Original Article

# The Prognostic Value of UHRF-1 and p53 in Gastric Cancer

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## ABSTRACT

**Background/Aims:** This study aimed to examine whether UHRF-1 and p53 overexpression is a prognostic marker for gastric cancer. **Patients and Methods:** Sixty-four patients with gastric cancer (study group) and 23 patients with gastritis (control group) were evaluated. Immunohistochemistry was used to examine expression of UHRF-1 and p53 in gastric cancers and a control group diagnosed with gastritis. **Results:** The median age was 63 years (18-83 years) in the study group. UHRF-1 was positive in 15 (23%) patients with gastric cancer and fi ve (21.7%) patients with gastritis ($P = 0.559$). UHRF1 expression level in gastric cancer is more powerful than in gastritis ($P = 0.046$). Thirty-seven (61%) patients with gastric cancer and only one patient with gastritis were p53 positive ($P < 0.001$). After a median follow-up of 12 months (1–110), the 2-year overall survival rates were 55% and 30% in negative and positive p53, respectively ($P = 0.084$). Also, the 2-year overall survival rates were 45% and 53% in negative and positive UHRF-1, respectively ($P = 0.132$). **Conclusion:** According to this study, UHRF-1and p53 were not prognostic factors for gastric cancer, whereas they may have a diagnostic value for differantiating between gastric cancer and gastritis.

**Key Words:** Gastric cancer, p53 genes, prognosis, survival, UHRF-1 protein

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Gastric cancer is one of the most frequent cancers in the world, for both men and women. Gastric cancer is the fourth most frequent cancer and is the second leading cause of cancer-related death worldwide. Nearly two-thirds of stomach cancers occur in developing countries.[1] Despite innovations in treatment modalities, gastric cancer still remains a mortal disease.[2] Gastric cancer is more frequent in the Eastern population and in Turkey than in the European population. It is the second leading cause of cancer death in men, and the third leading cause in women in Turkey.[3] Unfortunately, diagnostic and prognostic markers are insufficient for this mortal and frequent tumor.

Epigenetic silencing of tumor suppressor genes is a hallmark in human cancers affecting multiple cellular pathways, such as cell signaling, adhesion and invasion, cell cycle, angiogenesis, DNA repair, and apoptosis.[4,5] Epigenetic alterations contribute significantly to the development and progression of gastric cancer.[6] Ubiquitin-like, containing PHD and RING finger domains 1 (UHRF1) is a newly discovered gene reported to have a function in maintaining DNA methylation by helping recruit DNA methyltransferase 1 (DNMT1) to hemimethylated DNA.[7] UHRF1 is a putative oncogenic factor and, is overexpressed in numerous cancers.[8-12] There is insufficient data on the role of UHRF-1 in gastric carcinoma in the literature.

P53 is a well-known tumor suppressor gene and many human cancers are found to have a mutant p53 gene. In gastric carcinomas, p53 expression frequency has been reported to vary from 25% to 60%.[13-16] Its prognostic role in gastric cancer has remained controversial.[17]

In this study, p53 and UHRF-1 expression were investigated in gastric carcinoma and a control group. We aimed to investigate the diagnostic and prognostic value of these parameters for gastric carcinoma.

## PATIENTS AND METHODS

This investigation was conducted in the Medical Oncology and Pathology Department of Cumhuriyet University

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Hospital in Sivas, Turkey. The Institutional Review Board of Cumhuriyet University approved the study design.

From 2008 to 2012, a total of 64 gastric cancer patients as a "study group" and 23 patients with gastritis as a "control group" were admitted. Their formalin-fixed, paraffin-embedded tissue samples were obtained from the files of the Pathology Department. For p53 MoAb, a mouse anti-human antibody, clone DO-7 (Dako, Glostrup, Denmark, Labvision Laboratories) and for UHRF-1 polyclonal rabbit, clone bs-6427R (Woburn, Massachusetts, USA, Bioss Laboratories were used for immunohistochemistry. Immunohistochemically, the avidin–biotin peroxidase complex technique was employed. 3-Amino-9-ethyl-carbazole (AEC) was used as the chromogen and Mayer's hematoxylin was used for counterstaining. Negative controls were prepared by replacing the primary antibodies with normal mouse serum. Positive controls for UHRF-1 were prepared from breast carcinoma and colorectal carcinoma for p53. The p53 and UHRF-1 positive cases were semiquantitatively categorized. A staining of more than 5% of tumor cells was accepted as positive expression. The percentage of positive cells was divided into 5 grades (percentage cores): <5%, 0; 5%–25%, 1; 26%–50%, 2; 51%–75%, 3; and >75%, 4. Gastric cancer patients' cilinical data were obtained from hospital files.

Staging and grading were referred to the 7th edition of American Joint Committee on Cancer (AJCC) tumour-node metastasis (TNM) staging and classification for carcinoma of the stomach (2010). All patients underwent surgery/biopsy and postoperative treatment at the same hospital. Fifty-eight patients (91%) underwent surgery and six patients (9%) underwent only diagnostic biopsy and were admitted as inoperable. The surgical technique in this group was as follows: 28 (48%) patients subtotal gastrectomy, 28 (48%) patients total gastrectomy, and 2 (4%) patients palliative passage surgery. Thirty-nine (61%) patients were treated with surgery plus adjuvant chemo-radiotherapy. As a chemotherapy regimen, 5-fluorouracil and folinic acid were used for adjuvant treatment and docetexal–cisplatin and 5-fluorouracil (DCF) were used for metastatic disease.

Statistical analysis of clinicopathological parameters was done with frequency analysis, Chi-square test, or Fisher's exact test using a significance level of <0.05. The survival rates of the patients were estimated by the Kaplan–Meier method, and Cox regression analysis was used for all prognostic parameters. The SPSS 14.0 program (Chicago, IL, USA) for Windows was used for statistical analyses. A written informed consent was obtained from each subject and the study protocol was approved by the Human Ethics Committee of our university.

## RESULTS

The study was performed on 64 patients (study group) with primary gastric carcinoma and 23 patients (control group) with gastritis. Forty-nine patients (77%) were male and 15 (23%) were female. The median age was 63 years (18–83 years). All of the patients had adenocarcinoma and seven (11%) of them had signet ring cell morphology. According to morphologic classification, 38 (59%) patients were diagnosed intestinal type and 26 (41%) patients were diffuse type. Perineural invasion (PNI) was positive in 29 patients (56%), lymphovascular invasion (LVI) was positive in 36 (68%) patients, and extracapsular involvement was positive in 22 (41%) patients. The stages of disease were: Two patients (3%) stage I, 13 (13%) patients stage II, 37 (58%) patients stage III, and 12 (19%) patients stage IV. The histopathological grades were 10 (16%) patients grade I, 19 (31%) patients grade II, 28 (47%) patients grade III, and 4 (6%) patients grade IV. Sixteen (25%) patients had at least one comorbid disease.

UHRF-1 was positive in 15 (23%) patients with gastric cancer and in five (21.7%) patients with gastritis ($P = 0.559$) [Figures 1 and 2]. UHRF-1 expression level in gastric cancer is more powerful than in gastritis ($P = 0.046$) [Table 1]. p53 was positive in 37 (61%) patients with gastric cancer [Figure 3] and in only one patient with gastritis ($P < 0.001$).

There was no correlation between p53 positivity and lymph node involvement, LV and PN invasion, stage or survival ($P > 0.05$). The p53 positivity was more common in metastatic patients than in nonmetastatic patients (89% vs 56%) but the difference was not statistically significant ($P = 0.061$). UHRF-1 positivity was more frequent in intestinal type (32% vs 11%), but the difference was not statistically significant ($P = 0.057$). Table 2 shows the

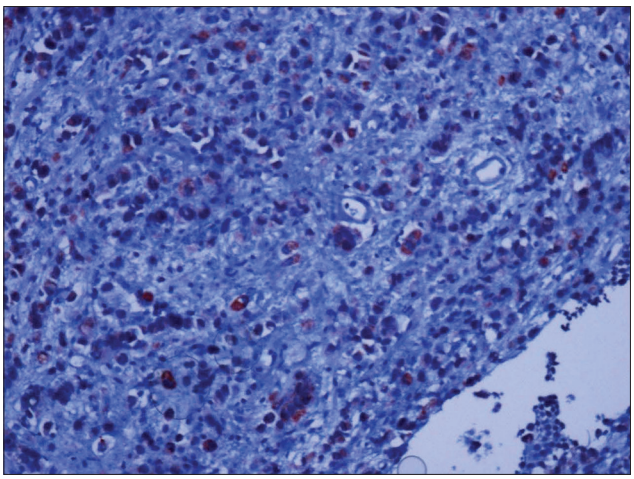

**Figure 1:** UHRF-1 positivity in gastric carcinoma (×40)

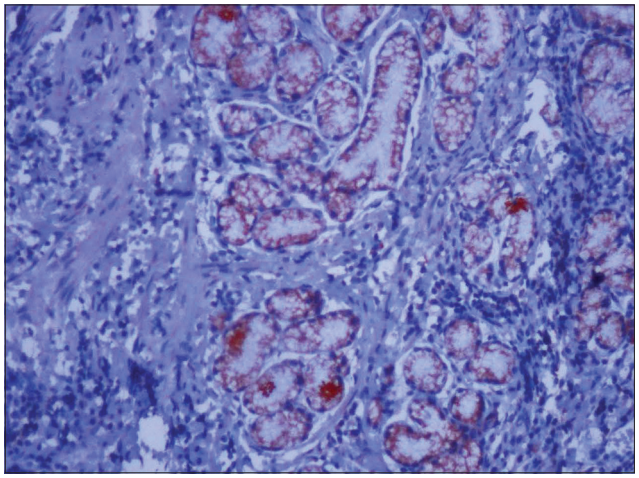

**Figure 2:** UHRF-1 positivity in gastritis (×40)

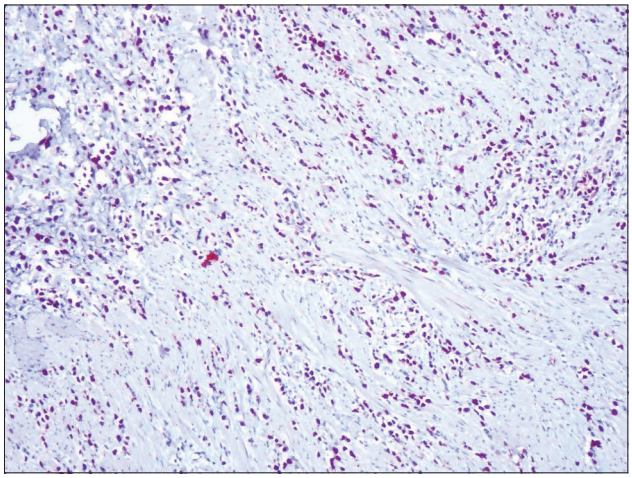

**Figure 3:** p53 positivity in gastric carcinoma (×40)

**Table 1: UHRF1 expression level in gastritis and gastric cancer**

| | *N* | Expression level of UHRF1 | | | | | *P* |
|---|---|---|---|---|---|---|---|
| **Grade** | | **0** | **1** | **2** | **3** | **4** | |
| Gastritis | 23 | 18 | 5 | 0 | 0 | 0 | 0.046 |
| Gastric cancer | 64 | 49 | 3 | 3 | 4 | 5 | |

relationship with p53, UHRF-1 and LVI, PNI, lymph node, stage, histopatologic type, and metastasis status.

The median follow-up of gastric cancer patients was 12 months (1–110). The median survival was 20 months and, the 2 years overall survival rate was 47% in all gastric cancer patients. According to morphologic subtype, the 2 years overall survival rate and the median survival of patients with intestinal type were 59% and 22 months; the 2 years overall survival rate and the median survival of patients with diffuse type were 41% and 11 months, respectivelly ($P = 0.116$). For positive p53 expression 2 years overall survival rate was 30%, whereas for negative expression it was 55% ($P = 0.084$). Also, for positive UHRF-1 expression 2 years overall survival rate was 53%, whereas for negative expression it was 45% ($P = 0.132$). There was no correlation between survival and both UHRF-1 and p53. Table 3 demonstrates the survival rates according to p53 and UHRF-1.

## DISCUSSION

Despite advances in treatment, gastric cancer still has a high mortality.[2] The curative resection, depth of invasion, lymph node metastasis, HER2 status, stage, and carcinoembryonic antigen (CEA) level are important prognostic factors for gastric cancer.[2,18,19]

Epigenetic modifications play a central role in gastric carcinogenesis.[20-22] UHRF-1, as an epigenetic regulator, has been shown to be overexpressed and to coordinate tumor suppressor gene silencing in several cancers.[7] UHRF-1 has been suggested to be an important biomarker to discriminate between cervical high-grade and low-grade cancer lesions.[23] Another study has highlighted the efficiency of UHRF-1 as a marker to differentially diagnose pancreatic adenocarcinoma, chronic pancreatitis, and normal pancreas.[24] UHRF-1 overexpression was also found in bladder cancer and the intensity of its overexpression appears to be related to the stage of the cancer suggesting that the presence of UHRF1 in urine sediment or surgical specimens could be a useful diagnostic marker and may improve the diagnosis of bladder cancer.[11] UHRF-1 overpression has also been described in lung cancer cells, particularly in nonadenocarcinomas.[10] This alteration in UHRF-1 expression could be linked to the degree of lung cancer aggressiveness and was detectable in half of the patients in an early pathological stage. This suggests therefore that UHRF-1 could be a novel diagnostic tool for lung cancer.[10] On the other hand, according to Babbio and colleagues, UHRF-1 expression is correlated with a higher risk of fatality in prostate cancer patients.[25] According to Geng *et al*., the UHRF-1 DNA level in plasma is highly correlated with breast cancer and its stage, and may be a potential independent diagnostic and prognostic factor for breast cancer patients.[12]

In our study, we compared patients with gastritis and with gastric cancer in order to look for the diagnostic value of UHRF-1 found to be a nondiagnostic marker. The patients with chronic active gastritis in the control group may have led to this negative result. It is known that stomach epithelium proliferation is frequent and its regeneration is continuous. UHRF-1 positivity in the control group may be secondary to the existence of active inflammation and proliferation, as in gastritis. Moreover, the success of the immunohistochemical test may vary according to the time that histopathologic slices are in the formaldehyde solution.

**Table 2: The relationship with P53, UHRF-1 and LVI, PNI, lymph node, stage, metastases status**

| Variable | *N* (%) | | *P* | *N* (%) | | *P* |
|---|---|---|---|---|---|---|
| | P53 (−) | P53 (+) | | UHRF-1 (−) | UHRF-1 (+) | |
| LVI[1] | | | | | | |
| Negative | 5 (24) | 16 (76) | 0.254 | 11 (28) | 28 (72) | 0.247 |
| Positive | 11 (37) | 19 (63) | | 6 (43) | 8 (57) | |
| PNI[2] | | | | | | |
| Negative | 12 (57) | 9 (43) | 0.145 | 15 (65) | 8 (35) | 0.205 |
| Positive | 11 (38) | 18 (62) | | 23 (79) | 6 (21) | |
| Lymph node | | | | | | |
| Negative | 5 (21) | 19 (79) | 0.246 | 15 (31) | 34 (69) | 0.161 |
| Positive | 12 (32) | 25 (68) | | 2 (13) | 13 (87) | |
| Stage | | | | | | |
| Stage 1-2 | 9 (60) | 6 (40) | 0.090 | 12 (80) | 3 (20) | 0.715 |
| Stage 3 | 13 (37) | 22 (63) | | 27 (73) | 10 (27) | |
| Stage 4 | 2 (18) | 9 (82) | | 10 (83) | 2 (17) | |
| Lauren classification | | | | | | |
| Intestinal type | 12 (33) | 24 (68) | 0.188 | 26 (68) | 12 (32) | 0.057 |
| Diffuse type | 12 (48) | 13 (35) | | 23 (89) | 3 (11) | |
| Distant metastases | | | | | | |
| No | 23 (96) | 1 (4) | 0.061 | 41 (84) | 8 (16) | 0.570 |
| Yes | 29 (78) | 8 (22) | | 13 (87) | 2 (13) | |

[1]LVI: Lymphovascular invasion, [2]PNI: Perineural invasion

**Table 3: The survival rates according to p53 and UHRF-1**

| p53/UHRF-1 | No. of patients | The 2 years OS[1] rate (%) | Median survival (month) | *P* |
|---|---|---|---|---|
| P53 | | | | |
| Negative | 24 | 55 | 26 | 0.084 |
| Positive | 37 | 30 | 11 | |
| UHRF-1 | | | | |
| Negative | 49 | 45 | 20 | 0.132 |
| Positive | 15 | 53 | N/A[2] | |

[1]OS: Overall survival, [2]N/A: Not available

If we examined fresh tissue and used a real-time PCR method or monoclonal antibody for paraffin-embedded tissue, we may have obtained positive findings. There is very little information in the literature about the relationship between UHRF-1 and gastric cancer. Zhou *et al*. demonstrated that UHRF-1 was overexpressed in GC tissues and a high level of UHRF-1 expression predicted poor survival.[26] Conversely, there was no correlation between UHRF-1 positivity and lymph node positivity, LVI positivity, PNI positivity, stage, and survival in our trial.

Mutations in the p53 gene belong to the most common genetic alterations in human cancer that have been implicated in tumorigenesis and tumor progression. In the literature, there are many studies about the importance of p53 in gastric cancer. Fondevila *et al*. demonstrated that p53 expression is an independent prognostic factor for both disease-free survival and overall survival in patients with curatively resected gastric cancer, and that p53 status may also influence response to chemotherapy.[13] In gastric carcinomas, p53 expression frequency has been reported to vary from 25% to 60%.[13-16] In this study, the p53 frequency for gastric carcinoma was 61% and was higher than the control group.

Kakeji *et al*. Xiao *et al*., and Starzynska *et al*. reported a positive correlation between p53 expression and metastasis.[27-29] Maehara *et al*. highlighted that the expression of p53 was closely related to the potential for tumor advancement and a poorer postoperative prognosis for patients with gastric cancer.[30] Deveci demonstrated that p53 positivity was a poor prognostic factor for more than five metastatic lymph nodes involved in gastric carcinoma patients.[14] On the other hand, some studies did not support the prognostic value of p53 in gastric cancer. Hurlimann and Saraga, Fukunaga *et al*., and Gabbert *et al*. have found no relationship of p53 expression with liver metastasis or lymph node involvement.[15,17,31] Gabbert *et al*. reported that p53 expression had no influence on survival, in either the lymph node-positive or -negative groups for gastric cancer.[17] Correspondingly, in this study no correlation was found between p53 positivity and lymph node, LVI, PNI, stage, or survival. In fact, the p53 positivity was more common in metastatic patients than in nonmetastatic patients, but the difference was not statistically significant in this trial.

## CONCLUSION

This trial demonstrated that p53 might be helpful in differentiating gastric cancer and gastritis. Although UHRF1 expression positivity was similar in both gastric cancer and gastritis, expression level in gastric cancer was found to be more powerful. Both of them were not prognostic for gastric cancer in this study. The small patient number, the features of the control group, and the immunohistochemical method may be restrictive sides of this trial. It is necessary to perform an extensive study on this subject in the future.

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