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# DETERMINATION OF ELEMENTS WITH PROTON PUMP INHIBITORS IN THE BIOLOGICAL SAMPLES OF GASTROINTESTINAL DISTURBED PATIENTS RESIDING IN DIFFERENT CITIES OF SINDH, PAKISTAN

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**Abstract**

**Background:** prolonged bloating, abdominal discomfort, reflux, nausea, vomiting, diarrhea, stomach pain, and discomfort characterize a collection of digestive conditions known as gastric distress. Proton pump inhibitors (PPIs) remain the superior choice worldwide in antisecretory therapy in the evidence-based treatment of upper gastrointestinal disorders including gastroesophageal reflux disease, erosive esophagitis, dyspepsia and peptic ulcer disease.

**Methods:** To digest the materials, a 2:1 ratio of $HNO_3$ (65%) and $H_2O_2$ (30%) has been employed in a microwave oven-assisted digestion process. Elemental concentrations were determined by atomic absorption spectrometry.

**Result:** The goal of this research was to evaluate the link between critical trace elements particularly iron" with proton pump inhibitors in samples of biological material, namely blood, serum, and urine of 350 gastrointestinal disturbance patients, who were inhabitants of different cities in Pakistan and ranged in age from 30-45 to 46 years. The biological specimens of reference subjects (n = 135) in the same age ranges have been analyzed for comparison analysis.

**Conclusion:** In samples from individuals with gastrointestinal disturbances, the mean amount of critical elements (Fe) was observed to be reduced in blood and serum than healthy values, however, these elements were observed to be greater in urine when compared to subjects ($p$<0.001). The collected information will be useful in the treatment of gastrointestinal disturbance patients, improving their chances of survival.

**Keywords:** Iron, Gastrointestinal disturbed patients, proton pump inhibitors

## Introduction

Gastric discomfort refers to a group of digestive disorders that include persistent constipation, bloating, reflux, nausea, vomiting, diarrhea, stomach pain, and cramping. Changes in the oral cavity, which is a part of the digestive system, can be the first sign of both central and digestive problems, Proton pump inhibitors (PPIs) remain the superior choice worldwide in antisecretory therapy in the evidence-based treatment of upper gastrointestinal disorders including gastroesophageal reflux disease, erosive esophagitis, dyspepsia and peptic ulcer disease.[1]. Along with bowel inflammation, a variety of degenerative gastrointestinal diseases and liver problems are linked to iron inadequacy anemia (IDA) [2]. IDA may be influenced by a variety of variables including prolonged bleeding, absorption problems, and infection.

Because it enables the proper operation of numerous essential enzymes, copper is crucial to our metabolism [03]. The body's connective tissue, blood vessels, skin, and epithelial tissues all depend on copper to be strong. The formation of myelin, melanin, and hemoglobin all depend on copper, which additionally keeps the gland that produces thyroid hormone functioning normally. A source of antioxidants and a pro-oxidant, copper may function. Free radicals are organic components of the body that can harm cell walls, communicate with human DNA, and play a role in the emergence of a variety of illnesses and health issues [04].

## Material and Methods

*Recruitment*

To start this study prior permission was obtained from the ethical review committee of the University of Sindh, Jamshoro – Pakistan.

### *Study design and pretreatment*

Male patients between the ages of 30-45, and 46-60 years old with any sort of IBS, Gastritis, GERD, or Gastric Ulcer were gathered between March 2022 and June 2023 at the Isra Hospital Sindh and the public hospital in Jamshoro, Pakistan. In general, it was challenging to acquire biological samples, albeit we were successful in doing so for the 245 patients listed in **Table 1**. For informational purposes, an oral discussion also took place at several hospitals, where participants and subjects received information about the objective and design of the study. Before collecting the samples, individuals were given an official consent form to fill out and sign. Questions on the participants' way of life, dietary habits, and employment history were answered in another descriptive form.

Physical analysis and biochemical data have been collected from a variety of individuals. In **Table 2**, biochemical factors were listed. After the discussion, it became evident that the patient's long-standing chronic illness, lack of knowledge about the situation, lack of consciousness, and malnutrition had worsened conditions. In rural locations, the death rate was found to be higher. Due to the aforementioned factors, the disease's discovery in Pakistan is concerning. A healthy reference was chosen if they met the following criteria: i) they did not have any recognized physiological disorders (such as hypertension, diabetes, gastrointestinal disorder, etc.) or infections at their time of choice; and ii) they had not been diagnosed with or had symptoms of any sort of illness over at least a year at their time of taking the samples. They were all family members of the sufferer and all were in good health. They weren't supplementing with any minerals. Before collecting biological

specimens, a certified physician performed a medical examination on all 135 healthy referents. All participants (controls as well as siblings of sufferers) had personal interviews by the writers, who also administered an inquiry form (questionnaire) to respondents to collect details such as race, physical attributes, nutritional habits, fitness, age, place of employment, as well as compliance.

### *Ethical consideration*

Before taking samples, ethical authorization was also acquired from various hospitals in Sindh, Pakistan. To get participants' and/or their official guardians' agreement, the Declaration of Helsinki code was scrupulously followed. Volunteers' identities were concealed by encoding them with secret identifiers. Only the project's principal investigator and qualified medical professionals had access to each of the individual's gastrointestinal disturbance data, which was kept strictly private.

### Sample collection

### *Blood and Serum sample collection*

Eventually, a needle was used to draw blood through the veins of every volunteer. A male certified nurse conducted the whole blood collection procedure with the aid of a 5mL uncontaminated, one-time-use injection. The region of the sample had been scrubbed with an alcohol-soaked swab, which had no contamination and was sealed in polyethylene. The only reason this particular alcohol swab had been selected was to avoid illness and contamination of any sort. Blood specimens for trace element analysis were collected using a BD (Oxford, UK) Vacutainer (Trace Element tube with potassium-EDTA) [05]. Every participant was instructed to fast before taking possession of 5ml of blood. To prevent any potential solar contact with specimens of blood, specimen tubes were stored out of the path of the sun and container tubes stuffed with aluminum foil. After being transported to the testing laboratory, the blood specimens were left to remain at ambient temperature for 1 hour before being centrifuged at 3000 rpm for 15 minutes to separate the serum from cells in the blood. The hemolytic antibodies were thrown away. During the centrifugation, the obtained samples were stored for further examination in a refrigerator at -20°C [06].

### Urine *sample collection*

In 100 mL disinfected polythene tubes (Milan, Italy) early urine specimens were taken. The receptacle is covered in a fresh plastic bag for sample sessions. Samples of urine were maintained at 4°C after being treated with ultra-pure concentrated sulfuric acid (l% v/v). The sample should be forcefully agitated for 1 minute to ensure a uniform suspension before sub-sampling for analysis [06].

### *Apparatus*

A dual radiate Perkin-Elmer atomic-absorbing spectrometer model 700, USA), outfitted using a graphite oven via incorporated device, an automatic sampler AS-800, as well as a deuterium lamp that served as a background modification framework, was used to analyze the elements. **Table 3** displays the operational parameters for analyte determination. The samples were digested using a home microwave machine (Pel PMO23, Japan) that could be programmed for time and had a microwave power range of 100-900 W [07]. To prepare and store responses, plastic (polypropylene) receptacles are acid-washed.

### *Reagents and glassware*

The highly pure water employed during the procedure was transfused using Millipore-type (USA). Chemicals for analysis made by E. Merck in Germany were utilized, along with nitric acid and hydrogen peroxide. Before usage, all samples had been tested against contamination with metals. Certified 1000 ppm conventional solutions of Fe, from working standard (stock) concentrations were serially diluted. In polyethylene containers, the produced solutions were stored in the refrigerator at 4°C for subsequent investigation [08].

***Microwave-assisted Acid Digestion Method***

In this elemental evaluation, replicates of each specimen were made using a digestion technique using a microwave device. Six replication samples of referential and other sorts of gastro-intestinally disturbed individuals were collected from authorized sources (human urine, serum, and blood). Regarding each sample, the digestion process involved mixing 0.2 mL of freshly generated $H_2O_2$-$HNO_3$ solution (1:2, v/v) with 1 mL of hemoglobin mentioned in Table 4. To ensure complete sample absorption, the mixture vial was set in a MW oven and exposed for three minutes to microwaves with a power of 950 MW [09]. After being brought to room climate, the flask comprising the samples that had been digested was diluted with Mili-Q freshwater to attain the intended final volume figure of 10mL. Following that, these samples were given to individual FAAS analyses for Fe. An identical procedure was employed to prepare the blank specimens.

***Data and Statistical Analyses***

The Minitab program (version 13.2) was used to analyze the data, which were presented as mean SD. The T-test was used to compare the essential elements contents between patients with gastrointestinal disturbances and controls. The findings of the EEs in the physiological samples of competent persons (whole blood, serum, and urine) showed statistically significant deviations (p 0.05). A significance level of 0.05 or lower was regarded as being significant.

**RESULTS**

The changes in key components were inversely correlated with biochemical measures in patients with gastrointestinal disturbances. **Table 5** details the mean concentrations and standard deviation for each critical trace element in blood, serum, and other biological materials. The vital trace element found in biological samples from patients with gastrointestinal disorders compared to reference persons. With a confidence level of 95% (CI: 409, 385, 362, 342), it was discovered that the iron levels in blood samples from males between the ages of 30-45 who had various forms of IBS, Gastritis, GERD, and Gastric Ulcer were reduced. Male participants with gastrointestinal disturbances who were between the ages of 46 and 60 had Fe levels that ranged between (CI: 415.8, 398, 379, and 350 mg/L), which were lower compared to the referents' values of (CI: 465.3 and 482) mg/L. However, the iron levels in specimens of blood from females with various forms of IBS, Gastritis, GERD, and Gastric Ulcer have been identified to be reduced at the confidence level of 95% (CI: 405, 375, 357, 335). Fe levels were lower in female patients with gastrointestinal disturbances who were 46 to 60 years old (CI: 410, 390, 364, 343 mg/L) compared to the values found in blood specimens from referents (CI: 452 and 474 mg/L). With a 95% confidence interval (CI: 3.48, 3.16, 2.85, 2.69), it was discovered that the iron levels in serum samples from males between the ages of 30-45 who had various forms of IBS, Gastritis, GERD, and Gastric Ulcer were lower mentioned in **Table 6**. Male patients with gastrointestinal disturbances who were between the ages of 46 and 60 had Fe levels that ranged between (CI: 3.54, 3.25, 3.04, and 2.85 mg/L), which was lower compared to the referents' values of 3.72 and 3.89 mg/L. However, the iron levels in serum samples from females with various forms of IBS, Gastritis, GERD, and Gastric Ulcer were found to be lower at the 95% confidence interval (CI: 3.35, 3.03, 2.72). Fe levels were lower in females with gastrointestinal disturbances who were 46 to 60 years old (CI: 3.43, 3.12, 2.95, 2.70 mg/L) than they had in serum samples from healthy (CI: 3.65 and 3.72 mg/L).

The amount of Fe in men with a mean age of 46-60 with gastrointestinal disturbed individuals sub kinds was in the vicinity of (CI: 3.54, 3.95, 4.38, 4.89 mg/L), versus referents CI: 2.52 and 2.75. The Iron levels in specimens of urine of various types of Ibs, Gastritis, GERD, and Gastric Ulcers having doses of Omeprazole and Esomeprazole 20 and 40 mg for up to four weeks in the male of 30-45 mentioned in **Table 7**. However, the amount of iron in a specimen of urine from women with various forms of IBS, Gastritis, GERD, and Gastric Ulcer is elevated at the level of confidence equivalent to 95% (CI: 2.95, 3.37, 3.82, 4.19). Fe levels were higher in female patients with gastrointestinal disturbances who were between the ages of 46 and 60 (CI: 3.75, 4.27, 4.55, 5.45

mg/L) than they were in urine samples from referents (CI: 2.75 and 2.95 mg/L). According to the 95% confidence interval (CI: 0.89, 0.75, 0.63, 0.50), the copper levels in blood samples from people with various types of IBS, Gastritis, GERD, and gastric ulcers who took doses of omeprazole and esomeprazole 20 and 40 mg for 2 to 4 weeks were shown to be lower.

**Table 1.** Shows the study population

| Age group (Years) | Gender | Gastric Ulcer | IBS | Gerd | Gastritis |
|---|---|---|---|---|---|
| **30-45** | Male | 58 | 20 | 44 | 39 |
| | Female | 26 | 30 | 23 | 22 |
| **46-60** | Male | 29 | 09 | 17 | 14 |
| | Female | 15 | 04 | 10 | 07 |
| | Number of Cases | 128 | 46 | 94 | 82 |
| | n (Total Count) | 350 | | | |

**Table 2** Hematological characteristics of control and gastrointestinal Disturbed patients' referents and normal range

| | | | Gastrointestinal Disturbed patients | | | |
|---|---|---|---|---|---|---|
| **Controls** | | | **IBS** | | **Gastritis** | |
| **Biochemical Parameters** | **Male** | **Female** | **Male** | **Female** | **Male** | **Female** |
| **BMI** | 25.3±1.02 | 25.7±0.44 | 26.2±0.35 | 28.9±0.52 | 29.8±1.02 | 31.0±0.85 |
| **Haematocrit % 41.9-48.7** | 16.2±0.69 | 15.4±0.78 | 14.5±0.53 | 14.2±0.50 | 13.6±0.35 | 12.8±0.25 |
| **Hemoglobin 13.7-16.3** | 46.9±1.09 | 48.2±0.39 | 47.3±0.57 | 48.5±0.40 | 48.9±0.72 | 51.6±1.33 |
| **RBC (XlOE$_{12}$/L) 4.5-6.5** | 5.52±0.52 | 5.60±0.35 | 4.70±0.38 | 4.59±0.56 | 4.26±0.48 | 4.03±0.70 |
| **WBC (XlOE g/l)** | 8.95±1.05 | 9.78±0.80 | 12.8±0.80 | 13.4±0.55 | 14.8±0.95 | 16.0±0.89 |
| **Platelets (XlOE g/l)** | 310±29.6 | 325±24.0 | 395±25.0 | 430±26.0 | 545±27.5 | 584±50.3 |
| **Helicobacter Pylori (IgG) (U/ml)** | 0.15±0.03 | 0.19±0.03 | 2.30±0.25 | 2.7s5±0.39 | 4.53±0.69 | 5.33±052 |
| **Less than 0.9 U/ml** | 0.13-0.17 | 0.17-0.21 | 2.18-2.40 | 2.67-2.88 | 4.20-4.90 | 5.08-5.60 |

**Table 3. Measurement conditions of elements in electro atomic absorption spectrometer A.Analyst 700.**

| Parameters | Fe |
|---|---|
| **Lamp current (mA)** | 7.5 |
| **Wavelength (nm)** | 213.8 |
| **Slit width (nm)** | 0.7 |
| **Burner height (nm)** | 7.5 |
| **Oxidant (Air) L/min** | 17.0 |
| **Fuel(acetylene ) L/min** | 2.0 |

Sample volume (10µl), Cuvette = Cup, Carrier gas = (200ml/min), Background correction (D2 Lamp)

**Table 4.** ***Determination of Fe in certified reference material of whole blood AND serum (ClinCheck control-lyophilized®) and Urine (mg/L) by the proposed method (n = 6)a***

| Elements | Certified values | MWD Mean ± SD | (%) Recovery | Paired t-test[a] t Experimental |
|---|---|---|---|---|
| **Certified Sample of Whole Blood (mg/*L*)** | | | | |
| Fe | 14.2±3.24 | 14.07±1.03 (7.24)c | 99.1b | 0.035a |
| **Certified Sample of Serum (mg/L)** | | | | |
| Fe | 1.3±0.3 | 1.29±0.13(8.52) | 99.2 | 0.32 |
| **Certified Sample of urine (mg/L)** | | | | |
| Fe | 39±10 | 38.6±1.4 (3.62) | 98.9 | 0.994 |

**Table 5.** Iron concentration in the blood of gastrointestinal Disturbed patients and referents

| Age Group | Treatment Duration | Generic Name | Referents | Ibs | Gastritis | Gerd | Gastric Ulcer |
|---|---|---|---|---|---|---|---|
| Male **(Fe In Blood )** | | | | | | | |
| 30-45 | **2 To 4 Weeks** | **Omeprazole and Esomeprazole 20 and 40 Mg** | **465.3±25.0** | **409±20.7** | **385±19.2** | **362±27.0** | **342±18.0** |
| 46-60 | | | **482±31.9** | **415.8±17.2** | **398±23.5** | **379±26.3** | **350±23.0** |
| Female Male **(Fe In Blood )** | | | | | | | |
| 30-45 | **2 To 4 Weeks** | **Omeprazole and Esomeprazole 20 and 40 Mg** | **452.5±33.0** | **405±26.3** | **375±22.0** | **357.5±19.9** | **335±21.0** |
| 46-60 | | | **474±26.9** | **410.2±24.5** | **390±18.5** | **364±24.0** | **343±25.5** |

**Table 6** Iron concentration Serum of gastrointestinal Disturbed patients and referents

| Age Group | Treatment Duration | Generic Name | Referents | IBS | GASTRITIS | GERD | GASTRIC ULCER |
|---|---|---|---|---|---|---|---|
| Male **(Fe in Serum)** | | | | | | | |
| 30-45 | **2 to 4 weeks** | **Omeprazole and Esomeprazole 20 and 40 mg** | **3.72±0.65** | **3.48±0.42** | **3.16±0.51** | **2.85±0.45** | **2.69±0.36** |
| 46-60 | | | **3.89±0.52** | **3.54±0.35** | **3.25±0.43** | **3.04±0.35** | **2.85±0.25** |
| Female Male **(Fe in Serum)** | | | | | | | |
| 30-45 | **2 to 4 weeks** | **Omeprazole and Esomeprazole 20 and 40 mg** | **3.65±0.70** | **3.35±0.39** | **3.03±0.37** | **2.72±0.34** | **2.51±0.31** |
| 46-60 | | | **3.72±0.39** | **3.43±0.27** | **3.12±0.29** | **2.95±0.24** | **2.70±0.19** |

**Table 7** Iron concentration Urine of gastrointestinal Disturbed patients and referents

| Age Group | Treatment Duration | Generic Name | Referents | Ibs | Gastritis | GERD | Gastric Ulcer |
|---|---|---|---|---|---|---|---|
| Male **(Fe in Urine )** | | | | | | | |
| 30-45 | **2 to 4 weeks** | **Omeprazole and Esomeprazole 20 and 40 mg** | **2.52±0.27** | **2.76±0.30** | **3.25±0.42** | **3.69±0.45** | **3.97±0.52** |
| 46-60 | | | **2.75±0.19** | **3.54±0.41** | **3.95±0.47** | **4.38±0.65** | **4.89±0.49** |
| Female Male **(Fe in Urine )** | | | | | | | |
| 30-45 | **2 to 4 weeks** | **Omeprazole and Esomeprazole 20 and 40 mg** | **2.75±0.33** | **2.95±0.25** | **3.37±0.29** | **3.82±0.39** | **4.19±0.40** |
| 46-60 | | | **2.95±0.22** | **3.75±0.50** | **4.27±0.35** | **4.55±0.53** | **5.45±0.66** |

## Discussion

This study's objective is to determine the level of critical traces of iron, copper, and zinc in tissue specimens from patients with gastrointestinal disorders and healthy controls in Hyderabad, Sindh, Pakistan. In addition to being necessary for developmental purposes, Fe is also important for the immune system and the absorption of antioxidants [10]. According to a study, blood copper levels could be used to gauge the extent of protein-calorie malnutrition (11). Humans who are malnourished have lower serum concentrations of Cu and Zn, therefore this must be considered while treating them (12). Human development, immunity, the density of bones, red and white blood cell number and quality, Fe transportation the utilization of glucose, and neurological growth all depend on copper. Clinical signs of acquired copper insufficiency are most frequently seen [13].

Changes in trace elements were associated with healthy referents in patients with gastrointestinal disturbances. Numerous trace elements are important constituents and are closely related to hydroxyapatite [14]. Fe, concentrations were shown to be lower in the blood and serum of patients with gastrointestinal disturbances (GDP) compared to referents, but they were found to be somewhat higher in urine samples [15].

Various essential elements particularly Fe, are known to play a variety of roles in human clinical prevention as well as various roles as antioxidants [10, 17]. GI suppressor protein p53, a Zn-binding transcription factor that plays a crucial role in regulating cell growth and survival in the face of

many types of cellular stress, is one of the more than 100 proteins that actively bind DNA [18]. Half of the human tumors have p53 mutations, and metals and redox processes tightly control their activity [19]. The etiology of H. pylori-related IDA has several mechanisms that have been discussed, including occult long-term bleeding from the GI tract caused by gastric intestinal micro erosions, bacterial rivalries for eating habits iron, decreased level of ascorbic acid in the stomach contents, which affects dietary iron incorporation, and elevated levels of cytokines that cause inflammation and hepcidin, the main regulator of iron equilibrium [20]. There is mounting evidence that the metabolic and pharmacological effects of losing weight could be serious, perhaps even fatal [21]. Following bariatric surgery, ID and IDA can be caused by intestinal bleeding (for example, from borderline ulcers) or decreased consumption of iron resulting from postoperative aversion for red meat, decreased stomach acid production of oil, or restriction of the duodenal from the gastrointestinal canal [22].

Cofactors of superoxide dismutase components are essential elements that prevent GDP from starting and moving through cell defense against substances that lead to the creation of free development and free radical oxygen species [23]. Fe and Zinc serves as layer stabilizer and is interested in providing antioxidative protection and corrosive pressure resistance. For gastrointestinal individuals, the liver, the kidneys, the uterus, the prostate, the lung, and the stomach have all been impacted by low Fe levels, according to published research [24]. These findings imply that decreased levels of essential elements particularly Fe, in patients with gastrointestinal disorders are likely among the factors contributing to the etiology of adverse changes in numerous tissues since deficiency has been linked to a significant lack of immune system capacity and an interruption in T-cell function. Lack of suppressor protein, which has been linked to several illnesses, results in P53 deactivation. [25].

## Conclusion

The study evaluated biological specimens from patients with digestive disorders to referents and demonstrated a significant departure for the trace elements. The data gathered demonstrates the enormous impact of necessary trace elements along with their methodologies, as well as how they balance out to hurt people. According to the current data, the levels in biological specimens like blood and serum have been determined to be lower, however, they were slightly greater in urine specimens. Therefore, repeated evaluation of biochemical markers reveals that these are crucial elements for individuals with gastrointestinal disturbances and that maintaining their balance will be beneficial to health. Therefore, more research is needed to determine their significance in gastrointestinal disturbance patients as well as their vulnerability to underlying trace elements.

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## Conflict of Interest

The authors state that there are no concerns at odds with one another.

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