

# Gastric Fluid Calprotectin Levels

## Gastrik Sıvı Kalprotektin Değerleri

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### ABSTRACT

**Objective:** This study aims to prove the presence of calprotectin in gastric fluid, and to evaluate the use of gastric fluid calprotectin in the diagnosis of gastric cancer.

**Materials and Methods:** Included in this prospective study were 60 patients with upper gastrointestinal system complaints. Gastric fluid samples obtained during gastroscopy procedures through an ERCP cannula were stored at -20 degrees centigrade for six months, after which the calprotectin levels were measured. Standard endoscopic biopsies were also taken from the patients included in the study for tissue diagnosis.

**Results:** A statistically significant difference was found between the groups ( $p=0.0001$ ), with significant differences identified between the three groups. The  $p$  values identified in comparisons of group 1 and group 2; group 1 and group 3; and group 2 and group 3 were found to be  $p=0.043$ ,  $p=0.001$  and  $p=0.0001$ .

**Conclusion:** Calprotectin, which is found in the gastric fluid, may be used in the differential diagnosis of benign and malignant ulcers. Prospective studies are needed to support the use of calprotectin in early diagnoses of gastric cancer.

**Keywords:** Calprotectin, gastric fluid, diagnosis

### ÖZ

**Amaç:** Kalprotektinin gastrik sıvıda varlığını ispatlayarak, gastrik kanser tanısında gastrik sıvı kalprotektinin kullanılabilirliğini değerlendirmektir.

**Gereç ve Yöntemler:** Bu prospektif çalışmaya üst gastrointestinal sistem şikayetleri bulunan 60 hasta dahil edildi. Gastroskopi işlemi esnasında ERCP kanülü ile alınan gastrik sıvı örnekleri -20 derecede 6 ay saklanarak kalprotektin değerleri ölçüldü. Çalışmaya dahil edilen hastalardan doku tanısı amacıyla standart endoskopik biyopsiler alındı.

**Bulgular:** Gruplar arasında istatistiksel olarak anlamlı fark tespit edilmiştir ( $p=0,0001$ ). Her üç grup arasında da anlamlı fark olduğu görüldü. Buna göre grup 1 ile grup 2 karşılaştırıldığında  $p=0,043$  olarak, grup 1 ile grup 3 karşılaştırıldığında  $p=0,001$  olarak ve grup 2 ile grup 3 karşılaştırıldığında  $p=0,0001$  olarak tespit edildi.

**Sonuç:** Gastrik sıvıda da mevcut olan kalprotektin, benign-malign ülser ayırıcı tanısında kullanılabilir. Gastrik kanser erken tanısında da kullanılabilirliğini ispatlamak için prospektif çalışmalara ihtiyaç vardır.

**Anahtar kelimeler:** Kalprotektin, gastrik sıvı, tanı

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## INTRODUCTION

Calprotectin, which is formed of light (MRP 8) and heavy (MRP 14) chains, and is present in the cell cytoplasm at the rate of 60 percent, is a 36 kD protein that belongs to a family of calcium-binding proteins (1). Calprotectin is more commonly found in the cytoplasm of neutrophils and has antimicrobial properties (2). Stimulated neutrophils and leukocytes present in the medium release calprotectin during cell death and rupture, leading to an increase in calprotectin levels in the urine, synovial fluid, plasma and feces (3-8). Calprotectin is a marker that is specific to acute inflammation (9). Fecal calprotectin values increase in colorectal cancer, inflammatory bowel disease, necrotizing enterocolitis and celiac disease, and so can be used as a non-invasive and non-specific marker for the diagnosis of these diseases (10-14). Furthermore, fecal calprotectin values also allow the observation of the activation and remission phases of ulcerative colitis and Crohn's disease (15-17). Another finding that supports the role of plasma calprotectin as a marker that increases in inflammation is the increased level of plasma calprotectin in cystic fibrosis, lung infections and rheumatoid diseases (18-23).

This prospective study aims to prove the presence of calprotectin in gastric fluid and to evaluate its utilization in diagnoses of gastric cancer.

## MATERIALS AND METHODS

A total of 60 patients who presented to the endoscopy unit of our hospital with epigastric pain, dyspepsia, lack of appetite and weight loss, were included in the study. The patients were divided into three groups according to the results of an upper gastrointestinal endoscopy and a biopsy that were performed following a fasting period of 8-12 hours. The groups were classified as normal (group 1, n=20), gastric ulcer (group 2, n=20) and gastric cancer (group 3, n=20).

Gastric fluid samples of 5 cc, obtained during gastroscopy procedures using an ERCP cannula, were stored at -20 degrees centigrade for around six months. Multiple biopsies were taken from the prepyloric region, antrum-lesser curvature, incisura angularis and corpus-greater curvature.

A PhiCal test micro ELISA kit was used to measure calprotectin levels after the gastric fluid samples had been allowed to thaw at room temperature. The gastric fluid samples were then diluted to a ratio of 1/20 using a

diluent solution that was diluted with distilled water in a ratio of 1/10. A 900 µL dilution solution, diluted at a ratio of 1/10, was added to 100 µL of gastric fluid in order to bring the pH level of the samples to 7.4. The samples, standard solutions and control solutions were added to special micro wells coated with 100 µL of calprotectin antibody. These were then incubated at room temperature for 45 minutes, and following incubation, they were rinsed five times with a 5% rinsing solution, pre-diluted with distilled water. An enzyme solution with immunoaffinity was added to 100 µL of anticalprotectin after rinsing. The solution was then covered and incubated for 45 minutes at room temperature, and then rinsed for five more times following incubation. An enzyme substrate of 10 µL was added to each well, and the samples were incubated for 30 minutes at room temperature. Their optic density values were then read by an ELISA reader at 450 nanometers.

## Statistical Analysis

Gastric fluid calprotectin levels were compared between the groups using a One-Way variance analysis, and a  $p < 0.05$  was considered significant. A post hoc Scheffe test was used to identify which group was the origin of the difference.

## RESULTS

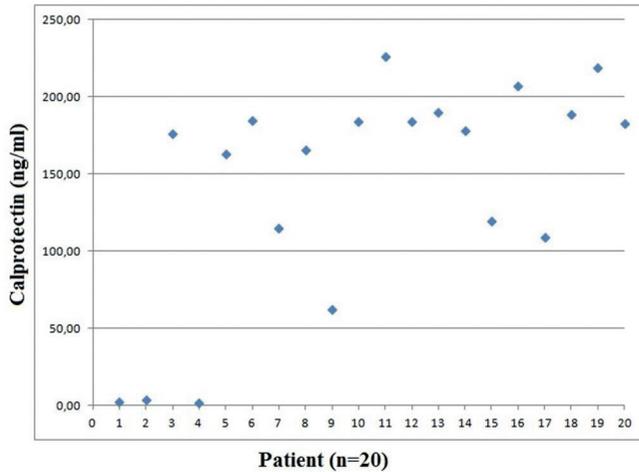
The mean age and mean calprotectin level was found to be 39 (21-54) years and  $73.58 \pm 20.06$  ng/mL (Graphic 1), respectively in group 1, compared to 47 (23-72) years and  $16.96 \pm 7.71$  ng/mL (Graphic 2), respectively in group 2 and 54 (23-77) years and  $143.07 \pm 16.10$  ng/mL (Graphic 3), respectively in group 3.

A statistically significant difference was found between the groups when  $p < 0.05$  was accepted as significant ( $p = 0.0001$ ), and the differences between all three groups were observed to be significant when a Scheffe test was applied to identify the source of the difference. The  $p$  values were found to be  $p = 0.043$ ,  $p = 0.01$  and  $p = 0.0001$ , respectively when groups 1 and 2; groups 1 and 3; and groups 2 and 3 were compared.

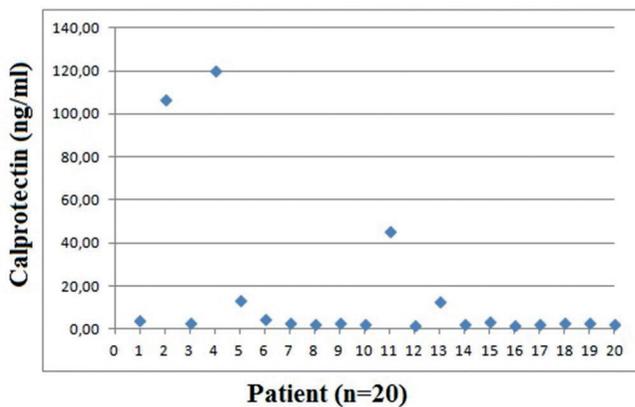
## DISCUSSION

Plasma levels in calprotectin have been shown to increase in acute inflammation in previous studies (24,25). Calprotectin, as a promising new marker for diagnoses of inflammatory pathologies, having been detected in various body fluids, and was found to be present in gastric fluid

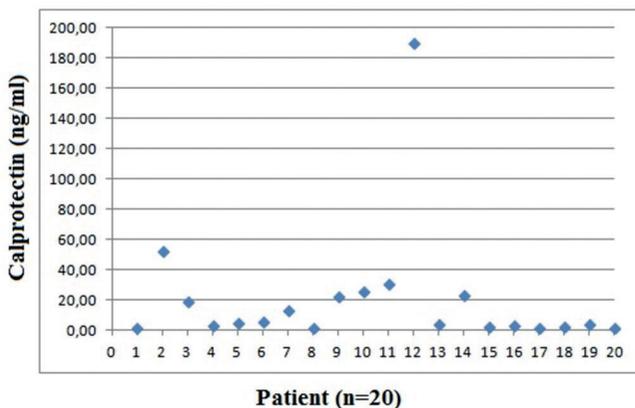
in the present study. The highest increase in calprotectin level was found in the gastric cancer group, which may



**Graphic 1:** Normal group calprotectin levels



**Graphic 2:** Gastric ulcer calprotectin levels



**Graphic 3:** Gastric cancer calprotectin levels

be attributed to the amount of calprotectin released into the medium from the leukocytes present in the tissues as a result of inflammation developing in the cancer tissue. In the gastritis-ulcer group, the gastric fluid calprotectin level was detected to be lower due to the denaturation of calprotectin, which is present in protein structure, due to increased acid amount. Levels of calprotectin in the gastric fluid are increased in the presence of a gastric ulcer with a malignant pathology, while calprotectin levels are low in gastric ulcers with a benign pathology.

Calprotectin levels were found to be statistically significantly higher in the cancer group than in the ulcer group, which supports the use of calprotectin in diagnoses of gastric cancer. Gastric cancer develops on the basis of gastric ulcers, and the level of calprotectin in gastric fluid can be useful when making a differential diagnosis of an ulcer in define whether or not it is malignant. This result suggests that the level of calprotectin in gastric can be used in diagnoses of early-stage gastric cancer, although prospective studies are required to further determine its use in this regard.

**Ethics Committee Approval:** For this study, nothing more than routine work of endoscopy unit and no intervention to patient has been done.

**Informed Consent:** There is no intervention to the patient for the study.

#### Authorship Contributions

Surgical and Medical Practices: M.Ç., Concept: M.Ç., Design: M.Ç., Data Collection or Processing: M.Ç., Analysis or Interpretation: M.Ç., Literature Search: E.G., Writing: M.Ç., E.G.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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