

# Microplastic in Gastric Fasting Liquid and Associated Gastric Pathology

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## ABSTRACT

**Objective:** To determine the presence of microplastics in the stomach, and the relationship between pathological changes in stomach tissue and microplastics.

**Study Design:** An analytical study.

**Place and Duration of the Study:** Department of Internal Medicine, Sorgun State Hospital, Yozgat, Turkiye, from December 2022 to November 2023.

**Methodology:** Fasting gastric fluid sampling and endoscopic sampling including mucosal and submucosal layers from the antrum were performed. The pH values of the gastric fluids were recorded. Samples were analysed gradually by adding iron solution, hydrogen peroxide, and sodium chloride (NaCl) in a beaker at 75 degrees for 30 minutes. Biopsy materials obtained from antrum were examined histopathologically and reported according to the Sydney classification. The relationship between gastric biopsy results and the presence of microplastic was evaluated using Chi-square test. The significance level was taken as  $p < 0.005$ .

**Results:** The study included 61 individuals. The presence of microplastics was detected in 17 (27.86%) gastric fluid samples obtained from the individuals. A significant correlation was found between increased activity and inflammation in antrum biopsy and the presence of microplastic ( $\chi^2 = 8.55$ ,  $p = 0.014$ ;  $\chi^2 = 25.75$ ,  $p = 0.001$ ). The relationship between atrophy, metaplasia, and *Helicobacter pylori* in gastric tissue and the presence of microplastic was statistically insignificant ( $p > 0.05$ ).

**Conclusion:** Microplastics were detected in gastric fasting fluid. These materials can cause histopathologic changes and inflammation in the gastric antrum.

**Key Words:** *H. pylori*, Intestinal metaplasia, Inflammation, Microplastic, Plastic, Sydney classification.

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## INTRODUCTION

Plastics are composed of polymeric particles existing in macro, micro, and nano sizes. Plastic parts with particle sizes smaller than 5 millimetres are called microplastics.<sup>1</sup> Plastic parts become micro-sized by the breakdown of large plastic parts in nature. These small plastic particles enter into human body through contact and inhalation as well as through food and drinking water.<sup>2</sup>

Plastics have a great place in human lives due to their ease of use, easy accessibility, and low cost.<sup>3</sup> Plastic waste, of which an estimated 10% is recyclable, is no longer just a waste due to the high consumption rate and uncontrolled disposal. It is now one of the factors of environmental pollution and a threat to human health.<sup>4</sup>

Approximately 2.4 mg of microplastics originating only from liquid cleaning materials have been detected in sewage per person.<sup>5</sup> Due to uncontrolled use it is possible to find microplastics even in the poles, uninhabited islands, and ocean depths.<sup>6</sup> Water resources are under grave threat,<sup>7</sup> with plastics reaching humans through aquatic organisms such as plankton from the lowest level of the ecosystem.<sup>8</sup> The lowest amount of microplastics was detected in rock salt located in the region farthest away from humans; while the highest amount was detected in sea salt. Microplastic density was also measured high in salts taken from places close to residential areas.<sup>9</sup>

Precipitation method and infrared spectroscopy are the main ones used in microplastic detection. Another method of analysis is the determination of organic carbon in liquids, which is performed via carbon dioxide released at high temperature in liquid substances.<sup>10-12</sup>

There is a paucity of data about the presence of microplastics in living human body. This study aimed to confirm the presence of microplastics in the gastric fluid and examine the possible effects of microplastics on gastric tissue.

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## METHODOLOGY

The study was approved by the Yozgat Bozok University Clinical Research Ethics Committee with the protocol code number 2017-KAEK-189\_2022.12.29\_01. Between December 2022 and November 2023, 61 individuals living in the same region who were scheduled for endoscopy with the indication of dyspepsia were included in the study. Consent was obtained from all individuals.

Patients were excluded if they had used medication that may affect gastric pH in the last three months, were receiving chemotherapy, had undergone surgery affecting the integrity of the gastrointestinal system, or had diseases affecting gastric acidity, such as atrophic gastritis, gastrinoma, and rheumatologic diseases, with reduced secretions such as Sjogren's disease. Patients with a body mass index (BMI) of 35 and above were excluded. Patients with habits such as smoking, use of other tobacco products, and alcohol were excluded.

Age, gender, background information; habits and medications were questioned. Individuals were questioned about their drinking water preferences, packaged food use, liquid soap use, and whether they used cosmetic products. In the selection of individuals, attention was paid to the same type of nutrition and drinking water useage in the same region.

During the endoscopy procedure, gastric fasting fluid sampling, and endoscopic sampling (bx) including mucosal and submucosal layers from the antrum were performed.

Haemotoxylin-eosin and Giemsa stains were performed on bx materials to evaluate *H. pylori* colonisation. Short, dark blue sticks, or S-shaped bacteria were detected. Endoscopic samples from the antrum including mucosal and submucosal layers were stained with Haemotoxylene-eosin stain for activity, inflammation, metaplasia, and atrophy. These data were collected retrospectively.

The potential Hydrogen (pH) value indicates the acidic or alkaline situation of the environment or material. The values of the gastric fluids measured with a pH meter were recorded.

In 10 ml of gastric fluid sample, 2 ml of iron solution, 2 ml of 30% hydrogen peroxide, and 60 mg sodium chloride (NaCl) to increase the density were added and analysed in a beaker at 75 degrees for 30 minutes. Precipitation occurs in the presence of microplastic particles in the mixture.<sup>13</sup>

The evaluation of microplastics was performed by an expert chemist in a special chemistry laboratory. Endoscopic samples were evaluated pathologically according to the Sidney classification by an expert pathologist in the pathology laboratory of the health institution. The relationship between the gastric biopsy results of the patients and microplastic status was evaluated.

The data obtained from the study were loaded into the SPSS (version 22.0) programme and since the parametric test assumptions were fulfilled in the evaluation of the data (Shapiro-Wilk test), a significance test of the difference between the two means was used to compare the measurements obtained from two independent groups. Independent samples t-test was used to determine whether the means obtained from two related groups and measurements differed significantly from each other. Cramer's V was used to determine the relationships between the measurements, and the Chi-square test was used in 2 x 2 and multivariate layouts in the evaluation of qualitative data obtained by counting. Data were shown in tables as the arithmetic mean and standard deviation for the data obtained by measurement, and as a percentage of the data obtained by counting, and the error level was taken as <0.05.

## RESULTS

Sixty-one individuals were included in the study. Forty-three (70.5%) were females and 18 (29.5%) were males. The minimum age was 21 years and the maximum age was 87 years with a mean age of 48.21 years. Mean BMI of the patients was 28.35 kg/m<sup>2</sup>. Thirty-seven of the patients were using plastic bottles and 24 of them preferred municipal water as a drinking water resource.

The pH of the fasting gastric fluid samples taken from 61 individuals before endoscopy was recorded with a pH meter. The presence of microplastics in the fasting gastric fluids was analysed. In the analysis of gastric fluid for the presence of microplastics, 17 (27.86%) individuals were found to have microplastics. When the relationship between microplastic positivity and the pathology result sent from the gastric tissue was evaluated; the relationship between the activity in the gastric tissue and the presence of microplastics was found to be significant ( $p=0.001$ ). The presence of microplastic was correlated with inflammation in the antrum. Microplastic increases inflammation in the antrum ( $R = 0.64$ ). The relationship between atrophy and metaplasia in gastric tissue and the presence of microplastic was not statistically significant ( $p=0.307$ ,  $p=0.203$ , respectively, Table I).

Minimum pH was 1.10; maximum pH was 3.50. Mean pH was calculated as  $1.92 \pm 0.65$ . The relationship between the presence of *H. pylori* and gastric pH was statistically significant ( $p=0.001$ ). The relationship between pH and the presence of microplastic was found to be significant ( $p=0.001$ , Table II).

When the dietary habits of individuals with microplastics were questioned, microplastics were detected in 70% of individuals who used drinking water from plastic bottles. Microplastics were detected in 12 (32.4%) of patients using plastic-bottled water, while microplastics were detected in 5 (20.8%) of patients using tap water. Out of these individuals, 66.6% consumed carboy water and 33.3% consumed disposable plastic water. Of the remaining five individuals, three stated that they use purified water and two stated that they use direct tap water.

**Table I: The relationship between the presence of microplastics in fasting gastric fluid and gastric tissue pathology.**

| Biopsy data             | Microplastics exist |           | Microplastics do not exist |           | Total |           | Result           |
|-------------------------|---------------------|-----------|----------------------------|-----------|-------|-----------|------------------|
|                         | n                   | Percent % | n                          | Percent % | n     | Percent % |                  |
| <i>H. pylori</i> exists | 15                  | 88.2      | 18                         | 40.9      | 33    | 54.1      | $\chi^2 = 11.06$ |
| <i>H. pylori</i> none   | 2                   | 11.8      | 26                         | 59.1      | 28    | 45.9      | $p = 0.001$      |
| Activity-low            | 5                   | 31.3      | 15                         | 71.4      | 20    | 54.1      | $\chi^2 = 8.55$  |
| Activity-medium         | 7                   | 43.8      | 6                          | 28.6      | 13    | 35.1      | $p = 0.014$      |
| Activity-intense        | 4                   | 25.0      | 0                          | 0         | 4     | 10.8      |                  |
| Metaplasia exists       | 3                   | 17.6      | 3                          | 6.8       | 6     | 9.8       | $\chi^2 = 1.62$  |
| Metaplasia none         | 14                  | 82.4      | 41                         | 93.2      | 55    | 90.2      | $p = 0.203$      |
| Inflammation low        | 2                   | 11.8      | 31                         | 70.5      | 33    | 54.1      | $\chi^2 = 25.75$ |
| Inflammation medium     | 4                   | 23.5      | 10                         | 22.7      | 14    | 23.0      | $p = 0.001$      |
| Inflammation intense    | 11                  | 64.7      | 3                          | 6.8       | 14    | 23.0      | $R = 0.64$       |
| Atrophy exists          | 2                   | 11.8      | 2                          | 4.5       | 4     | 6.6       | $\chi^2 = 1.43$  |
| Atrophy none            | 15                  | 88.2      | 42                         | 95.5      | 57    | 93.4      | $p = 0.307$      |

$\chi^2$  (Chi-square), n: Individual,  $p < 0.05$  significant\*,  $p > 0.05$  insignificant.

**Table II: The relationship between gastric pH and the presence of *H. pylori* and microplastic.**

|                  | Fasting gastric juice pH value<br>(mean $\pm$ standard deviation) |                             | Analysis<br>results     |
|------------------|---|-----------------------------|-------------------------|
| <i>H. pylori</i> | Yes   | 1.69 $\pm$ 0.3<br>(n = 33)  | t = 6.71<br>$p = 0.001$ |
|                  | No  | 2.58 $\pm$ 0.66             |                         |
| Microplastics    | Yes   | 1.51 $\pm$ 0.23<br>(n = 17) | t = 7.08<br>$p = 0.001$ |
|                  | No  | 2.32 $\pm$ 0.65             |                         |

t = Independent t-test, n= Number of individuals,  $p < 0.05$  significant,  $p > 0.05$  insignificant.

Although the percentage of plastic bottle use was higher in patients with microplastics in gastric fluid, there was no statistically significant relationship between the presence of microplastics and daily drinking water use from plastic bottles ( $p=0.324$ ). It can be thought that this may be due to many factors. Since plastic bottles with different contents are used in packaged waters or plastic pipes are used in the transportation of municipal water and the characteristics of the water tank, if any, are not known, there is a need for analysis and evaluation of drinking water.

## DISCUSSION

Microplastics threaten all living entities in the world. In a study conducted in the USA, 1,033 birds were examined and plastics were detected in the digestive system of 55% birds.<sup>14</sup> In a study of 611 sea turtles in Western countries, such as Italy, Spain, France, and Turkiye, microplastics were detected in the digestive system of 63% turtles and microplastics were detected in the faeces of 31% turtles.<sup>15</sup> In a study conducted on seals, plastic residues were found in the feeding chain and digestive systems.<sup>16</sup> In all of them, marine plastic wastes were shown as the causative factor. Results of this research indicates that microplastic particles were found in 27.86% of the digestive system of humans. It reveals the microplastic exposure of humans, the last step of the food chain.

In the histopathological examination of the damage caused by microplastics exposed through digestion, it was observed that it caused inflammation. Although it cannot be directly associated with metaplasia in the stomach, it may cause

malignancy as a result of long-term inflammation and irritation. As a result of inflammation, it may react with gastric contents such as food and drugs and may cause adverse effects and digestive problems. For a long time, attention has been drawn to the negative effects of microplastics in human lives. An example of this is the study by Lang *et al.* It was thought that the excessive amount of biphenol-A level in plastic contents may cause breast and ovarian malignancies by creating estrogenic effect and prostate malignancies by androgenic effect.<sup>17</sup> People may be exposed to these small particles *via* respiratory and dermal routes in addition to the digestive tract. Similar to the results of this research, there are studies suggesting that microplastics cause inflammation in the respiratory system as well as the digestive system and on the skin. Prata *et al.* detected microplastic particles in the atmosphere and concluded that since they are in respirable sizes, they may cause respiratory disorders in susceptible individuals by causing interstitial inflammatory response by inhalation.<sup>18</sup> Similarly, Proshad *et al.* demonstrated that polyvinyl chloride in plastic contents may cause chronic bronchitis when ingested by inhalation and allergic skin reactions when ingested by contact.<sup>19</sup>

Water is the basic source of human life. Studies on the presence of microplastics in drinking and utility water have intensified. In a large-scale study in which tap water from many different countries was analysed, microplastics were detected in 83% of the water samples. Among the countries, America ranked first with 94% and Lebanon ranked second. The lowest rate was in European countries with 72%, which is quite high.<sup>13,20</sup> In the study conducted by Ozkor *et al.* in the Kizilirmak River, which is Turkiye's one of the main source used as both drinking water and tap water, microplastics were found in water samples analysed with microscope.<sup>21</sup> Of the individuals, in whom the authors detected microplastics in fasting gastric juice, 70.6% did not consume water from plastics, while 29.4% used tap water; statistically, the authors found no difference between individuals who used plastic bottles and those who did not. The biggest reason for this is that the tap water people of Turkiye drink is exposed to plastics both during transportation and storage, and the study by Ozkor *et al.* unfortunately revealed that the main

source of water, Kizilirmak River, is full of microplastics. Even if treatment is carried out, it is not known to what extent these microparticles are eliminated.

Macroplastics can decompose into small particles by chemical dissolution in nature and in human digestive organs and dissolve in the stomach at the appropriate temperature and pH, or on the contrary, they can become rigid and adhere to the surface they find. They may even prepare an environment for *H.pylori* by forming a bio-film layer.<sup>22</sup> They reach effective colonisation at low gastric pH, penetrate the epithelium by overcoming gastric mucus secretion, and increase the pH with the urease they secrete. Thus, it adapts to the gastric mucosa and as a result, it may play a role as a facilitating factor for microplastics to remain on the gastric surface. With this vicious cycle, *H.pylori* eradication becomes difficult; it is obvious that microplastics should be avoided to get rid of this micro-organism, which is considered carcinogenic by WHO.<sup>23,24</sup> According to the results of this study, the presence of microplastic could not be directly associated with *H.pylori* positivity. However, the presence of microplastic may affect fasting pH of gastric fluid. pH changing of gastric fluid may be effect of *H.pylori* colonisation or microplastic presence.

The results of this study are based on the examination of 61 patients. The limited number of patients may not be enough to make assumption about microplastics and its affects on all human beings. Besides, subjects under evaluation in this study have upper gastrointestinal complaints. Therefore, the presence and effects of microplastics cannot be attributed to the entire population. By examining healthy and non-complained individuals, there may have a better and comprehensive conclusion about microplastic prevalence and its effects on gastric tissue.

## CONCLUSION

The authors investigated the histopathological effects of microplastics in the stomach. It was observed that changes in stomach pH were affected by the presence of microplastic in the antrum, and it was concluded that *H.pylori*, which affects gastric pH, may also be affected by this situation. While it was observed that microplastics increased inflammation in the antrum, they proportionally increased atrophy and intestinal metaplasia, but statistically sufficient evidence was not obtained.

## ETHICAL APPROVAL:

This research complies with all relevant national regulations, institutional policies, and the principles of the Declaration of Helsinki. Approval was obtained with the decision of the Yozgat Bozok University Ethics Committee (Dated: 29.12.2022 and Numbered: 01). (Clinical trial protocol code 2017-KAEK-189\_2022.12.29\_01).

## PARTICIPANTS' CONSENT:

Informed consent was obtained from all participants.

## COMPETING INTEREST:

The authors declared no conflict of interest.

## AUTHORS' CONTRIBUTION:

DF, OID, EC: Design, execution, and analysis of the paper. All authors approved the final version of the manuscript to be published.

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