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ORIGINAL RESEARCH

Urine Phthalate Metabolites are Elevated in Patients with Esophageal Squamous Cell Carcinoma and Associated with Advanced Cancer Stage and Poor Survival

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Background: The aim of this study was to investigate the role of phthalate in patients with esophageal squamous cell carcinoma (ESCC).

Methods: A total of 116 ESCC patients and 58 controls without any known histories of malignancies were enrolled. All eight urine phthalate metabolites were measured to assess phthalate levels. Clinical and urine phthalate metabolite profiles were compared between subgroups to identify differences, and the effects of phthalates on clinical ESCC outcomes were also examined.

Results: The concentrations of some urine phthalate metabolites were higher in the ESCC group than in the control group, including mono-(3-carboxypropyl) phthalate (MCPP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-n-butyl phthalate (MnBP). Higher concentrations of urine phthalate metabolites were associated with clinical T3–T4 status. Patients with higher concentration of mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-2-ethylhexyl phthalate (MEHP), and MEOHP had lower 1-year and 2-year overall survival (OS) rates than those with lower concentrations of these metabolites in our univariate analysis. Multivariate analysis showed that urinary MEHP of ≥3 μg/L and clinical stage IVB were independent prognostic factors for worse OS.

Conclusion: The results of our study showed that urine phthalate metabolites are elevated in ESCC patients and associated with advanced tumor stage, and that a high urinary concentration of MEHP is an independent prognostic factor of worse OS.

Keywords: phthalate, esophageal cancer, squamous cell carcinoma, survival

Introduction

Esophageal squamous cell carcinoma (ESCC) is an aggressive and life-threatening malignancy that ranks as the 9th leading cause of cancer-related mortality in Taiwan.[1] Some well-known risk factors for ESCC included cigarette smoking, alcohol drinking, betel nut chewing, achalasia, history of head and neck malignancy, and consumption of hot beverages/food.[2–8] In Taiwan, the incidence of esophageal cancer has been increased gradually and is more common in men than women. Despite recent developments in surgical techniques and systemic therapies, the prognosis of ESCC population is still poor.[9–11] Therefore, identification of other associated risk factors to prevent carcinogenesis or ESCC disease progression is crucial.

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Many chemical additives are hazardous to humans, and exposure to them may be caused through the production of plastics or due to leeching into products from plastic packaging.[12–14] Phthalates are plastic additives that are used to increase material transparency, durability, flexibility, and longevity. Phthalates readily leach out of products due to being incapable of covalent binding to other materials, contributing to human exposure to phthalates via ingestion, inhalation, absorption via dermal contact through food storage containers, children's toys, plastic medical tubing, cosmetics, furniture, clothing, and personal care products.[15,16] The National Health and Nutrition Examination Survey showed that more than 75% of adults in USA experienced phthalate exposure, and at least one kind of urine phthalate metabolite could be detected.[17] The biology of phthalates mimics various hormones and is involved in several endocrine pathways, resulting in their interference in health processes including child development, fertility, obesity, type 2 diabetes mellitus, cardiovascular disorders, pregnancy, and cancer.[13,15] Phthalates function as endocrine disruptors and mimic xenoestrogens, exhibiting reproductive, developmental, and carcinogenic toxicity in both human and animal studies.[18–22] One potential mechanism is that phthalates influence cancer cell proliferation by generating reactive oxygen species (ROS), leading to the accumulation of oncogenic changes that foster cancer development through DNA damage and genomic instability, such as colon tumorigenesis or hepatic tumorigenesis.[23–27] Another possible mechanism is that phthalates could promote cancer cells invasion through activating matrix metalloproteinases-9 overexpression.[28] Phthalates also mimic estradiol and enhance the development of reproductive cancers via estrogen receptor signaling, including breast cancer and uterine malignancies.[13,29]

Growing evidence has focused on the association between phthalates and cancer. Breast cancer is the most studied cancer type in this regard. Ahern et al demonstrated that a high exposure level to dibutyl phthalate was related to an approximately two-fold increase in the incidence of hormone receptor (HR)-positive breast cancer.[12] Another study also revealed that exposure to phthalates was associated with an increased risk of not only HR-positive breast cancer but also HR-negative breast cancer.[30] However, not all metabolites of phthalates are associated with an increased risk of breast cancer. One systematic review showed that a significantly positive association between the metabolites of di(2-ethylhexyl) phthalate (DEHP) and the risk of breast cancer risk existed, particularly in the case of mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP).[31] However, another meta-analysis reported that mono-benzyl phthalate (MBzP) and mono-2-isobutyl phthalate (MiBP), two metabolites of phthalates, were negatively associated with breast cancer, whereas there was no association between other metabolites and breast cancer, including mono-ethyl phthalate (MEP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(3-carboxypropyl) phthalate (MCPP), and mono-n-butyl phthalate (MnBP).[32] There have been several studies that have explored the effects of phthalates in other cancer types, such as urothelial carcinoma, gastric cancer, and prostate cancer.[33–35] However, to the best of our knowledge, the association between phthalates and ESCC remains unclear. This study aimed to investigate the role of phthalates in patients with ESCC.

Materials and Methods

Study Population

Patients with ESCC at Kaohsiung Chang Gung Memorial Hospital between January 2018 and December 2022 were enrolled. Clinical tumor staging was determined according to the 8th edition of the American Joint Committee on Cancer staging system.[36] We excluded patients with other pathologies other than squamous cell carcinoma (eg adenocarcinoma, neuroendocrine tumor, small cell carcinoma, etc). Moreover, patients with histories of second primary cancers, whether before or after ESCC diagnosis, were excluded. Only patients who were capable of cooperating with the procedures, including regular laboratory blood/urine tests, imaging examinations, and survival follow-ups were included. Another 58 subjects without any known histories of malignancies were enrolled as a control group. In the end, a total of 116 patients with ESCC who met the inclusion criteria were recruited.

Sample Collection and Analysis

For urine samples pre-screened urine sampling collection devices were used, to rule out external contamination with target analytes from the sampling procedures. We sub-aliquoted the samples in 1.5 mL tubes and stored them at −80°C until extraction. We followed the manufacturer's instructions for sample preparation and ultra performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) analysis.

Materials

The concentrations of eight urinary phthalate metabolites were measured, including MBzP, MECPP, MEHHP, MEHP, MEOHP, MEP, mono-methyl phthalate (MMP), and MnBP. Calibration analytes, deuterated internal standards (IS), β-Glucuronidase, LC/MS grade methanol, acetonitrile, formic acid, ammonium hydroxide and ammonium acetate will be purchase from Sigma–Aldrich (St. Louis, MO, USA). Deionized water (18.2 MΩ cm, organic carbon content ≤4 μg L^{-1}) will be obtained from a PURELAB Classic system (ELGA LabWater).

The urine specimens were allowed to reach room temperature and then subjected to centrifugation at 3000g for 10 minutes. The resulting supernatants were harvested for subsequent analysis. Each 300 μL urine sample was combined with 50 μL of an internal standard (IS) solution (200 ng/mL), 25 μL of β-glucuronidase solution (100 U/mL) adjusted to pH 6.5, and 275 μL of an ammonium acetate solution (1 mmol/L). This mixture was thoroughly mixed by vortexing for 1 minute and subsequently placed in a dry bath for incubation at 37°C for 2 hours. Following the incubation period, 1 mL of a 4% formic acid buffer solution was added to each deconjugated urine sample to halt enzymatic activity. Subsequently, the deconjugated urine samples were processed using an Oasis MAX Plate (30 mg, 30 μm) and extracted according to the recommended protocol for Oasis solid-phase extraction (SPE) by Waters, USA. The SPE cartridge was conditioned by successive washes with 1 mL of methanol and 1 mL of deionized water, followed by the loading of either deconjugated urine samples or quality control (QC) samples. After washing with 1 mL of 5% ammonium hydroxide in water, elution was performed using 300 μL of 2% formic acid in methanol.

The LC–MS/MS analysis was conducted utilizing a Waters Acquity UPLC™ system (Waters Co., UK), comprising a binary solvent manager, an automatic liquid chromatographic sampler, and a Waters Xevo™ tandem quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. Separation was achieved using a Waters Acquity UPLC® CSHTM Phenyl-Hexyl 1.7 μm 2.1×100 mm analytical column, which was maintained at 40°C within a column oven. A 5 μL injection volume was employed. The mobile phase consisted of methanol and 10 mM ammonium acetate (90:10; v/v), with a flow rate set at 0.3 mL/min. The MS/MS mode operated in the positive ion mode, with ESI parameters set as follows: capillary voltage, 3.20 kV; source temperature, 150°C; desolvation temperature, 400°C; desolvation gas flow, 800 L/h. The cone voltage was maintained at 40 V, and the collision energy at 39 V. Data acquisition, presentation, and peak quantification were facilitated by the MassLynx 4.1 software package.

Internal Standards and Standard Curve Preparation

Isotopically labeled phthalates were spiked in the sample before extraction to evaluate variation of extraction yields and changes. The standard mixture was prepared by pooling the commercial stock solutions, which were serially diluted down (at least 9 dilutions) to create calibration curves.

Method Validations

Linearity, lower limit of quantification (LLOQ), intra- and inter-assay accuracy and precision, matrix effects, and long-term storage stability were evaluated in this study. The linearity of the calibration curve was verified over a span of ten days within the concentration range of 0.01 to 200.0 ng/mL. Peak area ratios of analytes to their corresponding internal standards (IS) were utilized to compute the correlation coefficient, intercept, and slope. Accuracy and precision were assessed by spiking quality control (QC) samples at three concentrations (low, medium, and high) for each analyte, with each concentration tested in five replicates. Recovery rates were determined by comparing analyte responses from pre-extraction spiked samples to those from post-extraction spiked samples. Additionally, triplicate QC samples at three concentrations of reference standards were prepared in extracted biofluid from 10 healthy volunteers for each sample source. The matrix effect was evaluated by comparing IS response in the calibration matrix to IS response in the biological matrix post-extraction. LOQ was defined as the concentration at which the signal-to-noise ratio reached 10. All linear curves were required to have a coefficient of estimation of at least >0.995. The measured concentrations were reported as a percentage of the expected value along with the relative standard deviation (RSD). Results were deemed acceptable if the RSD of measurements was below 15% and the mean measurement fell within 85−115% of the expected values.

Ethics Statement

The present study received approval from the Chang Gung Medical Foundation Institutional Review Board (202001029B0). All procedures were conducted in compliance with the ethical principles outlined by the Institutional Research Committee and the World Medical Association Declaration of Helsinki. Prior to participation, written informed consent was obtained from each patient, and all methodologies were executed in accordance with the approved protocol.

Statistical Analysis

S For all statistical analyses, SPSS software version 26 (International Business Machines Corp., Armonk, NY, USA) was employed. The Chi-square test and Student's *t*-test were utilized to assess differences in categorical and continuous variables, respectively. Survival times were compared using the Kaplan-Meier method, with differences examined via the Log rank test. A multivariate Cox proportional hazard regression model was applied to identify independent prognostic factors. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to evaluate the strength of associations between prognostic factors and survival outcomes. Overall survival (OS) was defined as the duration from the diagnosis of ESCC to the date of death or the last living contact. All statistical tests were two-sided, and significance was set at $P < 0.05$.

Results

Patients

Between January 2018 and December 2022, we enrolled 116 patients with ESCC and another 58 subjects without histories of malignancies as a control group, at Kaohsiung Chang Gung Memorial Hospital. The mean age of the ESCC group was older than that of the control group, but the sex distribution was similar in both groups. Higher concentrations of MECCP, MEHHP, MEOHP and MnBP were found in the ESCC group compared to the control group, but there was no statistical difference in urine phthalate metabolites between both groups, including MBzP, MEHP, MEP and MMP. A comparison of baseline characteristics between these two groups is shown in Table 1.

Correlation Between Urine Phthalate Metabolites and Clinical Parameters in ESCC

Although patients with stage I–IVA had higher percentages of MBzP ≥0.15 than that in patients with stage IVB ($P = 0.013$), there were no statistical differences between all measured clinical parameters between the group with MBzP ≥0.15 μg/L and the group with MBzP <0.15 μg/L, including clinical T status, clinical N status, tumor grade, tumor location, and the presence of smoking, alcohol, or betel nut chewing (Table 2).

In the analysis of MECPP, there were no significant differences in any of the measured clinical parameters between the group with MECPP ≥18 μg/L and the group with MECPP <18 μg/L. In addition, patients with T3-T4 had higher concentrations of MECPP than those with T1-T2 (54.857 μg/L versus 16.221 μg/L, $P < 0.001$). A comparison of other parameters between groups with MECPP ≥ 18 μg/L or <18 μg/L did not reveal statistically significant differences (Table 2).

Table 2 also shows that the distribution of all clinical parameters was not different between groups with MEHHP ≥12 μg/L or <12 μg/L. Moreover, patients with clinical T3-T4 statuses had higher concentrations of MEHHP than those with clinical T1-T2 statuses (35.973 μg/L versus 11.700 μg/L, $P = 0.001$). The levels of MEHP were similar to those of MEHHP; higher concentrations of MEHP were observed in patients with clinical T3-T4 status compared to those with clinical T1-T2 status (6.993 μg/L versus 3.379 μg/L, $P = 0.010$).

The presentation of MEOHP, MEP and MnBP were similar; there were no significant differences in any clinical parameters between the groups with MEOHP ≥8 μg/L or <8 μg/L, those with MEP ≥ 10 μg/L or <10 μg/L and patients with MnBP ≥15 μg/L or MnBP <15 μg/L (Table 3). For MMP, a higher percentage of MMP ≥ 14 μg/L was found in patients with clinical T3-T4 statuses compared to those with clinical T1-T2 statuses ($P = 0.013$). Furthermore, higher concentrations of MMP were found in patients with clinical T3-T4 statuses (20.070 μg/L versus 14.488 μg/L, $P = 0.019$) compared to those with clinical T1-T2 statuses (Table 3).

Table 1 Baseline Characteristics and Urinary Concentrations of Phthalates in 116 ESCC Patients and 58 Sex/Age Matched Control Participants

	ESCC (n=116)	Control (n=58)	*P* value
Age (years)			
Mean	60.12±8.91	55.55±10.75	0.006*
Median	53.19	59.73	
Range	37.28~83.10	39.27~84.66	
Sex			
Male	112	56	1
Female	4	2	
MBzP (μg/L)			
Mean±SD	0.423±1.101	1.183±7.351	0.44
Median	0.15	0.15	
MECPP (μg/L)			
Mean±SD	50.902±86.980	14.290±25.964	<0.001*
Median	16.5	8.025	
MEHHP (μg/L)			
Mean±SD	33.583±63.241	11.966±11.001	<0.001*
Median	12.5	9.15	
MEHP (μg/L)			
Mean±SD	6.700±9.976	6.490±14.141	0.91
Median	2.85	2.55	
MEOHP (μg/L)			
Mean±SD	21.647±48.543	5.608±4.658	0.001*
Median	7.15	3.875	
MEP (μg/L)			
Mean±SD	30.817±80.229	16.191±38.954	0.19
Median	10.75	6.05	
MMP (μg/L)			
Mean±SD	19.396±15.872	15.326±9.895	0.08
Median	14.825	12.45	
MnBP (μg/L)			
Mean±SD	29.575±47.105	16.460±21.371	0.045*
Median	15.25	9	

Note: *Statistically significant.

Abbreviations: ESCC, esophageal squamous cell carcinoma; MBzP, mono-benzyl phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEP, mono-ethyl phthalate; MMP, mono-methyl phthalate; MnBP, mono-n-butyl phthalate; SD, standard deviation.

Urine Phthalate Metabolites and Clinical Outcomes

In our univariate analysis of OS, age, tumor grade, tumor location, smoking, alcohol consumption, betel nut chewing, and five urine phthalate metabolites (MBzP, MEHHP, MEP, MMP and MnBP), no statistical significances were found. Worse 1-year or 2-year OS rates were observed in patients with clinical T3-T4 statuses compared to those with clinical T1-T2 statuses (P = 0.045), and patients with clinical N2-N3 statuses had lower 1-year and 2-year OS rates compared to those with clinical N0-N1 statuses ($P = 0.012$). Advanced tumor stage was associated with worse OS than early tumor stage, stage IVB versus stage I–IVA ($P < 0.001$). In addition, a higher concentration of urine phthalate metabolites was related to shorter OS. Patients with MECPP ≥ 18 μg/L had worse 1-year and 2-year OS rates compared to those with MECPP < 18 μg/L ($P = 0.035$, Figure 1A). Lower 1-year and 2-year OS rates were found in the MEHP ≥ 3 μg/L group than in the MEHP < 3 μg/L group ($P = 0.005$, Figure 1B). Patients with MEOHP ≥ 8 μg/L had shorter OSs in comparison to those with MEOHP < 8 μg/L ($P = 0.030$, Figure 1C). Moreover, a multivariate analysis showed that MEHP ≥ 3 μg/L (OR:

Table 2 The Correlation Between Urinary MBzP, MECPP, MEHHP, MEHP and Clinicopathological Parameters in 116 Patients with ESCC

Parameters	MBzP (μg/L)			MBzP (μg/L)		MECPP (μg/L)			MECPP (μg/L)		MEHHP (μg/L)			MEHHP (μg/L)		MEHP (μg/L)			MEHP (μg/L)	
	< 0.15	≥ 0.15	*P* value	Mean±SD	*P* value	< 18	≥ 18	*P* value	Mean±SD	*P* value	< 12	≥ 12	*P* value	Mean±SD	*P* value	< 3	≥ 3	*P* value	Mean±SD	*P* value
Clinical T classification																				
T1-2 (n=14)	5	9	0.51	0.436±0.717	0.97	10	4	0.10	16.221±15.869	< 0.001*	8	6	0.40	11.700±10.547	0.001*	9	5	0.32	3.379±3.302	0.010*
T3-4 (n=102)	46	56		0.422±1.147		49	53		54.857±91.132		46	56		35.973±66.538		51	51		6.993±10.367	
Clinical N classification																				
N0-1 (n=48)	20	28	0.68	0.337±0.532	0.48	28	20	0.18	52.854±99.060	0.78	22	26	0.90	35.413±70.031	0.74	24	24	0.76	7.488±11.918	0.39
N2-3 (n=68)	37	31		0.484±1.369		31	37		48.316±77.138		32	36		31.372±57.937		36	32		5.899±8.113	
8th clinical AJCC staging																				
Stage I–IVA (n=84)	31	53	0.013*	0.481±1.245	0.36	46	38	0.17	47.573±89.960	0.60	40	44	0.71	33.094±69.948	0.99	46	38	0.29	6.609±10.503	0.93
Stage IVB (n=32)	20	12		0.271±0.565		13	19		57.073±77.598		14	18		32.911±40.068		14	18		6.417±8.030	
Tumor grade																				
Grade 1+2 (n=107)	47	60	1.00	0419±1.127	0.88	54	53	1.00	51.529±89.354	0.57	49	58	0.73	33.972±65.142	0.59	54	53	0.49	6.538±9.700	0.95
Grade 3 (n=9)	4	5		0.476±0.781		5	4		34.322±38.210		5	4		22.006±24.662		6	3		6.772±12.159	
Primary tumor location																				
Upper (n=30)	15	15	0.44	0.206±0.251	0.21	19	11	0.11	49.342±79.505	0.95	17	13	0.20	29.832±57.077	0.75	17	13	0.53	5.644±9.331	0.56
Middle + Lower (n=86)	36	50		0.499±1.264		40	46		50.491±89.253		37	49		34.164±65.167		43	43		6.875±10.057	
Alcohol																				
Absence (n=2)	0	2	0.50	0.565±0.262	0.86	1	1	0.98	67.925±74.706	0.77	0	2	0.50	41.650±32.527	0.85	0	2	0.23	7.015±1.011	0.95
Presence (n=114)	51	63		0.421±1.111		58	56		49.883±86.946		54	60		32.893±63.431		60	54		6.548±9.936	
Smoking																				
Absence (n=8)	2	6	0.46	0.541±0.669	0.76	5	3	0.72	32.956±42.223	0.56	3	5	0.72	16.331±20.305	0.44	5	3	0.72	3.251±2.565	0.33
Presence (n=108)	49	59		0.415±1.129		54	54		51.471±88.888		51	57		34.282±64.892		55	53		6.801±10.147	
Betel-nut chewing																				
Absence (n=25)	12	13	0.65	0.457±0.782	0.86	13	12	0.90	41.550±58.817	0.58	12	13	0.87	23.971±35.663	0.42	13	12	0.97	4.545±6.505	0.25
Presence (n=91)	39	52		0.414±1.178		46	45		52.569±92.799		42	49		35.536±68.533		47	44		7.109±10.547	

Note: *Statistically significant.

Abbreviations: ESCC, esophageal squamous cell carcinoma; MBzP, mono-benzyl phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono-2-ethylhexyl phthalate; SD, standard deviation; AJCC, American Joint Committee on Cancer.

Table 3 The Correlation Between Urinary MEOHP, MEP, MMP, MnBP and Clinicopathological Parameters in 116 Patients with ESCC

Parameters	MEOHP (μg/L)			MEOHP (μg/L)		MEP (μg/L)			MEP (μg/L)		MMP (μg/L)			MMP (μg/L)		MnBP (μg/L)			MnBP (μg/L)	
	< 8	≥ 8	P value	Mean±SD	P value	< 10	≥ 10	P value	Mean±SD	P value	< 14	≥ 14	P value	Mean±SD	P value	< 15	≥ 15	P value	Mean±SD	P value
Clinical T classification																				
T1-2 (n=14)	10	4	0.24	6.014±5.415	0.21	7	7	0.89	25.307±28.768	0.79	11	3	0.013*	14.488±5.946	0.019*	6	8	0.67	20.413±19.742	0.44
T3-4 (n=102)	56	46		23.134±50.804		49	53		31.573±84.956		44	58		20.070±16.689		50	52		30.823±49.634	
Clinical N classification																				
N0-1 (n=48)	30	18	0.31	20.995±48.363	0.99	26	22	0.29	21.442±28.082	0.29	24	24	0.64	19.465±12.375	0.97	18	30	0.05	27.857±30.354	0.74
N2-3 (n=68)	36	32		21.119±48.059		30	38		37.435±101.919		31	37		19.347±18.028		38	30		30.773±56.208	
8th clinical AJCC staging																				
Stage I–IVA (n=84)	51	33	0.18	21.108±53.223	0.99	43	41	0.31	22.620±33.600	0.25	41	43	0.63	18.809±14.899	0.52	40	44	0.82	23.987±25.746	0.16
Stage IVB (n=32)	15	17		20.963±30.881		13	19		52.334±142.110		14	18		20.935±19.212		16	16		44.212±78.436	
Tumor grade																				
Grade 1+2 (n=107)	60	47	0.73	21.985±49.776	0.48	52	55	1.00	31.589±83.233	0.72	54	53	0.034*	18.886±15.971	0.23	52	55	1.00	28.452±46.246	0.38
Grade 3 (n=9)	6	3		10.161±10.539		4	5		21.642±25.162		1	8		25.461±14.045		4	5		42.817±57.881	
Primary tumor location																				
Upper (n=30)	18	12	0.69	20.131±36.128	0.90	15	15	0.83	16.903±20.856	0.27	13	17	0.60	16.266±9.078	0.10	16	14	0.52	23.986±31.118	0.45
Middle + Lower (n=86)	48	38		21.395±51.654		41	45		35.671±92.021		42	44		20.487±17.552		40	46		31.513±51.550	
Alcohol																				
Absence (n=2)	1	1	1.00	28.215±28.730	0.83	0	2	0.50	20.375±4.137	0.85	0	2	0.50	34.765±20.103	0.17	0	2	0.50	79.840±2.814	0.13
Presence (n=114)	65	49		20.943±48.312		56	58		31.000±80.923		55	59		19.126±15.766		56	58		28.684±47.042	
Smoking																				
Absence (n=8)	5	3	1.00	10.660±15.696	0.53	5	3	0.48	12.894±13.602	0.52	3	5	0.72	20.748±13.660	0.80	4	4	1.00	21.160±26.796	0.60
Presence (n=108)	61	47		21.839±49.486		51	57		32.145±82.946		52	56		19.296±16.076		52	56		30.189±48.297	
Betel-nut chewing																				
Absence (n=25)	13	12	0.58	13.338±18.322	0.37	13	12	0.67	47.822±151.869	0.49	13	12	0.60	17.087±11.314	0.41	10	15	0.35	40.352±51.421	0.23
Presence (n=91)	53	38		23.191±53.200		43	48		26.145±44.405		42	49		20.030±19.909		46	45		26.603±45.711	

Note: *Statistically significant.

Abbreviations: ESCC, esophageal squamous cell carcinoma; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEP, mono-ethyl phthalate; MMP, mono-methyl phthalate; MnBP, mono-n-butyl phthalate; SD, standard deviation; AJCC, American Joint Committee on Cancer.

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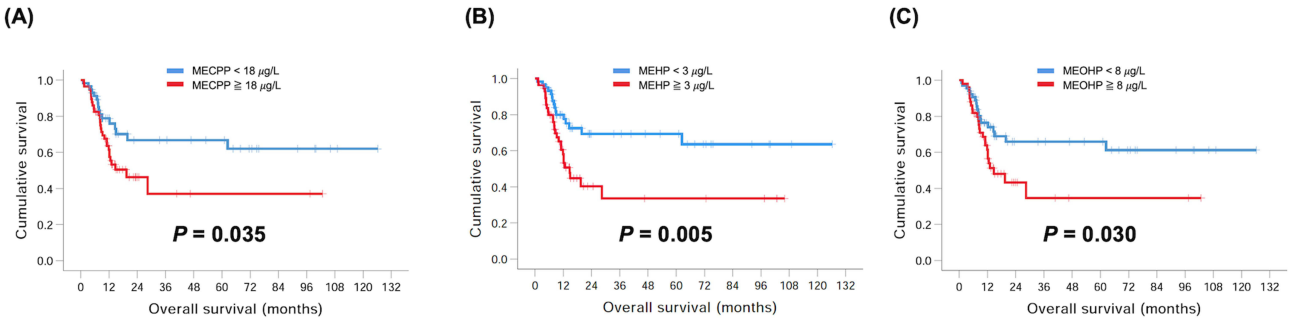

Figure 1 Comparison of Kaplan–Meier curves of overall survival in 116 patients with esophageal squamous cell carcinoma according to the concentration of urinary phthalate metabolites. (**A**) MECPP; (**B**) MEHP, and (**C**) MEOHP.
Abbreviations: MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate.

2.935, 95% CI: 1.256–6.681, P = 0.013) and tumor stage IVB (OR: 2.887, 95% CI: 1.220–6.835, P = 0.016) were independent prognostic factors for a worse OS. The survival outcomes of the univariate and multivariate analyses are shown in Tables 4 and 5, respectively.

Table 4 Results of Univariate Log-Rank Analysis of Prognostic Factors for Overall Survival in 116 Patients with ESCC

	Number of Patients	1-Year OS Rate (%)	2-Year OS Rate (%)	*P* value
Age (years)				
< 60	58	68%	57%	0.18
≥ 60	58	71%	54%	
MBzP (μg/L)				
< 0.15	51	64%	46%	0.34
≥ 0.15	65	74%	62%	
MECPP (μg/L)				
< 18	59	79%	67%	0.035*
≥ 18	57	62%	46%	
MEHHP (μg/L)				
< 12	54	75%	63%	0.17
≥ 12	62	64%	50%	
MEHP (μg/L)				
< 3	60	80%	69%	0.005*
≥ 3	56	58%	40%	
MEOHP (μg/L)				
< 8	66	76%	66%	0.030*
≥ 8	50	61%	43%	
MEP (μg/L)				
< 10	56	76%	60%	0.17
≥ 10	60	64%	51%	
MMP (μg/L)				
< 14	55	75%	62%	0.23
≥ 14	61	65%	51%	
MnBP (μg/L)				
< 15	56	63%	57%	0.38
≥ 15	60	75%	55%	
Clinical T classification				
T1-2	14	93%	93%	0.045*
T3-4	102	66%	51%	

(*Continued*)

Table 4 (Continued).

	Number of Patients	1-Year OS Rate (%)	2-Year OS Rate (%)	*P* value
Clinical N classification				
N0-1	48	83%	68%	0.012*
N2-3	68	59%	47%	
8th clinical AJCC staging				
Stage I–IVA	84	79%	66%	<0.001*
Stage IVB	32	44%	30%	
Tumor grade				
Grade 1+2	107	70%	55%	0.65
Grade 3	9	63%	63%	
Primary tumor location				
Upper	30	62%	55%	0.89
Middle + Lower	86	72%	56%	
Alcohol				
Absence	2	100%	100%	0.43
Presence	114	69%	55%	
Smoking				
Absence	8	88%	88%	0.20
Presence	108	68%	54%	
Betel nut chewing				
Absent	25	60%	47%	0.26
Present	91	72%	58%	

Note: *Statistically significant.
Abbreviations: ESCC, esophageal squamous cell carcinoma; OS, overall survival; MBzP, mono-benzyl phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEP, mono-ethyl phthalate; MMP, mono-methyl phthalate; MnBP, mono-n-butyl phthalate; SD, standard deviation; AJCC, American Joint Committee on Cancer.

Table 5 Results of Multivariate Cox Regression Analysis for Overall Survival in 116 Patients with Esophageal Squamous Cell Carcinoma

Factors	OR (95% CI)	*P* value
Age ≥ 60 years versus < 60 years	1.345 (0.646–2.801)	0.43
Clinical T3-4 versus T1-2	1.993 (0.470–8.448)	0.35
Clinical N2-3 versus N0-1	1.221 (0.479–3.111)	0.68
AJCC stage IVB versus stage I–IVA	2.887 (1.220–6.835)	0.016*
Tumor grade 3 versus 1+2	1.389 (0.443–4.352)	0.57
Tumor location upper versus middle + lower	1.297 (0.574–2.928)	0.53
MBzP ≥ 0.15 μg/L versus < 0.15 μg/L	1.036 (0.485–2.217)	0.93
MECPP ≥ 18 μg/L versus < 18 μg/L	1.342 (0.439–4.098)	0.61
MEHHP ≥ 12 μg/L versus < 12 μg/L	1.145 (0.369–3.559)	0.81
MEHP ≥ 3 μg/L versus < 3 μg/L	2.935 (1.256–6.681)	0.013*
MEOHP ≥ 8 μg/L versus < 8 μg/L	1.435 (0.413–4.985)	0.57
MEP ≥ 10 μg/L versus < 10 μg/L	1.337 (0.659–2.716)	0.42
MMP ≥ 14 μg/L versus < 14 μg/L	1.197 (0.561–2.555)	0.64
MnBP ≥ 15 μg/L versus < 15 μg/L	2.008 (0.966–4.184)	0.06
Smoking presence versus absence	6.236 (0.660–58.924)	0.11
Betel-nut chewing presence versus absence	2.217 (0.996–4.926)	0.05

Note: *Statistically significant.
Abbreviations: AJCC, American Joint Committee on Cancer; MBzP, mono-benzyl phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEP, mono-ethyl phthalate; MMP, mono-methyl phthalate; MnBP, mono-n-butyl phthalate; OR, odds ratio; 95% CI, 95% confidence interval.

Discussion

Phthalates have been reported to play crucial roles in the development of the reproductive system, and to exert carcinogenic effects.[37–39] Several studies have demonstrated that exposure to phthalates is related to increased risk of developing breast cancer, but the effect of phthalates on ESCC remains unclear.[31,40,41] In this case-control study, we showed that the concentrations of most urine phthalate metabolites were higher in ESCC patients than in a healthy control group. In addition, urine phthalate metabolites were elevated in ESCC patients with more advanced tumor stages, and a positive association between urinary concentration of MEHP and OS was also found. This is the first study to investigate the potential influences of phthalates in the pathogenesis and tumor progression of ESCC.

Regarding carcinogenesis, the association between phthalates and breast cancer has been explored widely, but the epidemiological information is still inconsistent. Several studies have confirmed the significance of phthalates in the increased risk of breast cancer, such as exposure to MECPP and MEHP.[31,40–42] However, two other studies revealed a different result and reported inverse associations between urinary concentrations of phthalate metabolites and breast cancer, as well as subsequent survival.[43,44] One meta-analysis, which focused on the association between phthalates and breast cancer, showed that MiBP and MBzP were negatively associated with breast cancer, whereas other metabolites such as MEHHP and MEOHP were unrelated to increased incidence of breast cancer.[32] One reason for the different findings of these studies may have been different study designs, sample sizes, ethnic compositions, ages, regions, and inclusion/exclusion criteria. In addition, phthalates are usually used as excipients in some drugs, and patients using phthalate-containing prescription drugs may have 50-fold higher urinary excretions of phthalate metabolites compared to non-users. A Danish study designed to examine the association between cumulative phthalate exposure through such drugs and the risk of gastric cancer did not find an increased risk of gastric cancer from the use of phthalate-containing drug products. On the other hand, DEHP is one of the most widely used phthalates, and may increase matrix metalloproteinase-9, which can contribute to the development of many cancer types such as breast cancer and urothelial cancer.[28] Chou et al demonstrated that MEHHP may be related to urothelial cancer in patients with chronic kidney disease, and the association was independent of other well-known risk factors for urothelial cancer.[33] In the past, the role of phthalates in ESCC was unclear, but the results of our study have revealed an association, showing that phthalates may be related to the disease progression of ESCC.

MEHP is one of the main metabolites of DEHP, which is widely used in the production of polyvinyl chloride materials and easily accumulates in the human body. Several studies have shown that MEHP may regulate the progression of various cancer types through multiple complex signal pathways, such as triggering tumor cell proliferation and migration via activation of NF-κB signals.[45–47] The direct exposure to MEHP during food and water digestion may contribute to the incidence of oral cavity cancers. Wang et al showed that MEHP promoted tumor proliferation and disease progression of oral cavity cancers by inducing the expression of c-Myc and subsequent down-regulation of miR-27b-5p and miR-372-5p.[45] On the other hand, phthalate exposure has been linked to reproductive dysfunction, resulting in adverse normal germ cell development. Yao et al showed that MEHP induces matrix metalloproteinase-2 and c-Myc expression in testicular embryonal carcinoma, contributing to tumor cell invasion, migration, and metastasis.[48] Furthermore, genome-wide gene expression profiles have also revealed that MEHP exposure primarily influenced genes in cell adhesion and transcription. Inhibitors of DNA-binding protein-1, vinculin, and Gap junction protein-alpha 1 were significantly down-regulated by MEHP treatment, while the expression of beta 1-catenin and claudin-6 were up-regulated.[48] In addition, Su et al also revealed that urinary concentrations of MEHP were significantly higher in patients with colorectal cancer compared to those with adenoma or healthy controls, suggesting that higher exposure to MEHP may result in higher incidences of colorectal cancer.[49] Detection of urinary MEHP may serve as a beneficial non-invasive biomarker of increased colorectal cancer risk.[49] In our study, MEHP ≥ 3 μg/L was an independent prognostic factor for a worse OS, consistent with the findings of previous studies.

There were several limitations in our study. First, the duration of follow-up may not have been long enough, resulting in the effect of some parameters potentially being insignificant. Second, the percentage of female patients in our study was relatively low, contributing to a difficulty examining the effect of sex in the presence of urine phthalate metabolites. In general, the prevalence of smoking, alcohol consumption, and betel nut chewing is more common in men than in women. However, to the best of our knowledge, the current study is the first research to investigate the role of phthalates in the carcinogenesis and disease progression of ESCC, and our findings may suggest an association between phthalates and ESCC.

Conclusions

The results of our study showed that urine phthalate metabolites were elevated in ESCC patients, and were associated with advanced tumor stages, and that a high concentration of MEHP was an independent prognostic factor of worse OS.

Institutional Review Board Statement

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Chang Gung Medical Foundation Institutional Review Board (202001029B0).

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Informed Consent Statement

Informed consent was obtained from each patient, and all methods were performed in accordance with approved protocol.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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