Associations Between Paraben In Human Serum And Rheumatoid Arthritis Risks

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Abstract

Parabens are widely used in consumer products resulting in frequent exposure to humans and some toxic effects in humans. However, little is known about the association between human paraben exposure and rheumatoid arthritis (RA). In this study, a case-control cohort ($n = 290$) were established in Hangzhou, China, aiming to quantify the residues of MeP, EtP, PrP, and BuP in serum samples and to determine their associations with RA risks. MeP (mean 4.7 ng/mL, < LOD–20 ng/mL) was the predominant paraben in human serum, followed by PrP (1.9 ng/mL, < LOD–24 ng/mL), EtP (1.4 ng/mL, < LOD–10 ng/mL), and BuP (1.09 ng/mL, < LOD–10 ng/mL). Human serum concentrations of MeP were correlated with the levels of rheumatoid factors [adjusted $\beta = 0.19$, 95% confidence interval (CI): 0.12, 0.46], anti-cyclic citrullinated peptide antibody (adjusted $\beta = 0.30$, 95% CI: 0.26, 0.58), immunoglobulin G (adjusted $\beta = 0.24$, 95% CI: 0.21, 0.30). Significant associations between MeP (adjusted $\beta = 0.15$, 95% CI: 0.037, 0.28) and PrP (adjusted $\beta = 0.23$, 95% CI: 0.080, 0.38) exposure were correlated with C-reactive protein levels. In addition, a correlation between MeP and odds ratios (OR) of RA ($OR_{\text{crude}} = 1.337$, CI: 1.110, 1.624, $p = 0.03$; $OR_{\text{adjusted}} = 1.866$, CI: 1.321, 2.636, $p = 0.02$) was positive and significant. To our knowledge, this study first finds the significant associations among parabens exposure, change of specific immune marker, and RA risks, indicating that human exposure to parabens may increase the risk of RA.

Introduction

Parabens (*p*-hydroxybenzoate) are a family of compounds with phenolic groups, mainly including methyl paraben (MeP), ethyl paraben (EtP), propyl paraben (PrP), and butyl paraben (BuP) (Fisher et al., 2020). Parabens have unique antibacterial properties with relatively low prices, hence they have been widely applied as antimicrobial preservatives and aromatics in various consumer products, such as food and beverages processing, personal care products, and drugs, for more than 90 years (Bledzka et al., 2014, Fisher et al., 2017, Snodin, 2017). Humans can be exposed to paraben in many ways, including cosmetics (Bledzka et al., 2014), diet (Zhao et al., 2021), indoor dust (Wang et al., 2012), and drinking water (Ferreira et al., 2011). The frequent detection of paraben in the blood (Li et al., 2020) and urine (Jurewicz et al., 2020) also verifies the high exposure of humans to this substance.

In 2008, Cosmetic Ingredient Review and US Food and Drug Administration conducted safety assessments on the use of parabens and concluded that their use in cosmetics was safe (Andersen, 2008, Fisher et al., 2017). However, parabens have long been considered as endocrine disruptors, and their estrogenic activities increased with the increase of the alkyl substituent length (Han and Washington, 2005). Moreover, parabens could cause a wide range of adverse health effects on animals and humans, such as significantly lower sperm counts and testosterone levels in rats (Kang et al., 2002, Oishi, 2001), decreased body weight and height in children (Wu et al., 2019), and decreased serum thyroid levels in humans (Aker et al., 2018). Hence, European Union imposed restrictions on the use of parabens in cosmetics from the year of 2009 (i.e., < 0.4% for single and < 0.8% for mixtures of parabens) (Zhu et al., 2020). China is the second-largest consumer of cosmetics in the world (Song et al., 2020), paraben exposure may pose a health risk to the Chinese population.

To date, there have been many studies on the association of paraben with immune diseases, most of which have focused on sensitive populations such as mothers and infants or children. For example, an epidemiologic study on 587 pregnant women in France suggested that exposure to EtP was correlated with the increased rate of asthma (Vernet et al., 2017). Only one study from the United States explored the relationships of paraben with adult autoimmune diseases, which reported that urinary MeP, PrP, EtP, and BuP levels were negatively associated with inflammatory bowel disease in people aged 20–80 years (de Silva et al., 2017). Therefore, studies between paraben

and adult autoimmune diseases in China are still lacking. Rheumatoid arthritis (RA), a systemic heterogeneous autoimmune disease, is characterized by symptoms such as joint destruction (Yarwood et al., 2016, Zeng et al., 2017). Some studies claimed that 0.5 to 1.0% of adults worldwide are affected by this disease (Tian et al., 2021). Although information on demographic trends in the prevalence of RA is scant, concern exists that there may be an increased risk of developing RA due to the increasingly frequent exposure to organic pollutants in the environment. The underlying pathogenic mechanism of RA is considered to be a disturbed immune response inducing increased susceptibility in the host in response to single or multiple environmental risk factors. Rheumatoid factors (RF), anti-cyclic citrullinated peptide antibody (ACPA), C-reactive protein (C-RP), and erythrocyte sedimentation rate (ESR) are commonly used as diagnostic markers of RA. Watkins et al. (2015) explored the relationship between paraben exposure and markers of oxidative stress and inflammation and reported that BuP concentrations were associated with C-RP levels. Therefore, it is necessary to quantify the parabens residuals in serum and to determine the relationship between RA and parabens exposure in humans.

Serum is one of the major matrix used for human biological monitoring studies (Angerer et al., 2007). Indicator values related to RA, including ACPA, RF, C-RP, ESR, and immunoglobulin G (IgG), are also determined in the human serum. Although parabens in the human body have a short half-life of about 24 to 72 hours, parabens can still be detected in human serum and blood samples (Boberg et al., 2010, Janjua et al., 2008). A domestic biomonitoring study has suggested that the detected frequencies of MeP, EtP, PrP, and BuP in young adult blood were 82%, 57%, 77%, and 41%, respectively (Zhang et al., 2020). Moreover, paraben exposure is widespread in different populations from various countries (Adoamnei et al., 2018, Iribarne-Duran et al., 2020, Vela-Soria et al., 2013).

In the present work, we established a case-control cohort, including 152 RA cases and 138 controls, and collected their whole blood samples in Hangzhou, China. Four parabens, including MeP, EtP, PrP, and BuP, were quantified in these serum samples. The main purposes of this study were to determine the profile of target parabens in human serum and to explore the relationship among paraben levels in human serum, immune markers, and incidence of RA by adjusting the covariates. This study contributes to our better understanding of the risk of human immunotoxicity from exposure to parabens.

Methods And Materials

Study Population and Sample Collection. Between August 2018 and November 2020, a case-control cohort, including 152 RA cases and 138 non-RA people (controls), was established in Hangzhou, China. After signing a written informed consent, all participants were received questionnaires regarding their demographic and health-related characteristics. The minimum eligibility criteria for RA patients included long-term residence in Hangzhou, with no occupational exposure, without the experience of infection in the last two months, and without pregnant or breastfeeding women. Recruitment criteria for controls were comparable to those described above, with the addition of no self-reported history of cancer and tumors. We extracted data on immune markers of subjects from medical records, including ACPA, RF, C-RP, ESR, and three types of immunoglobulins (IgA, IgG, and IgM). The study protocol was authorized by the Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine.

With the help of nurses in the hospital, the blood samples were collected from the study population in BD-Vacutainer collection tubes (Becton, Dickinson and Company, NJ, USA). After that, all blood samples were centrifuged at 4500 r/min for 15 min. The serums were transferred to new PP vials and stored in freezers at -80°C until extraction. During the sampling campaign, field blanks (5 mL of Milli-Q water) were transported along with the real human serum samples.

Sample Extraction. Serum paraben concentrations were analyzed using the method of Li et al. (2020), with slight modification. In brief, 500 μ L of serum spiked with 10 ng internal standards was added into 15 mL polypropylene (PP) tubes. Then, 6 mL of acetonitrile containing 1% formic acid was added to serum samples. The mixture was treated by sonication (53 kHz) for 30 min and centrifuged at 5000 r/min for 10 min. The supernatant was transferred to a new 15 mL PP tube, and the above extraction process was repeated one more time. The supernatants were combined and evaporated to 3 mL with mild nitrogen. The concentrated mixture was further processed by Oasis MCX SPE cartridge (100 mg/3 mL; Waters, USA). Before loading, the cartridge was pretreated with 5 mL of methanol and 5 mL of Milli-Q water. Target analytes eluted with 5 mL of methanol were evaporated to dryness with nitrogen and reconstituted with 100 μ L of methanol for instrumental analysis.

Instrumental Analysis. Analysis of parabens was conducted by high-performance liquid chromatography (HPLC) equipped with a triple quadrupole mass spectrometer (MS/MS), using an ACQUITY UPLC BEH C₁₈ column (1.7 μ m, 2.1 mm $\times$ 50 mm; Waters, Milford, USA) maintained at 40 $^{\circ}$ C. The mobile phase was pure water containing 2 mM ammonium acetate (A) and methanol (B), with a flow rate of 0.3 mL/min. The elution gradient started at 75% A and 25% B, then ramped up to 45% B by 1.5 min, increased to 100% B at 8 min, held for 1 min, and returned to the initial condition within 0.5 min. The MS/MS was operated in negative ionization mode and a chromatogram was recorded using multiple reaction monitoring (MRM). The ionization source temperature was 450 $^{\circ}$ C. Parent and product ions of four parabens are listed in the Supporting Information (SI, Table S1).

Quality Assurance and Quality Control. In this study, all solvents and reagents used were examined for paraben contamination, and no detectable parabens were observed. Field blank sample analysis was performed before the detection of real samples, and no measurable parabens were detected. After every ten samples, 10 μ L of methanol was analyzed to monitor the background contamination. At the same time as extracting real samples, procedural blank samples were also extracted. No detectable MeP, EtP, PrP, and BuP were detected in procedural blanks, so LODs of all target parabens were set as the concentrations corresponding to the three-times of signal-to-noise ratio. LODs of MeP, EtP, PrP, and BuP were 0.05, 0.09, 0.12, and 0.10 ng/mL, respectively (SI, Table S2). The internal-standard method was used for the quantification of parabens in human serum. Calibration curves of parabens (at least five concentration points) were highly linear with correlation coefficients higher than 0.995. Extraction recoveries of all compounds were determined by analyzing real human serum samples ($n = 3$) in this study and commercial fetal bovine serum ($n = 3$) spiked with the mixture of native standards (each spiked at 10 ng/mL). The mean extraction recoveries of parabens in human serum ranged from 97–123%. The intra-day relative standard deviation (RSD) of human serum samples ($n = 5$) spiked at 10 ng/mL parabens ranged from 8.6–12.8% and inter-day RSD ($n = 5$) within 3 weeks was 12–29%.

Statistical Analysis. We performed preliminary descriptive statistics to assess the demographic characteristics, immune markers, and serum paraben concentrations. Associations between individual serum paraben concentrations and serum immune marker levels were evaluated using Spearman's rank correlation analysis (r_s). We used LOD/ $\sqrt{2}$ to calculate the concentrations of samples below the LOD. The ln-transformed paraben concentrations were deemed as independent variables in the regression model. To satisfy the normal distribution of the residuals, all serum immune marker levels were ln-transformed. The multiple linear regression analysis was used to evaluate relationships of paraben concentrations in serum with immune marker levels. The stepwise selection method was adopted in this model to find the most suitable final model. Besides, non-linear trends were explored by dividing the natural ln-transformed paraben concentrations into quartiles, with the lowest quartile as the reference group. The logistic regression was conducted to further determine the association of paraben exposure with the incidence of RA. The models were adjusted for sex (male and female; categorical), age at enrollment (< 40, 40–50, 51–60, 61–70, >

70; categorical), education level (> 12 , $9-12 < 9$; categorical), BMI (< 18.5 , $18.5-24.9$, > 24.9 ; categorical), annual household income (< 50000 , $50000-100000$, > 100000 ; categorical), and parity (0, 1, 2, ≥ 3 ; categorical). All statistical analysis was performed using IBM SPSS Statistics (version 25; Chicago, IL, USA). The statistical significance was set as $p < 0.05$.

Results

Demographic Characteristics. Table 1 provides immune markers (mean $\pm$ SD) and descriptive data [n (%)] of all participants ($n = 290$). The study population ranged in the age from < 40 to > 70 , which is divided into 5 grades. The mean (range) age of the study population was 55 (28–84) years. More than half of the study population had BMI between 18.5 and 24.9 and had been educated for more than 9 years. 72% of RA cases never smoke, which is comparable to the controls (70%). Overall, there was no obvious difference in the characteristics of the RA cases and controls.

Table 1
Demographic Characteristics in This Study [$n = 290$; n (%) or mean $\pm$ SD].

Characteristic	Controls ($n = 138$)	RA cases ($n = 152$)
<i>Age (years)</i>		
< 40	20 (14)	21 (14)
40–50	48 (34)	37 (25)
51–60	38 (27)	35 (24)
61–70	22 (16)	30 (20)
> 70	13 (9)	26 (17)
<i>Education level (years)</i>		
> 12	34 (24)	37 (25)
9–12	76 (54)	75 (51)
< 9	32 (22)	36 (24)
<i>Body mass index (BMI, kg/m^3)</i>		
Underweight < 18.5	30 (22)	26 (17)
Normal 18.5 to < 24.9	98 (73)	110 (70)
Overweight > 24.9	6 (5)	20 (13)
<i>Annual household income (RMB/year)</i>		
< 50000	46 (32)	52 (36)
50000–100000	66 (45)	62 (43)
> 100000	33 (23)	31 (21)
<i>Medical history</i>		
Yes	35 (25)	24 (16)
No	103 (75)	128 (84)
<i>Smoking habit</i>		
Never	99 (72)	107 (70)
Current	20 (14)	21 (14)
Ever	19 (14)	24 (16)
<i>Parity</i>		
0	4 (3)	8 (5)
1	50 (34)	48 (32)
2	61 (41)	58 (38)

Characteristic	Controls (<i>n</i> = 138)	RA cases (<i>n</i> = 152)
≥ 3	33 (22)	38 (25)
<i>Sex</i>		
Male	70 (46)	71 (52)
Female	83 (54)	66 (48)
<i>Immune markers</i>		
ESR (mm/h)	15 $\pm$ 8.0	50 $\pm$ 33
C-RP (mg/L)	2.2 $\pm$ 1.0	31 $\pm$ 27
RF (IU/mL)	9.8 $\pm$ 33	316 $\pm$ 286
ACPA (IU/mL)	11 $\pm$ 1.8	449 $\pm$ 304
IgA (g/L)	2.1 $\pm$ 0.6	3.3 $\pm$ 2.4
IgG (g/L)	12 $\pm$ 2.2	25 $\pm$ 5.3
IgM (g/L)	1.0 $\pm$ 0.5	1.4 $\pm$ 1.5

Human Serum Concentrations of Parabens. Table 2 displays the concentrations of parabens in human serum samples. Among 4 parabens, MeP had the highest detection frequency (100% and 97%, respectively) in both RA cases and controls, followed by EtP (63% and 50%), and PrP (71% and 53%). BuP was detected in 55% and 43% of RA cases and controls serum samples, respectively. The mean serum concentrations of parabens ranked in the order of MeP (5.8 ng/mL) > PrP (2.0 ng/mL) > EtP (1.6 ng/mL) > BuP (1.4 ng/mL), accounting for 52.7%, 18.8%, 14.9%, and 13.6% of the total paraben burden in RA human samples, respectively (Fig. 1). Serum concentrations of MeP in RA cases (mean $\pm$ SD, 5.8 $\pm$ 4.6 ng/mL) were significantly ($p < 0.05$) higher than that in controls (4.0 $\pm$ 4.2 ng/mL).

Table 2
Distribution of Parabens Concentrations (ng/mL) in Human Serum from RA cases ($n = 152$) and controls ($n = 138$). The DR mean detection rate (%).

Analyte	DR (%)	Min	Mean ± SD	Max	Quartile		
					25th	50th	75th
RA Cases (n = 152)							
MeP	100	0.10	5.8 ± 4.6	20	2.0	4.7	8.1
EtP	63	< LOD	1.6 ± 1.8	10	< LOD	0.96	2.2
PrP	71	< LOD	2.0 ± 3.8	24	< LOD	0.74	2.1
BuP	55	< LOD	1.4 ± 1.5	10	< LOD	0.98	1.7
Controls (n = 138)							
MeP	97	< LOD	4.0 ± 4.2	18	1.2	2.6	5.4
EtP	50	< LOD	1.0 ± 1.7	8.3	< LOD	0.33	1.0
PrP	53	< LOD	1.6 ± 3.4	18	< LOD	0.49	1.4
BuP	43	< LOD	0.66 ± 0.53	3.2	< LOD	< LOD	0.77

Associations Between Serum Parabens and Immune Markers. Associations between concentrations of individual parabens in human serum, as well as between paraben concentrations and immune markers levels in human serum are displayed in the Table S3. Spearman correlation analysis showed that levels of MeP in human serum were significantly correlated with RF ($r_s = 0.48$, $p < 0.05$), ACPA ($r_s = 0.56$, $p < 0.01$), C-RP ($r_s = 0.35$, $p < 0.05$), as well as IgG ($r_s = 0.43$, $p < 0.05$) levels, but were not correlated with ESR ($r_s = 0.14$, $p = 0.43$), IgA ($r_s = 0.089$, $p = 0.58$), and IgM ($r_s = 0.14$, $p = 0.45$). PrP concentrations in human serum samples were significantly ($r_s = 0.40$, $p < 0.05$) correlated with C-RP levels.

For all participants, one unit increase in serum natural log-transformed MeP concentrations was correlated with increased in natural log-transformed RF levels [crude $\beta = 0.16$, 95% confidence interval (CI): 0.094, 0.33, $p < 0.05$; adjusted $\beta = 0.19$, 95% confidence interval (CI): 0.12, 0.46, $p < 0.05$] (Table 3) in both crude and adjusted models. The coefficients of log-transformed MeP to the dependent variable ACPA (crude $\beta = 0.28$, CI: 0.11–0.44, $p < 0.05$; adjusted $\beta = 0.30$, CI: 0.26–0.58, $p < 0.05$), C-RP (crude $\beta = 0.22$, CI: 0.070–0.37, $p < 0.05$; adjusted $\beta = 0.23$, CI: 0.08–0.38, $p < 0.05$), and IgG (crude $\beta = 0.23$, CI: 0.20–0.26, $p < 0.05$; adjusted $\beta = 0.24$, CI: 0.21–0.30, $p < 0.05$) were positively and statistically significant. Besides, the parabens concentrations (ln-transformed) were divided into quartiles to explore the nonlinear trends with immune markers of RA (Table S4).

Table 3

Multivariable Linear Regression Coefficients β (95% Confidence Interval) for Immune Markers and Ln-Transformed Paraben Concentrations in Study Population.

Outcome	MeP		EtP		PrP		BuP	
	crude β	adjusted β	crude β	adjusted β	crude β	adjusted β	crude β	adjusted β
RF	0.16 (0.094, 0.33)	0.19 (0.12, 0.46)	0.011 (-0.19, 0.21)	0.011 (-0.14, 0.16)	0.17 (-0.032, 0.39)	0.18 (-0.038, 0.39)	0.32 (-0.067, 0.72)	0.33 (0.055, 0.60)
ACPA	0.28 (0.11, 0.44)	0.30 (0.26, 0.58)	0.18 (-0.028, 0.39)	0.12 (-1.3, 1.5)	0.24 (0.050, 0.44)	0.14 (-1.3, 1.1)	0.40 (0.040, 0.58)	0.35 (-0.95, 1.4)
C-RP	0.13 (0.015, 0.25)	0.15 (0.037, 0.283)	0.23 (0.087, 0.38)	0.22 (-0.20, 0.64)	0.22 (0.070, 0.37)	0.23 (0.08, 0.38)	0.36 (0.084, 0.63)	0.36 (-0.30, 1.0)
ESR	0.19 (0.11, 0.27)	0.18 (0.051, 0.31)	0.10 (0.007, 0.19)	0.10 (-0.053, 0.26)	0.042 (-0.059, 0.14)	0.042 (-0.12, 0.29)	0.22 (0.039, 0.41)	0.24 (-0.078, 0.55)
IgA	0.12 (0.073, 0.18)	0.13 (-0.062, 0.32)	0.047 (-0.023, 0.11)	0.049 (-0.19, 0.29)	0.057 (-0.025, 0.13)	0.058 (-0.22, 0.34)	0.16 (0.045, 0.29)	0.17 (-0.25, 0.59)
IgG	0.23 (0.20, 0.26)	0.24 (0.21, 0.30)	0.093 (0.012, 0.083)	0.093 (-0.22, 0.41)	0.069 (0.023, 0.11)	0.074 (-0.33, 0.47)	0.036 (-0.030, 0.10)	-0.033 (-0.56, 0.49)
IgM	0.038 (-0.028, 0.10)	0.046 (-0.054, 0.14)	0.051 (-0.043, 0.14)	0.061 (-0.057, 0.18)	-0.064 (-0.16, 0.032)	-0.035 (-0.16, 0.095)	-0.10 (-0.23, 0.026)	-0.016 (-0.21, 0.18)
Note: Models adjusted for age, education level, BMI, annual household income, sex, smoking habit, parity, medical history. CI, confidence interval. The bold suggests the significance at $p < 0.05$.								

Significant associations of serum paraben concentration with an incidence of RA were found here (Table 4). The crude and adjusted odds ratio (OR) for RA was 1.337 and 1.866, respectively, for a unit increase in natural log-transformed MeP levels in human serum (95% CI_{crude}: 1.110–1.624, $P = 0.03$; 95% CI_{adjusted}: 1.321–2.636, $P = 0.02$). Apart from the above findings, we did not find a statistically significant association between other individual parabens and the incidence of RA.

Table 4

Crude and adjusted odds ratio (OR, 95% confidence intervals (CI)) for ln-transformed parabens exposure and risk of rheumatoid arthritis using a logistic regression model.

Analyte	Crude		Adjusted	
	OR (95% CI)	P _{trend}	OR (95% CI)	P _{trend}
MeP	1.337 (1.110, 1.624)	0.03	1.866 (1.321, 2.636)	0.02
EtP	1.255 (0.998, 1.562)	0.07	1.657 (0.976, 2.062)	0.1
PrP	1.466 (1.129, 1.905)	0.06	1.208 (0.564, 1.823)	0.07
BuP	0.918 (0.507, 1.452)	0.8	0.929 (0.518, 1.423)	0.9
Note: adjusted for age, education level, BMI, annual household income, sex, smoking habit, parity, medical history. MeP, methyl paraben; PrP, propyl paraben; EtP, ethyl paraben; BuP, butyl paraben; Model were adjusted P were used for trends and the bold means the significance at $p < 0.05$.				

Discussion

MeP (mean, 4.7 ng/mL) is the predominant paraben in human serum from all participants, with a detection frequency of 98%, which is consistent with the study on pregnant Chinese women (Li et al., 2020). This suggests that the Chinese population may be universally exposed to MeP. The median concentration of MeP (3.4 ng/mL) in serum of the study population is higher than that reported in Guangdong (0.21 ng/mL) (Song et al., 2020), Cambridge (1.6 ng/mL) (Fisher et al., 2020), and Granada (1.41 ng/mL) (Iribarne-Duran et al., 2020), but is much lower than that in Tamil Nadu (13.18 ng/mL) (Shekhar et al., 2017). This is probably attributed to the differences in lifestyle and use of individual care products.

PrP (mean, 1.9 ng/mL) was the secondary abundant paraben measured in human serum. Many studies have described the occurrence of PrP in human serum. For example, Yu et al. (2021) reported that PrP was detected in all samples ($n = 50$) at the geometric mean concentration of 0.31 ng/mL from Beijing, China. A biomonitoring study showed that 53% of PrP was measured in menstrual blood samples from Granada (Spain) at the mean concentration of 1.7 ng/mL (Iribarne-Duran et al., 2020). In this study, PrP was measured in 71% and 53% of serum samples in RA cases and healthy people, respectively. Previously, studies had demonstrated extremely high PrP concentration levels in personal care products, such as creams (mean 746 ng/mL) and body or hand lotion (mean 596 ng/mL) from China (Guo et al., 2014). As a result, the Hangzhou population may be exposed to extensive PrP through the daily use of personal care products.

EtP was detected in 52% of human serum samples with a mean concentration of 1.4 ng/mL. Previous studies showed that the detection frequencies and concentrations of EtP in foodstuffs were 84% and 11 ng/mL, respectively (Liao et al., 2013). Therefore, food intake may be one of the major sources of EtP exposure in Hangzhou, China. BuP was the least frequently detected paraben in human serum, with the detection frequencies lower than 55%. Interestingly, low concentrations and detection frequency of BuP had been reported in various matrixes, such as breast milk, indoor dust, river water, and soil, collected from several countries (Alshana et al., 2015, Wang et al., 2012, Zhao et al., 2020). The low detection frequency of BuP in serum maybe because of the low use of BuP in personal care products in China, and the rapid excretion of BuP in humans (due to its low hydrophobicity) (Guo et al., 2014).

To our knowledge, this is the first report to investigate the association between environmental paraben exposure and increased immune markers for RA in a general population cohort in Hangzhou. In this cohort, the patients were reviewed by medical records for ACPA, RA, immunoglobulins, and physical examination to verify disease, and approximately 85% agreed to a medical review, with an 80% efficiency rate. Our study showed that increased MeP exposure was significantly associated with increased levels of RF and ACPA. RF and ACPA are considered to be the specific autoantibody against the IgG Fc segment (Dobloug et al., 1980, Liou and Huang, 2020, Wang et al., 2017). Therefore, we examined the correlation between MeP concentrations in serum and levels of IgG in this study. The result denoted that a unit increase in serum MeP concentration levels was correlated with increased IgG levels. Despite that, whether MeP-induced IgG increase affects RF levels remains to be further exploited.

Further, a significant association of residual concentrations of MeP in serum with the incidence of RA was found. Plausible explanations for the association between MeP and RA include the initiation or exacerbation of the inflammatory response by affecting the oxidative homeostasis in the host. A toxicology experiment studied by Pishkari et al. found that increased levels of MeP in hemoglobin substrates resulted in earlier production of glycosylation end products and significantly increased the production of reactive oxygen species (ROS) (Pishkari et al., 2020). Another study on semen reported that exposure to 13mM MeP for 2 hours inhibited semen cell activity while causing an increase in ROS (Samarasinghe et al., 2018). Upregulation of ROS in the host may cause damage to biomolecules (e.g., proteins and lipids) by activating different signaling pathways (Phull et al., 2018). The oxidative stress may trigger an inflammatory response through the non-enzymatic degradation of proteins following amino acid oxidation, leading to the stable production of late glycosylation end products, which would allow the production of specific proteins associated with RA (Hitchon and El-Gabalawy, 2004). In addition, the study of Pishkari et al. also reported that lipid peroxidation (LPO) is enhanced with increasing concentrations of MeP (0-100 μ M) exposure (Pishkari et al., 2020). Polyunsaturated fatty acids generate lipid peroxy radicals under peroxidation, leading to cell membrane damage. Bereketolu and Pradhan et al. found that MeP exposure may trigger the secretion of inflammatory cytokines by inducing oxidative stress or altering fatty acid metabolism (Bereketoglu and Pradhan, 2019). In zebrafish experiments, the expression levels of two pro-inflammatory cytokines, tumor necrosis factor and interleukins, were significantly increased in the larval stage after exposure to MeP at concentrations of 1 and 10 μ M (Bereketoglu and Pradhan, 2019). IL-1 and TNF- α play important roles in RA by triggering multiple signaling pathways to activate transcription factors that affect relevant inflammatory mediator genes, leading to impaired host tolerance (Phull et al., 2018). Overall, these findings offer evidence for the relationship between PFOA and RA in the present study.

As for C-reactive protein, their relationships with higher serum MeP as well as PrP concentration levels were observed in the current study. Previously, the Puerto Rico Testsite for Exploring Contamination Threats study in pregnant women reported that there were significant associations between urinary BuP exposure (1.03 ng/mL), but not MeP and PrP, and levels of C-RP (Watkins et al., 2015), which was inconsistent with the results of this study. In another epidemiological study of paraben exposure to pregnant women in the US, MeP and PrP concentrations in urine were not related to C-RP levels (Aung et al., 2019). This may be explained by the change of exposure profiles and the difference between different target populations and the analysis matrix. The National Health and Nutrition Examination Survey between 2005 and 2006 years showed that the odds of aeroallergen sensitization increased with the urinary BuP exposure level (Savage et al., 2012), but in this study, we did not find a significant relationship between BuP and immune markers. Therefore, this study needs to be replicated.

This work also has some limitations. First, parabens are metabolized rapidly in the blood (at approximately 24–72 hours), which may bias the results of paraben and RA risk in this study. Therefore, the relationship between paraben

and the incidence of RA was also evaluated based on the exploration of the linear relationship between paraben and immunological indicators. Second, the limited sample size may also have influenced the results of this study. Finally, we were not aware of other potential environmental contaminants in the serum of the general population of Hangzhou, so we could not exclude confounding of paraben by exposure levels of other environmental toxicants.

Conclusions

We conducted paraben exposure measurements among 290 Chinese population and examined the connection between paraben levels in serum and immune markers for RA. MeP (4.7 ng/mL) is the most abundant detected paraben in human serum, followed by PrP (1.9 ng/mL), EtP (1.4 ng/mL), and BuP (1.09 ng/mL). This study first reports that increased human serum MeP concentrations are correlated with increased RF, ACPA, and IgG levels. We also identified that MeP and PrP concentrations in human serum were also significantly associated with levels of C-RP, which suggests the evidence that exposure to parabens can contribute to a higher risk of RA.

Declarations

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ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study procedures were approved by the Ethics Committee of the Second Affiliated Hospital of Zhejiang University School of Medicine. All subjects have signed a written informed consent form.

CONSENT TO PUBLISH

The work in this manuscript has not been previously published and is not under consideration of other journals.

AUTHOR STATEMENT

Yun Zhao: Methodology, Formal analysis, Writing- Original draft preparation. Writing-Reviewing and Editing.

Jianli Qu: Data curation, Investigation.

Meirong Zhao: Conceptualization, Methodology.

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DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Figures

Figure 1

Composition profiles of parabens in human serum ($n = 290$) between the RA cases and controls.

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