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# The distribution of telomerase activity in patients with *Helicobacter pylori* positive gastritis

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**Aim:** *Helicobacter pylori* is considered a class I carcinogen by the World Health Organization. We aimed to determine whether *H. pylori* has an effect on telomerase activity in patients with *H. pylori* related non-specific gastritis, atrophy, and intestinal metaplasia.

**Materials and methods:** One hundred and seventy-two adult patients who underwent upper gastroduodenoscopy were enrolled in the study. Three biopsy specimens were taken from the antrum: 1 from the incisura angularis and 2 from the mid-antrum. Biopsy specimens taken from the incisura angularis were evaluated using the urease test for detection of *H. pylori*. The mid-antrum specimens were sent for histopathology and tissue telomerase activity testing. The histopathologic evaluation was performed based on the updated Sydney system. Quantitative detection of hTERT mRNA was performed with the available method for telomerase activity.

**Results:** Of the 172 patients, 119 were eligible for the study. *H. pylori* was positive in 68 (57.14%) and negative in 51 (42.85%) of the cases ($P > 0.05$). Of the 119 patients, 6 had intestinal metaplasia, 27 had glandular atrophy, 62 had neutrophilic activation, and 102 had chronic inflammation. The telomerase activity of the *H. pylori* positive and negative groups did not show a statistically significant difference in patients with intestinal metaplasia, glandular atrophy, neutrophilic activation, and chronic inflammation ($P > 0.05$, for each). hTERT activity was higher in *H. pylori* positive patients who had glandular atrophy and intestinal metaplasia than the negative group. However, the differences were insignificant.

**Conclusion:** We could not find any significant relationship between telomerase activity and *H. pylori* related non-specific gastritis, atrophy, and intestinal metaplasia. hTERT activity was higher in patients who had glandular atrophy and intestinal metaplasia (early stages of gastric carcinogenesis) in the *H. pylori* positive group. However, these differences were not significant. *H. pylori*, which is considered an oncogenic agent, may influence telomerase activity of further stages of carcinogenesis, particularly those after intestinal metaplasia.

**Key words:** *Helicobacter pylori,* telomerase

## *Helicobacter pylori* positif gastritli hastalarda telomeraz aktivitesinin dağılımı

**Amaç:** *Helicobacter pylori* Dünya Sağlık Örgütü tarafından class I karsinojen olarak kabul edilmiştir. Biz *H. pylori* ile ilişkili non-specific gastrit, atrofi ve intestinal metaplazi tespit edilen hastalarda, *H. pylori*'nin telomeraz üzerine etkisi olup olmadığını araştırmayı amaçladık.

**Yöntem ve gereç:** Gastroduodenoscopy yapılıp gastirit tanısı konulmuş 172 hasta çalışmaya dahil edilmiştir. Bu hastalardan, 1 tanesi incisura angularis, diğer 2si de mid-antrumdan olmak üzere üç biyopsi örneği alınmıştır. İncisura angularisden alınan biyopsi örneklerinde *H. pylori* varlığını göstermek için üreaz testi kullanılmıştır. Mid-antrumdan alınan iki örneğin biri histopatolojik incelemede, diğeri de telomeraz aktivite tayininde kullanılmıştır. Histopatholojik değerlendirme için update edilmiş Sydney sistemi kullanılmıştır. hTERT'in kantitatif analizi ile telomeraz aktivite tayini yapılmıştır.

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**Bulgular:** Yüz yetmişiki hastanın 119 çalışmaya dahil edilmiştir. Vakaların 68 (57,14%) tanesinde *H. pylori* pozitif ve 51 (% 42,85)'inde negatif (P > 0,05) idi. Hastalardan, 119'unun 6'sında intestinal metaplazi, 27'sinde glandular atrofi, 62'sinde nötrofilik aktivasyon ve 102'sinde kronik inflamasyon görüldü. *H. pylori* positif ve negatif gruplar arasında telomeraz aktivitesinde istatistiksel olarak önemli bir farklılık yoktu (P > 0,05, herbiri için). hTERT aktivitesi, glandular atrofi ve intestinal metaplazisi olan *H. pylori* positif grubunda negatif olanlara gore daha yüksek olmasına rağmen istatistiksel açıdan önemli değildir.

**Sonuç:** Biz telomeraz aktivitesi ile, *H. pylori* ile ilişkili non-specific gastrit, atrofi ve intestinal metaplasia arasında istatistiksel açıdan önemli bir farklılık bulamadık. *H. pylori* pozitif glandular atrofi ve intestinal metaplazili hastalarda HTERT aktivitesini (gastrik karsinogenezin erken basamağı olan) daha yüksek bulduk. Fakat bu değişiklikler istatistiksel açıdan anlamlılık göstermedi. Onkojenik bir ajan olan *H. pylori*'nin karsinogenezin intestinal metaplaziden daha sonraki basamaklarını etkileyerek telomeraz aktivitesini artırdığı düşünülmektedir.

**Anahtar sözcükler:** *Helicobacter pylori*, telomeraz

## Introduction

Telomeres are specialized heterochromatic structures at the ends of vertebrate chromosomes that have been implicated in stabilizing, protecting, and anchoring chromosomes within the nucleus, as well as assisting the replication of linear DNA (4). Telomeric repeats are lost with each cell division because DNA polymerases cannot replicate the very end of a linear DNA molecule. In contrast, activation of telomerase, the enzyme that synthesizes telomeric DNA, is proposed to be an essential step in cancer cell immortalization and cancer progression (1).

Gastric cancer is thought to be a multistep progression from chronic gastritis, atrophy, and intestinal metaplasia ultimately to dysplasia and cancer (2). *Helicobacter pylori* (*H. pylori*) is a gram-negative spiral microorganism that occurs in the human stomach. It was first cultured in vitro in 1983. Epidemiologic evidence strongly supports a causal role for *H. pylori* in gastric carcinogenesis. In addition, the infection was recognized as a Class I human carcinogen by the International Agency for Research on Cancer in 1994. *H. pylori* infection predisposes to gastric cancer and contributes to the induction of chronic atrophic gastritis and precancerous lesions, such as intestinal metaplasia (3).

Little is known about the influence of *H. pylori* infection on genetic alterations and cell immortality in the gastric mucosa. In this study, we aimed to determine whether *H. pylori* has an effect on telomerase activity in patients with *H. pylori* related non-specific gastritis, atrophy and intestinal metaplasia. To our knowledge, there is no study in the literature reporting quantitative values in *H. pylori* gastritis patients. We also tried to evaluate the quantitative detection of hTERT mRNA by real-time fluorescence RT-PCR, a new molecular diagnostic parameter in the work-up of *H. pylori* gastritis.

## Materials and methods

This study was carried out at the Gastroenterology Endoscopy Unit and Clinical Biochemistry Department of the University Hospital in Diyarbakir, between September 2004 and January 2005. We studied 172 patients with dyspeptic symptoms who had undergone upper gastrointestinal endoscopy. The study was approved by the local ethics committee, and all subjects were recruited in a voluntary manner, giving their written informed consent. Endoscopy was performed using an Olympus GIF-V-70 device. The patients had not been treated for *H. pylori* before. A total of 3 biopsies were taken form each patient using Olympus FB-25K biopsy forceps: 1 from the incisura angularis, and 2 from the mid-antrum. Of the 2 specimens obtained from the mid-antrum, 1 was placed in a separate tube filled with 10% formalin, labeled, and sent to the pathology laboratory, while the other biopsy specimen taken from the antrum was frozen in liquid nitrogen and stored at −80 °C until the analysis of human telomerase reverse transcriptase (hTERT). The biopsy specimens taken from the incisura angularis were placed in tubes containing urease solution and incubated at 37 °C for 2 h. The turning of the solution's color to pink was accepted as a positive test for *H. pylori* while no changes in color were accepted as a negative test. Histopathology of gastritis was classified according to the updated Sydney classification system (4).

Total RNA from the frozen tissue specimens was isolated using "High Pure RNA Tissue Kit" (Roche Diagnostics, Germany). Tissue specimens were crushed using a porcelain mortar and homogenized with "Lysis/Binding Buffer". The obtained total RNA was stored at −80 °C until the analysis of hTERT. Quantitative detection of hTERT mRNA was performed with the commercially available "LightCycler TeloTAGGG hTERT Quantification Kit" (Roche Diagnostics, Germany), using the light-cycler instrument (Roche Diagnostics, Germany).

The recently introduced Light Cycler is a thermocycler with on-line monitoring of PCR. The amplicon is detected by fluorescence using 2 short oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the PCR cycles. One probe is labeled with a fluorescent dye at the 5th end, with another at the 3rd end. The probes are designed to hybridize to the target strand so that the 2 dyes can be in close proximity and fluorescence resonance energy transfer can take place between the 2 fluorophores. This leads to the emission of fluorescence, which is detected on-line during the PCR cycles.

Statistical analysis was carried out by using SPSS 10.0, and a P value lower than 0.05 was considered to be statistically significant.

## Results

Of the 172 patients, 119 were included in the study while 53 patients were excluded, 24 were not classified according to the Sydney classification system during histopathological evaluation, and 29 were not detected for RNA. In the study group, 75 were female and 44 were male and their mean ages were 40.29 ± 14.21 and 42.07 ± 15.72, respectively. *H. pylori* was positive in 68 (57.14%) and negative in 51 (42.85%) patients. Telomerase activity was positive in 83 (69.74%) and negative in 36 (30.25%) patients. Intestinal metaplasia was present in 6, glandular atrophy in 27, neutrophil activation in 62, and chronic inflammation in 102 of the 119 patients.

Telomerase activity of *H. pylori* positive and negative samples, and hTERT median values were evaluated by Mann-Whitney U test in chronic inflammation positive and negative patients. Median telomerase activity and hTERT values were not significantly different in *H. pylori* positive and negative samples in terms of chronic inflammation (P = 0.52 and P = 0.46, respectively).

Telomerase activity of *H. pylori* positive and negative samples, and hTERT median values were evaluated with the same test in neutrophil activity positive and negative patients. Telomerase activity and hTERT were not significantly different in *H. pylori* positive and negative samples in terms of neutrophil activation (P = 0.93 and P = 0.59, respectively).

Telomerase activity of *H. pylori* positive and negative samples and hTERT median values were evaluated in glandular atrophy positive and negative patients. Telomerase activity and hTERT values were higher in *H. pylori* positive than negative samples (telomerase activity 836 vs. 454; hTERT: 1433 vs. 1391). However, the difference was not statistically significant (P = 0.71 and P = 0.13, respectively) (summarized in Table 1).

Table 1. Median values of telomerase activity and hTERT in *H. pylori* positive and negative patients with atrophy and intestinal metaplasia.

| Atrophy | hTERT | TA (Median) | (Median) |
|---|---|---|---|
| *H. pylori* | Positive | 1433 | 836 |
| | Negative | 1391 | 454 |
| Intestinal Metaplasia | | | |
| *H. pylori* | Positive | 1862 | 1867 |
| | Negative | 1422 | 752 |

(TA: Telomerase activity)

Telomerase activity of *H. pylori* positive and negative samples and hTERT median values were also evaluated in intestinal metaplasia positive and negative patients. Telomerase activity and hTERT values were higher in all *H. pylori* positive metaplastic patients (telomerase activity 1867 vs. 752; hTERT: 1862 vs. 1422) (summarized in Table 1). We could not compare these results with the *H. pylori* negative ones since all intestinal metaplasia patients were *H. pylori* positive.

*H. pylori* positive and negative patients were also classified according to their telomerase activity.

Telomerase activity of chronic inflammation, neutrophil activity, and glandular atrophy positive and negative samples were not significantly different in the *H. pylori* positive group (P = 0.56; P = 0.63; P = 0.63, respectively). Telomerase activity of these 3 parameters was also insignificant in the *H. pylori* negative group (P = 0.37; P = 0.43; P = 0.84, respectively) (Table 2).

## Discussion

Recently, a sensitive polymerase chain reaction (PCR)-based telomerase assay, designed for the telomeric repeat amplification protocol (TRAP), has been used to investigate human telomerase activity for cancer and aging research (6). Telomerase activity appears in 85% of human cancers, including breast, bladder, colon, prostate, and liver, and so telomerase may be used as a molecular marker for cancer diagnosis and therapeutic strategies (7). Telomerase activity is also found in precancerous lesions (i.e. gastric intestinal metaplasia and adenomas in the colon mucosa) (8).

Recent epidemiologic evidence indicates that *H. pylori* infection increases the risk of gastric carcinoma. Infection with *H. pylori* leads to chronic atrophic gastritis, which frequently advances to intestinal metaplasia, occasionally to dysplasia, and rarely to carcinoma. *H. pylori* infection increases the rate of proliferation of the gastric epithelial cells and decreases the gastric secretion of ascorbic acid, processes that may modulate the process of carcinogenesis (9).

The presence of *H. pylori* in the gastric mucosa is almost always associated with intense submucosal inflammation with infiltration by neutrophils and monocytes. *H. pylori* induced gastric pathology shares the same proinflammatory mediator profiles as many other systemic diseases (10). The incriminating proinflammatory mediators include leukotriene-B4 (LT-B4), which promotes neutrophil migration and degranulation; interleukin-1β (IL-1 β), which mediates inflammation; tumor necrosis factor-α (TNF- α), which interacts synergistically with interleukin-1 (IL-1); and interleukin-8 (IL-8), which is one of the most potent chemoattractants that also degranulate neutrophils in different organs in the body (11). TNF-α, IL-1β, and IL-8 levels are all raised in gastric mucosa of patients with *H. pylori* infection compared with those who are not infected. Local production of inflammatory cytokines can induce telomerase activity in inflammatory mucosa. *H. pylori* stimulates lymphocytic infiltration of the mucosal stroma and this infiltration may act as a focus for cellular alteration and proliferation, ultimately resulting in neoplastic transformation of cells. It has been reported that infiltrating lymphocytes express telomerase activity in inflammatory tissues (12,13).

Telomerase activity was reported to be higher in intestinal-type gastric cancer than in the diffuse type. How *H. pylori* infection induces high telomerase activity is not clear. One explanation is that *H. pylori* infection may influence the negative regulator of telomerase activity during the early stages of stomach carcinogenesis (14). In a previous study, it was reported that telomerase activity was higher in intestinal metaplasia with *H. pylori* infection than in that without infection. Telomerase is associated with the severity and extent of intestinal metaplasia and *H. pylori* eradication may improve the endoscopic and

Table 2. Results in Sydney criteria of *H. pylori* positive and negative patients in terms of telomerase activity.

| | Telomerase Activity Positive | | |
|---|---|---|---|
| | *H. pylori* positive (n/%) | *H. pylori* negative (n/%) | P value |
| Chronic inflammation positive | 51 (70%) | 22 (30%) | 0.639 |
| Neutrophil activation positive | 40 (85%) | 7 (15%) | 0.059 |
| Glandular atrophy positive | 13 (72%) | 5 (28%) | 0.470 |
| Intestinal metaplasia positive | 6 (100%) | 0 (0%) | – |

histologic features of intestinal metaplasia, and decrease telomerase activity (3). Our study also indicated that *H. pylori* positive samples' telomerase activity and hTERT median values were higher in intestinal metaplasia positive patients than the negative samples.

Zhang et al. found higher telomerase activity in *H. pylori* positive patients with chronic atrophic gastritis and intestinal metaplasia (67.9%) than *H. pylori* negative patients (21.4%) (15). Yao et al. studied telomerase activity of 30 patients with chronic superficial gastritis (44 with precancerous lesions and 42 with gastric cancer) using in situ hybridization and found telomerase activities to be 0%, 36%, and 86%, respectively (16).

In our study, telomerase activity of *H. pylori* positive patients with glandular atrophy was not significantly different from that of *H. pylori* negative patients ($P > 0.05$). Both *H. pylori* infection and telomerase activity were positive in 44% of patients with glandular atrophy. *H. pylori* infection and telomerase activity were negative in 15% of patients with glandular atrophy. *H. pylori* infection was higher in intestinal metaplasia and telomerase activity positive patients. Suzuki et al. found the hTERT gene expression to be positive in 16% of chronic gastritis patients without intestinal metaplasia (17). In our study, we found telomerase activity to be higher in *H. pylori* positive (41%) patients than in *H. pylori* negative (16%) patients with gastritis but without intestinal metaplasia.

Chung et al. reported that telomerase activity increased in 18% of patients with type III intestinal metaplasia (18). In our intestinal metaplasia group, 4 of the 6 patients had high telomerase activity and *H. pylori* positivity. Two of the 6 patients had high telomerase activity but *H. pylori* was negative in them. It was also reported that hTERT expression was higher in the *H. pylori* infected group than in the non-infected group in chronic gastritis patients (19).

This study has additional value in terms of the study protocol. We provided the quantitative values of telomerase activity by real-time fluorescence RT-PCR, a new molecular diagnostic parameter in the workup of *H. pylori* gastritis.

## Conclusion

In our study, we did not find any relationship between *H. pylori* infection and telomerase activity in the presence of neutrophil activation and chronic inflammation in patients with gastritis. Although hTERT activation was higher in patients with glandular atrophy and intestinal metaplasia in *H. pylori* positive samples than in *H. pylori* negative samples, a statistical significance was not found. Our data suggest that *H. pylori*, as an oncogenic bacterium causing non-specific gastritis, intestinal metaplasia, and glandular atrophy, is not related to telomerase activity. These data support the hypothesis that *H. pylori* may increase the risk of gastric cancer after the step of intestinal metaplasia.

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