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Exploring alternatives for detecting microplastics in the human body: questionnaire survey

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Microplastics (MPs) can enter the body via plastic products. Given modern plastic exposure, we seek to assess MP exposure in large populations through epidemiological tools. In this quasi-experimental study, every participant filled out a questionnaire, and those who satisfied any of the following requirements were not allowed to continue in the study: Diabetes, ulcerative colitis, Crohn's disease, infectious diseases. Participants in the exposure and control groups were provided three hot meals in disposable plastic tableware (DPT) (n = 30) or non-DPT (n = 30), respectively. After a month of observation, individuals in the exposure group discontinued the three meals provided in DPT (n = 27) for 1 month as the post-exposure group. Each Participant in the three groups received a questionnaire survey and fecal sample collection. We compared the differences in MP levels between different groups and used the Bland–Altman analysis method to evaluate the consistency of the results obtained by different measurement methods. Statistically significant differences in the total quantity (*P* (0.80 matching degree) = 0.020; *P* (0.65 matching degree) < 0.001) and types (Polyethylene Terephthalate (EVA) (*P* = 0.039), Polyethylene Terephthalate (PET) (*P* = 0.022), Polyvinyl Butyral (PVB) (*P* = 0.013), Chlorinated Polyethylene (CPE) (*P* = 0.039), phenolic epoxy resin (*P* = 0.012)) of MPs were observed between the exposure and post-exposure groups. The Bland–Altman analysis results indicate that the two methods exhibit good consistency in the three groups (control group: mean difference = 0.54, agreement limits (95% CI) = − 0.44 ~ 1.54; exposure group: mean difference = 0.41, agreement limits (95% CI) = − 0.19 ~ 1.01; post-exposure group: mean difference = 0.19, agreement limits (95% CI) = − 0.63 ~ 1.02). The method based on questionnaire surveys can substitute the method of fecal sample detection to evaluate the exposure of MP particles.

Keywords Bland–Altman analysis, DPT, Questionnaire surveys, Fecal samples, Consistency

Abbreviations

ACR	Acrylic Resin
EVA	Ethylene Vinyl Acetate
PC	Polycarbonate
PET	Polyethylene Terephthalate
PLA	Polylactic Acid
PP	Polypropylene
PE	Polyethylene
PMMA	Polymethyl Methacrylate
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PU	Polyurethane
PVB	Polyvinyl Butyral
PVC	Polyvinyl Chloride

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CPE	Chlorinated Polyethylene
EAA	Ethylene Acrylic Acid Copolymer
ABS	Acrylonitrile Butadiene Styrene
BR	Butadiene Rubber
PIB	Polyisobutylene
SBR	Styrene Butadiene Rubber
MBS	Methyl Methacrylate Butadiene Styrene
POM	Polyoxymethylene
SBS	Styrene Butadiene Styrene
SIS	Styrene Isoprene Styrene

In the last century, the world's plastic production increased sharply, with an annual output exceeding 350 million tons[1,2]. Because of its enduring nature, resistance to corrosion, and low cost, plastic is frequently used as throwaway tableware in the food take-out sector[3,4]. There are more than 400 million users in China's online food delivery market, which is growing quickly[5]. According to a prior analysis, each order typically consumes 3.44 disposable plastic tableware (DPT)[5]. This suggests that over 60 million plastic food containers are used daily in China alone. It is worth noting that disposable tableware, due to direct food contact, represents a primary avenue of exposure to microplastics (MPs) apart from the food chain[6]. Studies have indicated that individuals consuming takeaway meals 4–7 times a week may ingest 12–203 MPs from DPT[7]. An increasing body of cellular, animal, and human studies suggest that harmful substances in leachates from plastic products can affect the intestinal microbiota and metabolic balance of mammals[8–10], thereby leading to abnormalities in reproductive[11], immune[12], cognitive functions, inflammatory bowel disease (IBD)[13], infertility[14], diabetes[15], and Alzheimer's disease[16]. So, scientists and public units have raised concerns about the ingestion of MPs in food and their impact on health[17–19].

The impact of MPs on health, with the primary challenge lying in effectively detecting the amount of MPs ingested within the human body[20,21]. Current research indicates that the features of MPs can be recognized using various methods, including direct observation, optical and electron microscopy, as well as chemical characterization methods such as Fourier-transform infrared spectroscopy and Raman spectroscopy[22,23]. Additionally, analytical methods encompass techniques such as differential scanning calorimetry, thermogravimetric analysis, and gas chromatography-mass spectrometry[24]. However, each of these conventional methods has its limitations, such as susceptibility to human error, time consumption, and high costs[25]. Therefore, there is an urgent need for a simpler, more feasible method to detect MP particles within the body. Questionnaire surveys are valuable tools commonly utilized by medical researchers and epidemiologists to assess various aspects of health and disease, including the attitudes and behaviors of specific populations. In this context, we require a convenient and viable tool for utilization in large-scale epidemiological surveys.

In this study, we collected questionnaires and fecal samples from each participant to understand and explore inter-group differences in the quantities and types of MPs intake in the body. Additionally, we performed Bland–Altman analysis on the questionnaire survey and fecal sample results to investigate the consistency. Our primary aim was to ascertain if questionnaire survey results could serve as substitutes for fecal sample results.

Methods

Study participants and design

This is a quasi-experimental study. The Ningxia Medical University ethics committee examined and authorized this study (grant no. 2021-N0100), and informed consent forms were signed by all eligible individuals. The research methods were carried out in accordance with relevant guidelines and regulations.

Each participant completed a questionnaire prior to the collection of fecal samples to investigate exposure to MPs over the past month. Individuals meeting any of the following criteria were excluded from the study: diabetes, ulcerative colitis, Crohn's disease, infectious diseases, radiation, chemotherapy, or surgery within three months before sampling, irregular bowel movements in the week preceding sampling, and menstruation females[10].

In all, sixty postgraduate students from China's Ningxia Medical University participated in the study. At the outset of the study, two groups of participants were created: an exposure group and a control group. The exposure group (n = 30) volunteered with a one-month COVID-19 survey and consumed three meals each day packaged in DPT boxes. In the control group (n = 30), people who were roommates or classmates were given preference when matching in a 1:1 ratio depending on age and sex. For one month, the control group received three meals daily from the university canteens, which were not packaged using the DPT method. After a one-month observational period, individuals who were previously receiving DPT meal packages will switch to non-DPT meals, which will be provided by the university cafeteria for a duration of one month. Participants during this phase will be designated as the post-exposure group (n = 27). One fecal sample was collected from each participant in three groups to analyze MP levels.

Questionnaire development

Before the fecal sample was collected, each participant got a self-developed questionnaire. There were two primary sections to the questionnaire: (1) basic personal information, including name, age, gender, height, and weight; (2) exposure to plastic products, with questions such as "How frequently do you consume food from plastic boxes, plastic bags, paper boxes, and paper food bags?" and "How often have you used disposable cups (including paper cups) to drink hot beverages over the past month?" Responses were categorized as always (four), often (three), occasionally (two), or almost never (one). The other responses were also assigned values of four, three, two, or one in order, and the skipped questions were assigned a value of zero. The total score was the sum of all questions in the second part of the questionnaire. Additional questions and answers are provided in supplementary material 1.

Fecal sample collection

Stainless steel spoons were used to gather stool samples in 90 mm glass culture dishes for the purpose of assessing MP levels. All physical contact with plastic items was carefully avoided during the sampling procedure. Every sample had a weight of at least 30 g. The samples were then preserved by being kept at − 80 °C.

MPs detection

To determine the MP levels, 87 fecal samples in total were examined. Fecal MP levels were measured using the Agilent 8700 LDIR Laser Infrared Imaging Spectrometer. The Supplementary Materials 2 provide the detailed testing protocol. The samples underwent pretreatment and analysis as outlined below[26]: (1) To eliminate proteins, the sample was treated for an hour with an excess of concentrated nitric acid (68%). (2) After being subjected to a large pore-size filter, the treated sample was sent to a vacuum filter for further filtration. The membrane was put in a clean petri dish to dry before the LDIR test and rinsed several times with ultrapure water and ethanol. (3) The MPs spectrum library building approach and particle analysis mode (offered by Microspectrum Company, China) were chosen. Using the automated test procedure, plastic particles with diameters between 20 and 500 μm were identified by a matching degree of more than 0.65[10].

Quality control (QC) of MP detection

QC procedures were meticulously integrated throughout the testing protocol to ascertain the absence of exogenous plastic particles. An initial step involved subjecting the instrument to specific testing conditions using an empty high reflective glass to verify its response without plastic debris, thus eliminating potential interference from the instrument itself. Before being used, all chemicals were also filtered through a 0.45-μm silver membrane to reduce the possibility of foreign particle contamination. A crucial aspect of quality control, the reagent blank test, regularly produced findings showing no more than three plastic messages and no more than thirty particle points, guaranteeing the validity of the testing procedure. Each experimental iteration was conducted three or more times to further bolster reliability. Moreover, to mitigate the risk of contamination, laboratory personnel adhered to strict protocols by donning laboratory clothing and cotton gloves crafted from plastic-free materials throughout the experimentation period. Prior to the collection of samples, all volunteers received standardized training to ensure that they were familiar with the experimental protocols, which included the careful collection of fecal samples. In addition, three ultrapure water samples were carefully prepared and examined in addition to the fecal samples in order to strengthen quality assurance protocols and prevent any possible experimental errors[27].

For the validation of Low-Density Impurity Recognition (LDIR), a comprehensive array of pure styrene MPs (PS MPs) and mixed MPs (Mix MPs) were meticulously employed for QC purposes. These MPs encompassed various plastic types, including polyethylene, polypropylene, polycarbonate, polyamide 6, polyethylene terephthalate, polyurethane, polyvinyl chloride, polystyrene, polymethyl methacrylate, and rubber[10]. Each MP particle's recognition was contingent upon a matching degree surpassing 0.65, thereby ensuring accurate identification as MPs.

Statistical analysis

Statistical analysis was conducted using R version 4.3.1. For continuous variables following a normal distribution, means and standard error (SE) were utilized, while skewed distributions were described using the median and interquartile range (IQR). Categorical variables were presented as counts and percentages. The chi-square test was applied to compare categorical variables.

Comparison between two groups for continuous data was performed using the Mann–Whitney U-test, Wilcoxon signed-rank test, and paired t-test. Fisher's exact test and McNemar's test were employed for categorical data comparison. Validity and reliability tests were conducted using Cronbach's alpha, Kaiser–Meyer–Olkin (KMO) measure, Bartlett's test of sphericity, and indicators from Principal Component Analysis. Bland–Altman analysis was utilized to evaluate the consistency of results obtained from two different measurement methods. All statistical tests were two-sided, with a significance level set at $p < 0.05$.

Results

Baseline characteristics of study participants

Table 1 displays the study participants' baseline characteristics. Age, gender, and body mass index (BMI) did not significantly differ between individuals in the exposure group, control group, or post-exposure group ($P < 0.05$).

Indices	Exposure (n = 30)	Control (n = 30)	*P* value
Age (year, mean ± SE)	26.15 ± 0.23	26.11 ± 0.36	0.590
Gender (male/female)	13/17	13/17	0.975
BMI (kg/m2, mean ± SE)	22.10 ± 0.55	20.83 ± 0.40	0.057

Table 1. Baseline characteristics of study participants. *P* value indicates the group difference between the three groups.

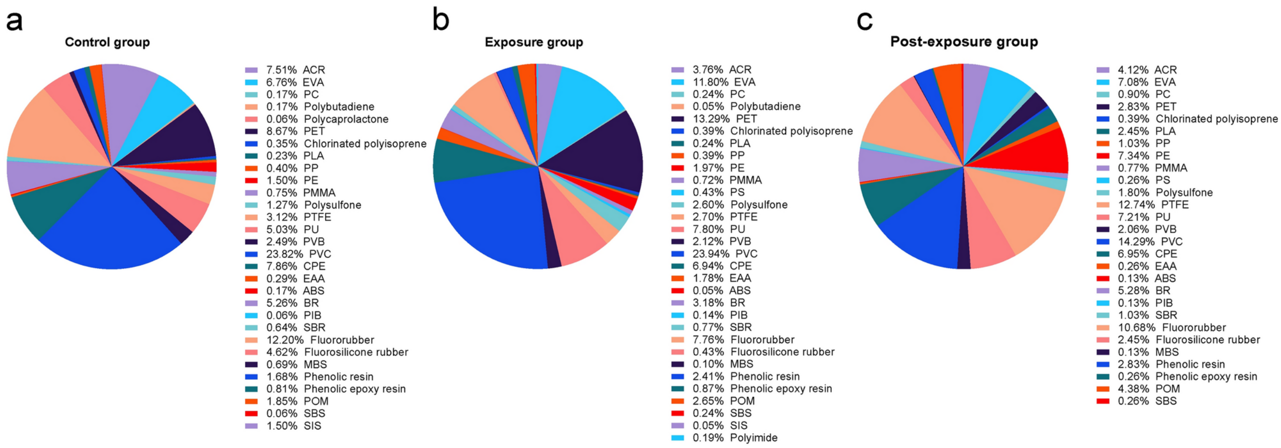

Fig. 1. Proportion of MPs in different groups.

Matching degree	Control VS Exposure	Exposure VS Post-Exposure
0.80	$P = 0.596$	$P = 0.020$
0.65	$P = 0.093$	$p < 0.001$

Table 2. The discrepancy in the total quantity of MPs among different groups. 0.80 means every MP particle is identified, with a matching degree > 0.80 being recognized as MPs; 0.65 means every MP particle is identified, with a matching degree > 0.65 being recognized as MPs.

Study participants and MP levels

Discrepancy in MPs between different groups.

In the fecal samples, As shown in Fig. 1, the most prevalent types of MPs in the control group are Polyvinyl Chloride (PVC) (23.82%), fluororubber (12.20%), and Polyethylene Terephthalate (PET) (8.67%). In the exposure group, the most predominant types are PVC (23.94%), PET (13.29%), and Ethylene Vinyl Acetate (EVA) (11.80%). In the post-exposure group, the most prevalent types are PVC (14.29%), Polytetrafluoroethylene (PTFE) (12.74%), and Polyethylene (PE) (7.34%).

The discrepancy in the quantity of MPs between different groups.

Statistically significant differences in the total quantity of MPs were observed between the exposure and post-exposure groups at both the 0.80 and 0.65 matching degree (P (0.80) = 0.020; P (0.65) < 0.001) (Table 2).

In the control and exposure group, at the 0.65 matching degree, statistically significant differences were observed in the quantities of Polystyrene (PS) ($P = 0.040$) and fluorosilicone rubber ($P = 0.049$) between the two groups. In the exposure and post-exposure groups, at the 0.65 matching degree, statistically significant differences were observed in the quantities of Acrylic Resin (ACR) ($P = 0.038$), EVA ($P < 0.001$), PET ($P = 0.004$), PVB ($P = 0.021$), PVC ($P < 0.001$), CPE ($P = 0.009$), and phenolic epoxy resin ($P = 0.005$) between the two groups. At the 0.80 matching degree, statistically significant differences were observed in the quantities of PET ($P = 0.001$), Polypropylene (PP) ($P = 0.021$), and CPE ($P = 0.039$) between the two groups (Supplementary Table 1).

The discrepancy in the types of MPs among different groups.

In the exposure and post-exposure groups, at the 0.65 matching degree, statistically significant differences were observed in the types of EVA ($P = 0.039$), PET ($P = 0.022$), Polyvinyl Butyral (PVB) ($P = 0.013$), Chlorinated Polyethylene (CPE) ($P = 0.039$), and phenolic epoxy resin ($P = 0.012$) between the two groups. (Supplementary Table 2).

Study participants and questionnaire scores

Validity and reliability testing of questionnaires.

The total scale demonstrated good reliability with a coefficient of 0.819. The significance probability of Bartlett's sphericity test was found to be $p < 0.001$, with a KMO value of 0.710, indicating acceptable validity.

Differences in total questionnaire scores among different groups.

The analysis of total scores from the questionnaire indicates significant differences between the control group and the exposure group ($P < 0.001$). Similarly, significant differences were observed between the exposure group and the post-exposure group ($P < 0.001$) (Table 3).

Bland–Altman analysis of questionnaire scores and MP levels

The Bland–Altman plot demonstrates that the mean difference line is close to zero, with a narrow 95% confidence interval (CI) for the agreement limits in three groups (Fig. 2).

Questionnaire	Control VS Exposure	Exposure VS Post-Exposure
Total score	$P<0.001$	$P<0.001$

Table 3. The discrepancy in the total scores of the questionnaire among different groups.

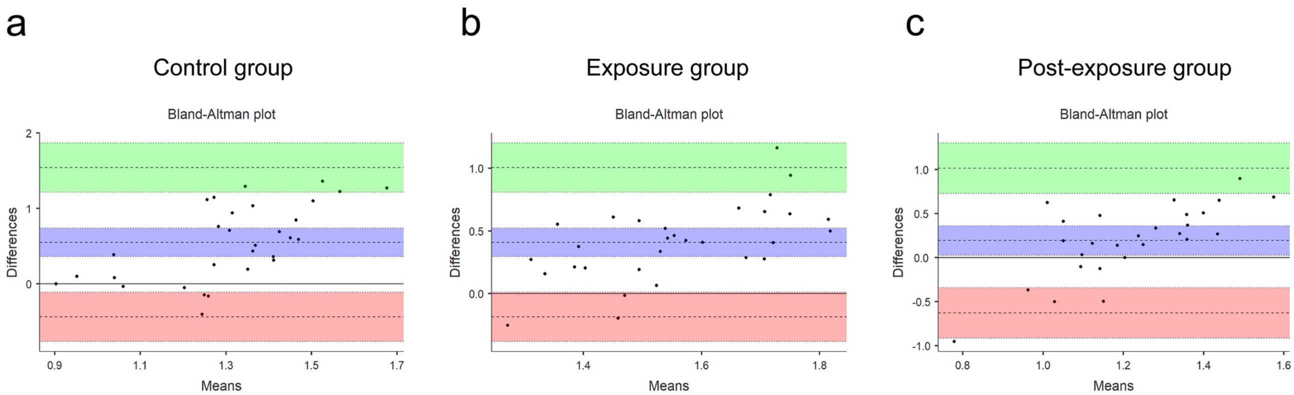

Fig. 2. The Bland–Altman analysis of questionnaire results and fecal sample results in the three groups. *Note* The control group has 30 participants; the exposure group has 30 participants; and the post-exposure group has 27 participants.

Group	Bias	Lower limit of agreement	Upper limit of agreement
Control	0.5499082	−0.4422501	1.5420664
Exposure	0.4096703	−0.1862236	1.0055641
Post-exposure	0.1933689	−0.6283611	1.0150989

Table 4. The Bland–Altman analysis of questionnaire results and fecal sample results in the three groups.

In the control group, the mean difference was 0.54, and the 95% CI for the agreement limits ranged from −0.44 to 1.54; In the exposure group, the mean difference was 0.41, and the 95% CI for the agreement limits ranged from −0.19 to 1.01; In the post-exposure group, the mean difference was 0.19, and the 95% CI for the agreement limits ranges from −0.63 to 1.02(Table 4). It is possible to say that the bias is not significant, and results measured by the questionnaire survey method maybe 0.44 (0.19 or 0.63) units below or 1.54 (1.01 or 1.02) units above the fecal sample method. Currently, there is no established clinical threshold for the detection of MPs. So, we consider this range acceptable. In conclusion, the two methods exhibit good consistency.

Discussion

The research findings suggest a positive correlation between the frequency of DPT usage and the quantity of MP particles present in the body. The cessation or reduction in the use of DPT would correspondingly lead to a decrease in the quantity of microplastic particles within the body. Application of the Bland–Altman consistency analysis method indicates that questionnaire-based approaches can to some extent substitute complex and costly laser infrared imaging spectroscopy detection methods.

In fact, the human ingestion of MPs via plastic containers for food use has been revealed in previous studies[28–30]. MPs can enter the human body through the consumption of food, as confirmed by their detection in human feces[31]. DPTs commonly consist of PS, PET, PVC, and PP[32]. Our research findings indicate a notable quantitative discrepancy in PS particles between participants who consumed food packaged in DPT and those who did not. Furthermore, a significant variation in the levels of PVC and PET was detected one month after stopping DPT-packaged food consumption. The consumption of DPT-packaged food may be a significant contributor to the intake of microplastics, aligning with the direction of previous research outcomes[10].

MPs can exert toxic effects on human health by impacting various aspects of the gastrointestinal tract, such as the physical barrier, immune defense, and gut microbiota, potentially leading to further health implications[33]. Policymakers have attempted to control the proliferation of plastic pollution by enacting legislation restricting plastic bag use. However, plastics continue to be extensively utilized in the food packaging industry, providing a significant source of microplastic exposure[34]. This widespread use has raised concerns about the potential health impacts associated with the ingestion of microplastics. Our research has introduced an innovative and more accessible method for assessing the level of people's exposure. A study conducted in Beijing utilized FTIR to detect microplastics in participants' fecal matter, and through quantitative and qualitative analysis of their content and types, complemented by surveys on drinking water and dietary habits, concluded that the consumption of bottled water may be a significant factor in increasing microplastic intake[35]. Our survey questionnaire detailed the participants' consumption of DPT-packaged foods and performed a consistency analysis with the detection results of fecal samples, concluding that the questionnaire has a certain degree of substitutability. A recent study assessed the frequency of plastic product usage among participants through a questionnaire and concluded that higher levels of plastic exposure are associated with an increased risk of mild cognitive impairment (MCI), particularly among individuals who frequently use plastic utensils[36]. The questionnaire survey is an important tool for assessing exposure, which aligns with our research approach and provides a promising alternative to traditional, more complex detection methods.

One limitation of our study is the relatively modest sample size, which constrained the thoroughness of validity and reliability assessments for the questionnaire used. Additionally, no assessment or comprehensive analysis was conducted on the diet outside of the three main meals and other potential sources of microplastic exposure for the exposed group, such as drinking water, toothbrushes, and toothpaste[35]. Furthermore, we only sampled the participants at the end of the intervention, without taking samples at multiple time points throughout the entire study.

Nonetheless, our study provides a novel perspective on the detection of MPs, thereby establishing a foundation for future advancements in this field. The questionnaire-based survey serves as an initial screening tool for MP detection, paving the way for large-scale epidemiological investigations and providing scientific evidence for policymaking and environmental management. However, further research requires larger sample sizes and more comprehensive methodologies to more accurately assess microplastic exposure and its potential impacts. Certainly, reducing exposure to microplastics is essential. It is recommended to use paper packaging that does not contaminate food or pose risks to human health, or traditional food packaging materials such as palm leaves, sugarcane bagasse, and cellulose paper from wood pulp[37]. Metal and glass materials, which do not react with packaged food, can also be effectively used for various food packaging applications[38].

Conclusion

Through this study, the questionnaire-based collection approach can substitute the intricate and costly laser infrared imaging spectral detection method for assessing the quantity of MP particles in fecal samples.

Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

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Author contributions
YW is responsible for collecting, analyzing, drafting, and writing materials. YZ and YW are responsible for data organization and form analysis, while YY and LS are responsible for writing reviews and editing. PC, JL, and SH are responsible for the research and design, data analysis, drafting, writing, and editing of the manuscript. All authors have read and approved the final manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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