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Chronic tamoxifen use is associated with a decreased risk of intestinal metaplasia in human gastric epithelium

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Abstract

Background—Intestinal metaplasia (IM), a premalignant lesion, is associated with an increased risk of gastric cancer. Although estrogen exposure, including tamoxifen, has been studied in correlation with gastric cancer, little has been investigated about its effects on IM.

Aims—Therefore, we investigated whether chronic tamoxifen use was associated with the risk of IM in human stomach.

Methods—We evaluated 512 gastric biopsies from 433 female breast cancer patients that underwent endoscopic gastroduodenoscopy (EGD) 6 months after breast surgery. Histopathological findings were scored according to the updated Sydney classification. Demographic and clinical characteristics were also included to identify predictive factors for IM.

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Results—In a multivariate logistic regression analysis, age at EGD (odds ratio [OR], 1.04; $P=0.002$), biopsies from antrum (OR, 2.08; $P<0.001$), and *H. pylori* positivity (OR, 1.68; $P=0.016$) were significantly associated with an increased risk of IM, whereas chronic tamoxifen use (≥ 3 months) was associated with a decreased risk of IM (OR, 0.59; $P=0.025$). After stratifying by biopsy site, association between tamoxifen use and IM persisted for corpus (OR, 0.42; $P=0.026$) but not for antrum (OR, 0.74; $P=0.327$). In analysis limited to patients with follow-up EGD, chronic tamoxifen use also correlated with improved IM score compared to no tamoxifen use (improved, 77.8% vs. 22.2%; no change, 65.4% vs. 34.6%; worsened, 30.0% vs. 70.0%; $P=0.019$).

Conclusions—This study suggests that chronic tamoxifen use can decrease the risk of IM in human stomach. The effect of tamoxifen is predominantly observed in the corpus.

Keywords

Tamoxifen; intestinal metaplasia; gastric epithelium; corpus

INTRODUCTION

Gastric cancer is the second most common cause of cancer-related deaths in the world [1]. Colonization by the bacteria *Helicobacter pylori* (*H. pylori*) is one of the principal causes of most gastric cancer. Chronic persistent inflammation caused by *H. pylori* in the gastric mucosa can result in atrophic gastritis and intestinal metaplasia (IM), which may progress to malignancy [2–4]. Atrophic/metaplastic changes have been considered as premalignant conditions that are significant risk factors for gastric cancer [5, 6]. However, whether *H. pylori* eradication can improve the regression of gastric precancerous lesions, especially IM, still remains debatable [7–14] and there is a clear need for investigation into alternate and/or supplemental medical strategies to reverse premalignant gastric lesions.

The strong male predominance in gastric cancer incidence has led to an interest in the potential protective effect of estrogen [15, 16]. It has been reported that the risk of gastric cancer was decreased among women using postmenopausal hormone replacement therapy (HRT) and among men with prostate cancer treated with estrogen [17, 18]. Tamoxifen is the most commonly used exogenous estrogenic agent with selective estrogen receptor modulator (SERM) properties: i.e., with anti-estrogenic effect in estrogen receptor-positive breast cancer but pro-estrogenic effect in other organs such as the endometrium and bone. It has been widely used clinically in hormone therapy for breast cancer, as an anti-osteoporotic, and in HRT [19, 20]. The effect of tamoxifen on the stomach and, specifically, its impact on gastric cancer is an open question. In observational studies of women with breast cancer, one study demonstrated that tamoxifen treatment was not associated with an increase of incidence of gastric cancer [21], whereas others reported that it increased the risk for subsequent development of gastric cancer [22, 23]. In experimental studies using mice, chronic tamoxifen administration prevented *H. pylori*-induced gastric cancer through attenuating neutrophil infiltration, chronic inflammation, and oncogenic signaling [24, 25]. Our previous study in mice has shown that high-dose tamoxifen treatment causes rapid loss of nearly all gastric parietal cells with concomitant, robust stimulation of gastric epithelial stem/progenitor cells, which in turn leads to restoration of nearly normal gastric epithelium

within three weeks in the corpus [26]. To our knowledge, however, there have not been any studies focusing on the impact of chronic tamoxifen exposure on precancerous lesions of the stomach in humans. Given the long lead-time and multistep processes required for development of gastric cancer in humans and given the lack of treatment options for precancerous lesions, we reasoned that IM in a population where this lesion is common, would be an appropriate end point to investigate chronic tamoxifen effects on the stomach and gastric carcinogenesis.

Therefore, the aim of the present study was to investigate (i) whether chronic tamoxifen use is associated with an increased or decreased risk of IM and (ii) whether continued tamoxifen use effects longitudinal change in presence of IM in human stomach.

MATERIALS AND METHODS

Study subjects

In this study, the electronic medical record system of Samsung Medical Center, Sungkyunkwan University School of Medicine and Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea, was used to select all breast cancer patients who underwent endoscopic gastroduodenoscopy (EGD) and biopsies after breast surgery between March 1999 and November 2011. The inclusion criteria were female patients with breast cancer that have: (1) followed anti-cancer therapies including chemo-, radio-, and hormone therapy according to the standard treatment strategy of each hospital; (2) subsequently undergone EGD 6 months postoperatively; (3) undergone EGD after taking tamoxifen or aromatase-inhibitors for more than 3 months (in the hormone therapy wings); (4) undergone EGD for the purpose of routine screening for gastric cancer or evaluating common symptoms such as chronic dyspepsia or gastroesophageal reflux; (5) had one or more gastric biopsy specimens obtained from corpus or antrum of the stomach during endoscopic examination. In contrast, subjects with the following attributes were excluded: (1) those undergoing EGD for acute illness including abdominal pain or gastrointestinal bleeding; (2) those diagnosed with peptic ulcer disease, gastric polyps or malignancies (dysplasia, mucosa-associated lymphoid tissue [MALT] lymphoma, or adenocarcinoma) at the time of EGD; (3) those undergoing EGD during the period of radiotherapy. (4) those with a history of gastrectomy. (5) those diagnosed with chronic inflammatory diseases (inflammatory bowel disease, rheumatoid arthritis, Behçet's disease, or systemic lupus erythematosus). Finally, a total of 512 gastric biopsies from 433 patients were included in this study. This study was approved by the ethics committees of the institutional review board of Samsung Medical Center and Yonsei University College of Medicine and was conducted in accordance with the ethical guidelines of the Declaration of Helsinki.

Clinical characteristics

We retrospectively collected the demographic and clinical characteristics of all patients including age at breast cancer surgery and EGD, duration between surgery and EGD, the use of hormone therapy, duration of hormone therapy, and history of chronic nonsteroidal anti-inflammatory drug (NSAID) and proton pump inhibitor (PPI) use (Table 1). Chronic hormone therapy including tamoxifen was defined as an intake of any kind of hormone

agents for breast cancer for more than 3 months before EGD. The duration of hormone therapy was defined as the interval between when patients began any hormone agents to the time of EGD. Hormone agents used in this study population were tamoxifen (20 mg/day) and aromatase-inhibiting agents (1 mg/day of anastrozole, 25 mg/day of exemestane, or 2.5 mg/day of letrozole). Chronic NSAID and PPI use was regarded as their intake for more than 3 months during the period between breast cancer surgery and EGD. The most common NSAID used was 100 mg/day of aspirin and the most common PPI was 20 or 40 mg/day of esomeprazole.

Endoscopic and histopathological characteristics

All specimens were routinely fixed in formalin and stained with hematoxylin-eosin (H&E). The histopathological characteristics of these slides were scored according to the updated Sydney classification system [27]. However, the grading of atrophy was not included in this study because a considerable number of specimens did not contain the full-thickness gastric mucosa. To reduce the interobserver variability, *H. pylori* and IM were scored as present (‘mild’ grade in the Sydney classification) or absent. *H. pylori* positivity was based on the histological assessment of *H. pylori* colonization on the gastric surface and accompanied by positive results of rapid urease test in some cases. IM was recognized by the replacement of native gastric epithelium with goblet cells, absorptive cells, and colonocyte-like cells in H&E slides [27]. Original histopathological assessments were performed by experienced pathologists at each institution at time of patient diagnosis. Pathologists were blind to a patient’s hormone therapy status. Furthermore, 138 biopsy tissue blocks were randomly selected, resectioned and reexamined by another pathologist (JCM) in the United States; there was no discordance between assessment at time of diagnosis and reexamination. Finally, IM diagnosis was reassessed in some selected biopsies by immunohistochemical (IHC) staining for CDX2 (1:50; Abcam, Cambridge, MA), which confirmed diagnosis and distinguished IM from other lesions (Fig. 1).

Statistical analysis

Statistical analysis was performed to find the significant clinical or histological characteristics associated with IM in gastric biopsies. In the univariate analysis, we compared all characteristics for cases with and without IM. A Student’s *t* test was used for comparison of continuous variables and χ^2 test or Fisher’s exact test for categorical variables. Variables with a statistical significance (*P* value < 0.05) on univariate analysis were subsequently entered into the multivariate analysis using logistic regression. Clinically important statistically insignificant variables were also included in the multivariate analysis. Correlation between variables and IM was expressed as an odds ratio (OR) and the 95% confidence interval (CI). We also carried out univariate and multivariate analysis after stratifying by biopsy site to identify significant associations on a per-patient level. In addition, we analyzed the diagnosis of IM longitudinally among cases that underwent follow-up EGD. Associations between the variables of interest and the IM change classified into 3 groups (improved, no change, and worsened) were statistically analyzed using One-Way analysis of variance (ANOVA) for continuous variables and linear by linear association for categorical variables.

All values are two-sided, and a P value < 0.05 was considered to indicate statistical significance. The management of data and all statistical analysis was carried out using SPSS version 13.0 (SPSS Inc., Chicago, IL).

RESULTS

Characteristics of the patients and biopsy samples

All of the 433 patients were female, and the mean ages at the surgery for breast cancer and at the EGD were 52.9 ± 9.3 and 56.2 ± 9.1 years, respectively., 179 (41.3%) of the patients were chronic tamoxifen users, 111 (25.6%) were chronic users of aromatase-inhibiting agents (anastrozole, exemestane, or letrozole) and 143 (33.0%) had never used any hormone therapeutic agents. Among the patients with any hormone therapy for breast cancer, the mean duration of hormone therapy use was 28.8 ± 19.7 months. Of these patients, 178 (41.1%) patients underwent endoscopic biopsies from only corpus, 176 (40.6%) from only antrum, and 79 (18.2%) from both. Of a total of 512 gastric biopsies, 169 (33.0%) were *H. pylori* positive, and 147 (28.7%) were found to have IM based on histological examination. Clinicohistological characteristics of the patients and biopsies are shown in Table 1.

Predictive factors of IM (a per-biopsy analysis)

To determine the variables associated with gastric IM, we initially analyzed the difference in various clinical and histological features between biopsies with and without IM (Table 2). Length of hormone therapy was one such variable, except in biopsies from patients who had received no hormone therapy, where the duration between breast surgery and EGD was analyzed instead. On univariate analysis, significant predictive factors for the presence of IM were age at the time of EGD ($P < 0.001$), biopsies from antrum ($P < 0.001$), and *H. pylori* positivity ($P = 0.029$). On the contrary, chronic tamoxifen use was closely related to a decreased risk of IM ($P = 0.001$). No significant differences were seen in the duration between breast surgery and EGD, chronic use of NSAIDs and PPIs, and the grade of inflammation of biopsied tissues between IM-positive and IM-negative groups.

In the multivariate analysis for the independent predictors associated with IM, variables with statistical significance ($P < 0.05$) in univariate analysis were incorporated (Table 3). We also included clinically important variables such as NSAIDs and PPI use in this analysis; however, the grade of inflammation was not included, because it was closely linked to *H. pylori* positivity. After multivariate analysis, three factors were significantly associated with an increased risk of IM: age at the time of EGD (OR [Odds ratio], 1.04; 95% CI [confidence interval], 1.01 – 1.06; $P = 0.002$), biopsies from antrum (vs. corpus; OR, 2.08; 95% CI, 1.39 – 3.13; $P < 0.001$), and *H. pylori* positivity (OR, 1.68; 95% CI, 1.10 – 2.57; $P = 0.016$). Additionally, chronic tamoxifen use was identified as a protective factor for IM (OR, 0.59; 95% CI, 0.37 – 0.94; $P = 0.025$).

To evaluate whether there was a different association with a risk of IM between aromatase-inhibitor use and no use of any hormone agents, we further performed a subgroup analysis among only patients without tamoxifen use. Chronic aromatase-inhibitor use was not associated with IM in univariate ($P = 0.584$) and multivariate analysis (OR, 1.02; 95% CI,

0.61 – 1.71; $P = 0.937$), whereas *H. pylori* positivity was a significant predictor for IM (OR, 1.80; 95% CI, 1.06 – 3.05; $P = 0.029$) (Supplementary table 1).

Predictive factors of IM when stratifying by biopsy site (a per-patient analysis)

To explore which clinical features were associated with IM in a per-patient analysis, we performed separate univariate and multivariate analyses after stratifying by biopsy site (Table 4). Among the patients who underwent biopsies from the corpus ($n = 257$), chronic tamoxifen use was correlated with a significantly reduced risk of IM (OR, 0.42; 95% CI, 0.20 – 0.90; $P = 0.026$). In addition, the age at the time of EGD was an independent predictor for IM (OR, 1.06; 95% CI, 1.02 – 1.10; $P = 0.005$). *H. pylori* positivity was more common in cases with IM, though this was of borderline statistical significance (OR, 1.94; 95% CI, 1.00 – 3.75; $P = 0.050$). However, among those with biopsies from antrum ($n = 255$), none of the clinical or histological factors were significantly associated with presence or absence of IM.

Analysis of the association of clinicohistological variables with the longitudinal change in IM status

We performed an analysis among the 80 biopsy samples from the patients in our cohort who had follow-up EGD to determine which characteristics affect longitudinal change in IM status. In this analysis, changes in IM were classified into three groups (improved, no change, or worsened) and were statistically analyzed for the association with clinical and histological characteristics. Our results showed that chronic tamoxifen use was significantly associated with improved IM score in follow-up compared to no tamoxifen use (improved IM, 77.8% vs. 22.2%; no change, 65.4% vs. 34.6%; worsened IM, 30.0% vs. 70.0%; $P = 0.019$). One representative case with improved IM was shown in Fig. 2. There were no significant differences among these three groups in terms of age at operation for breast cancer and at the time of initial EGD, duration between initial and follow-up EGD, NSAIDs and PPI use, biopsy site, *H. pylori* positivity and inflammation grade at initial EGD, and change of *H. pylori* positivity and inflammation grade (Table 5).

DISCUSSION

This study demonstrated, consistent with previous reports, that age at EGD, biopsies from antrum, and *H. pylori* positivity all correlate with an increased risk of IM (adjusted OR, 1.04, 2.08, and 1.68, respectively) in female breast cancer patients. The most remarkable finding of our study was the significant inverse association between chronic tamoxifen use and the risk of IM. Tamoxifen use was associated with a 41% reduced risk of IM (adjusted OR, 0.59) in the per-biopsy multivariate logistic regression analysis.

There have not been any previous observational or experimental studies identifying mechanisms of action for how tamoxifen would be protective against gastric IM. However, the relationship between estrogenic modulation, including tamoxifen, and gastric cancer has been investigated by previous studies. On the basis of these studies and tamoxifen's known action as a SERM, one could hypothesize that the effect of tamoxifen on IM depends on estrogen modulation. There is substantial biologic evidence that estrogen has a protective

effect on gastric cancer. One recent study reported that the expression of sex hormone receptors including estrogen receptor α and β was decreased in gastric cancers compared to normal tissues [28]. One study showed that estrogen receptor α (ER α) and ER β were expressed in parietal cells, whereas ER α was not detected in the antral epithelium [29]. It is possible the differential effects of tamoxifen on corpus vs. antrum in the current study could be due to differential expression of estrogen receptor. Another study has suggested that estrogens stimulate the expression of trefoil factor (TFF) genes, which encode secretory proteins that protect the gastric mucosa from various insults and contribute to mucosal healing [30, 31]. Decreased expression of these genes characterizes precancerous lesions and gastric cancer [32]. In addition, a recent meta-analysis including human observational studies has reported that the risk of gastric cancer was decreased in women with longer periods of fertility or with HRT exposure [33]. Finally, incidence of gastric cancer is much higher worldwide in men than in women [15, 16].

However, in contrast with pure pro-estrogenic agents, tamoxifen correlation with development of or protection from gastric cancer remains inconclusive in human observational studies [21–23]. These prior studies have limitations because they did not take into account *H. pylori* infection in their analyses. On this issue, a recent study examined whether chronic treatment with 17 β -estradiol and tamoxifen can affect *H. pylori*-induced carcinogenesis in male and female insulin-gastrin (INS-GAS) transgenic mice. They demonstrated that tamoxifen prevented gastric cancer in male mice infected with *H. pylori*, suggesting that tamoxifen may act as an agonist in this context [24]. They also suggested that this prevention of gastric cancer by both 17 β -estradiol and tamoxifen may be due to a pro-estrogen effect, decreasing the inflammatory response and oncogenic pathway activity [24]. However, in our subgroup analysis among only patients without tamoxifen use, there was no significant association between the risk of IM and aromatase-inhibitor use. Aromatase-inhibitors work by blocking the aromatase enzyme which converts androgen into estrogen in the body [34], so they lead to systemic loss of estrogen, which would affect stomach as well as other organs. Generally, tamoxifen is thought to be pro-estrogenic in most organs besides breast, where it antagonizes estrogen [19]. In light of the results in the current study showing lack of effect of aromatase-inhibitors, we speculate that tamoxifen may affect IM either by stimulating estrogen signaling and/or by its many modes of action that are estrogen independent, such as activation of protein kinase C and phospholipase D [35, 36].

Previous studies have examined the role of stem cells and metaplasia [37–39]. Recently, we, too, have made a link between stem cells and metaplasia in an animal model using tamoxifen. We found that treatment of wild type mice with a single dose of tamoxifen (at about a ten-fold higher dose equivalent to the human dosing regimen) leads to rapid parietal cell injury, and a near concomitant increase in proliferation in corpus gastric epithelial stem/progenitor cells as well as a reparative recruitment of chief cells into the cell cycle [26, 40]. Within three weeks, tamoxifen stimulates regeneration of all the normal gastric corpus lineages. In short, tamoxifen causes acute damage followed by a corpus-dominant regenerative response. We have not elucidated the mechanisms behind the injury-regeneration cycle induced by tamoxifen, but it is neither caused by nor rescued by estrogen

stimulation or blockade [26]. Thus, tamoxifen in this mouse model may work by modulating other signaling pathways to stimulate corpus-specific regeneration, which may provide another partial answer for why the protective effect of tamoxifen on IM was statistically significant only in the corpus (adjusted OR, 0.42) but not the antrum in the current study on human data. However, there are several important differences between the current study and the previous work in mice, other than the species and tamoxifen dose. In the mouse model, tamoxifen was delivered as a rapid bolus, and the endpoint was a few weeks after treatment. Also, IM does not normally form in wild type mice [41], and there are no easy models to assess tamoxifen effects on IM.

To date, few studies have suggested treatment strategies for improving IM, other than eradication of *H. pylori*. Although *H. pylori* has been identified as the main cause of gastric premalignant changes and gastric carcinogenesis, the beneficial effect of *H. pylori* eradication for reversing IM is still inconclusive [7–14]. In prior studies, chronic use of celecoxib, which has been promising for the treatment of gastric cancer through its chemopreventive effect [42], was significantly associated with a higher regression rate of gastric IM after *H. pylori* eradication [43] and 8-week celecoxib treatment also induced the regression of long-term persistent IM after *H. pylori* eradication [44]. In addition, a prospective, randomized study found that long-term administration of ascorbic acid following *H. pylori* eradication helps to regress IM in the stomach [45]. In the present study, we found that chronic tamoxifen use was significantly correlated with lower IM score compared to no tamoxifen use in the analysis of longitudinal change of IM. Our findings would suggest that chronic tamoxifen use may be a promising alternative therapy to improve IM. However, to verify our results, further, larger scale randomized controlled trials are necessary. Also, additional studies to better understand the mechanism of tamoxifen action *in vitro* or in animal models are certainly warranted.

Our study has several limitations. First, this study has been performed in a retrospective manner. Some confounding factors cannot totally be excluded although we have attempted to collect as much information as possible and adjust for confounders using multivariate logistic regression analysis. Selection bias is possible because we have included only breast cancer patients who underwent EGD and biopsies after surgery. An asymptomatic study population routinely screened by EGD for gastric cancer would help us reduce bias derived from severe GI disease co-morbid with breast cancer, but such a study design would be impractical because of chronic use of hormone therapeutic agents. Second, histological findings including IM, *H. pylori* positivity, and inflammation were diagnosed according to protocols used at the time by the surgical pathologists assessing the biopsy. Thus, only one or two biopsy specimens were taken from each patient in this study, whereas the updated Sydney classification recommends five or more specimens from the corpus, antrum, and incisura to assess the distribution and extent of IM and *H. pylori* colonization [27]. Thus, overall IM and *H. pylori* positivity in the current study may be underestimated. Especially, there may exist some bias arising from the different number and site of biopsy specimens per patients. To help obviate this potential limitation, we separately performed multivariate analyses in per-biopsy level and per-patient level after stratifying by biopsy site. Both analyses demonstrated a significant correlation between chronic tamoxifen use and a

reduced risk of IM. We also verified diagnosis in 53 biopsy samples that were randomly selected and immunostained for CDX2 to prove intestinal differentiation; however, we cannot control the initial sampling of biopsies that were obtained by the treating physicians and diagnostic pathologists in our retrospective analysis. Additionally, other assessments for *H. pylori* infection such as rapid urease or serologic test had not been performed in all patients. Determining *H. pylori* positivity mostly depended on the histological assessment accompanied by positive results of rapid urease test in only a fraction of the cases. That may explain the lower proportion of overall *H. pylori* positivity (33.0%) in our results compared to those of previous reports (54.3% and 56.9%) of the same ethnic and gender population [46, 47]. In Korean asymptomatic adults of either sex (age > 16 years) without a history of eradication therapy, the seroprevalence of *H. pylori* infection in 2005 was 59.6% [46]. Our lower fraction of positivity could also affect the results of association between *H. pylori* positivity and IM in our study. Our study also lacks information on some important environmental factors including alcohol and smoking habits. However, we included only female patients of the same ethnicity in this study. Given the quite low rates of smoking and high-risk drinking in this age group of Korean women [48–50], we do not anticipate those factors would have a large influence on the results.

Despite these limitations, this study is the first to uncover a significant association between chronic tamoxifen use and a reduced risk of IM. In addition, we identified a correlation between chronic tamoxifen use and improvement of IM, although this longitudinal study was of small sample size. Future studies that *prospectively* investigate the possible protective effect of tamoxifen for regression of IM through observation for a long-term period with multiple biopsies as per the Sydney classification and OLGA staging are warranted to confirm our current results. Eventually, studies of tamoxifen treatment in conjunction with *H. pylori* eradication or with other agents may prove to be especially useful.

In conclusion, the present study demonstrated that chronic tamoxifen use is significantly associated with a decreased risk of IM in human stomach. We also found that this possible protective effect of tamoxifen is dominantly identified in the corpus compared to antrum. On the basis of our results, tamoxifen use might be a promising approach in a management strategy for patients with gastric premalignant lesions such as IM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Histological assessment of intestinal metaplasia (IM) in gastric biopsy specimens from the study population. **(a, b)** Representative hematoxylin-eosin (H&E) staining images of gastric tissues from patients with **(a)** or without **(b)** chronic tamoxifen use (x200). **(c)** Representative images of gastric tissue from a patient without chronic tamoxifen use, with nuclear CDX2-positivity confirming IM (x400). **(d)** In some patients without tamoxifen use, there was incomplete-type IM, lacking both goblet cells and enterocytes with brush border. CDX2 confirmed IM and allowed us moreover to investigate the relationship between incomplete IM (orange dotted box) and SPEM (spasmolytic polypeptide expressing metaplasia; magenta dotted box). Nuclear CDX2 (arrowhead indicating an example positive cell; note also mitotic activity in cell just to the left) confirmed incomplete IM, whereas the SPEM lesion showed only background cytoplasmic staining for CDX2 (arrow). **(e)** A serial section from the patient in **(d)** is stained with the lectin GS-II that labels TFF2/Spasmolytic

Polypeptide-expressing cells. Staining confirms the incomplete intestinal metaplasia lesion in **(d)** is not SPEM (black dotted box), whereas the region outlined by the blue dotted box is.

Fig. 2.

Representative images showing improved gastric intestinal metaplasia (IM) in a patient with follow-up endoscopic gastroduodenoscopy (EGD) after chronic tamoxifen use. **(a)** Positive CDX2 immunostaining indicates the presence of IM in the biopsy specimen obtained from initial EGD (x200). **(b)** There were no cells stained positive with CDX2 in the biopsy specimen obtained from follow-up EGD (x200). The interval between initial and follow-up EGD was 27.6 months.

Table 1

Clinical and histological characteristics of the study population and biopsy samples

Patients (n = 433)	
Female (%)	433 (100)
Age at operation for breast cancer (years) ^a	52.9 ± 9.3 [24–80]
Age at EGD (years) ^a	56.2 ± 9.1 [28–82]
Duration of hormone therapy (months) ^{a,b}	28.8 ± 19.7 [3.0–72.0]
Hormone therapy for breast cancer (%)	
Tamoxifen	179 (41.3)
Other hormone therapeutic agents ^c	111 (25.6)
No hormone therapy	143 (33.0)
Chronic NSAIDs use (%)	51 (11.8)
Chronic PPI use (%)	74 (17.1)
Biopsy site (%)	
Corpus only	178 (41.1)
Antrum only	176 (40.6)
Both corpus and antrum	79 (18.2)
Biopsy samples (n=512)	
<i>H. pylori</i> positivity (%)	169 (33.0)
Inflammation (%)	
None	11 (2.1)
Mild	368 (71.9)
Moderate	129 (25.2)
Severe	4 (0.8)
Presence of intestinal metaplasia (%)	147 (28.7)

EGD, endoscopic gastroduodenoscopy; NSAIDs, non-steroidal anti-inflammatory drugs; PPI, proton-pump inhibitor;

^aMean ± standard deviation [range]^bThis was defined as the total period when patients had taken any hormone therapeutic agents before the time of EGD.^cAromatase-inhibiting agents (anastrozole, exemestane, or letrozole).

Table 2

Univariate analysis of clinical and histological characteristics associated with intestinal metaplasia (a per-biopsy analysis)

	Without IM (n=365)	With IM (n=147)	P value
Age at EGD (years) ^a	55.3 ± 9.2	58.8 ± 8.0	< 0.001
Interval between breast surgery and EGD (months) ^a	38.9 ± 27.0	38.2 ± 27.1	0.787
Chronic tamoxifen use (%) ^b	169 (46.3)	45 (30.6)	0.001
Chronic NSAIDs use (%) ^b	43 (11.8)	15 (10.2)	0.611
Chronic PPI use (%) ^b	57 (15.6)	26 (17.7)	0.565
Characteristics of biopsied gastric tissues			
Biopsy site (%) ^b			< 0.001
Corpus	202 (55.3)	55 (37.4)	
Antrum	163 (44.7)	92 (62.6)	
<i>H. pylori</i> positivity (%) ^b	110 (30.1)	59 (40.1)	0.029
Inflammation (%) ^b			0.195
None and mild	276 (75.6)	103 (70.1)	
Moderate and severe	89 (24.4)	44 (29.9)	

IM, intestinal metaplasia; EGD, endoscopic gastroduodenoscopy; NSAIDs, non-steroidal anti-inflammatory drugs; PPI, proton-pump inhibitor;

^aMean ± standard deviation

^bPercentage means (numbers of cases)/(numbers of each group [without IM or with IM])

Table 3

Independent predictors associated with intestinal metaplasia after multivariate logistic regression analysis (a per-biopsy analysis)

	OR	95% CI	P value
Age at EGD	1.04	1.01–1.06	0.002
Interval between breast surgery and EGD	1.00	0.99–1.01	0.904
Chronic tamoxifen use	0.59	0.37–0.94	0.025
Chronic NSAIDs use	0.66	0.34–1.26	0.204
Chronic PPI use	1.21	0.70–2.08	0.491
Biopsy site			
Corpus	1 (reference)		
Antrum	2.08	1.39–3.13	< 0.001
<i>H. pylori</i> positivity	1.68	1.10–2.57	0.016

OR, odds ratio; CI, confidence interval; EGD, endoscopic gastroduodenoscopy; NSAIDs, non-steroidal anti-inflammatory drugs; PPI, proton-pump inhibitor.

Table 4

Predictive factors associated with gastric intestinal metaplasia when stratifying by biopsy site (a per-patient analysis)

	Corpus				Antrum			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	Without IM (n=202)	With IM (n=55)	P value	OR (95% CI)	P value	With IM (n=92)	P value	OR (95% CI)
Age at EGD (years) ^a	54.7 ± 9.2	59.8 ± 7.8	<0.001	1.06 (1.02–1.10)	0.005	58.2 ± 8.1	0.057	1.03 (0.99–1.06)
Interval between breast surgery and EGD (months) ^a	37.2 ± 26.4	33.4 ± 24.9	0.349	0.995 (0.98–1.01)	0.449	41.0 ± 27.6	0.996	1.00 (0.99–1.01)
Chronic tamoxifen use (%) ^b	97 (48.0)	12 (21.8)	<0.001	0.42 (0.20–0.90)	0.026	72 (44.2)	0.196	0.74 (0.41–1.35)
Chronic NSAIDs use (%) ^b	19 (9.4)	7 (12.7)	0.469	0.84 (0.31–2.28)	0.732	24 (14.7)	0.163	0.51 (0.21–1.21)
Chronic PPI use (%) ^b	26 (12.9)	10 (18.2)	0.314	1.74 (0.74–4.10)	0.208	31 (19.0)	0.748	1.00 (0.50–2.01)
Characteristics of biopsied gastric tissues								
<i>H. pylori</i> positivity (%) ^b	63 (31.2)	23 (41.8)	0.139	1.94 (1.00–3.75)	0.050	47 (28.8)	0.092	1.54 (0.88–2.70)
Inflammation (%) ^b			0.170				0.492	
None and mild	151 (74.8)	36 (65.5)				125 (76.7)		
Moderate and severe	51 (25.2)	19 (34.5)				38 (23.3)		

IM, intestinal metaplasia; OR, odds ratio; CI, confidence interval; EGD, endoscopic gastroduodenoscopy; NSAIDs, non-steroidal anti-inflammatory drugs; PPI, proton-pump inhibitor;

^aMean ± standard deviation,

^bPercentage means (numbers of cases)/(numbers of each group [without IM or with IM])

Table 5

Clinical and histological variables associated with the longitudinal change of intestinal metaplasia among 80 biopsy samples from the patients with follow-up EGDs.

	Change of IM			P value
	Improved IM (n=18)	No change (n=52)	Worsened IM (n=10)	
Age at operation for breast cancer (years) ^a	52.6 ± 6.9	51.9 ± 7.8	55.6 ± 7.4	0.364 ^b
Age at initial EGD (years) ^a	54.5 ± 7.4	54.5 ± 8.3	59.9 ± 8.4	0.149 ^b
Interval between initial and follow-up EGD (months) ^a	32.8 ± 21.4	27.4 ± 23.5	26.1 ± 13.7	0.629 ^b
Hormone therapy for breast cancer (%)				0.019 ^c
Tamoxifen use	14 (77.8)	34 (65.4)	3 (30.0)	
No tamoxifen use	4 (22.2)	18 (34.6)	7 (70.0)	
Chronic NSAIDs use (%)	0 (0)	3 (5.8)	1 (10.0)	0.221 ^c
Chronic PPI use (%)	2 (11.1)	12 (23.1)	3 (30.0)	0.209 ^c
Characteristics of biopsied gastric tissues				
Biopsy site (%)				0.157 ^c
Corpus	5 (27.8)	27 (51.9)	5 (50.0)	
Antrum	13 (72.2)	25 (48.1)	5 (50.0)	
<i>H. pylori</i> positivity at initial EGD (%)	9 (50.0)	22 (42.3)	6 (60.0)	0.789 ^c
Change of <i>H. pylori</i> from initial to follow-up EGD (%)				0.540 ^c
Decreased colonization of <i>H. pylori</i>	6 (33.3)	8 (15.4)	5 (50.0)	
No change	9 (50.0)	38 (73.1)	4 (40.0)	
Increased colonization of <i>H. pylori</i>	3 (16.7)	6 (11.5)	1 (10.0)	
Inflammation at initial EGD (%)				0.392 ^c
None and Mild	12 (66.7)	31 (59.6)	5 (50.0)	
Moderate and severe	6 (33.3)	21 (40.4)	5 (50.0)	
Change of inflammation from initial to follow-up EGD (%)				0.290 ^c
Decreased inflammation	2 (11.1)	8 (15.4)	2 (20.0)	
No change	14 (77.8)	40 (76.9)	8 (80.0)	
Increased inflammation	2 (11.1)	4 (7.7)	0 (0)	

IM, intestinal metaplasia; EGD, endoscopic gastroduodenoscopy; NSAIDs, non-steroidal anti-inflammatory drugs; PPI, proton-pump inhibitor;

^aMean ± standard deviation

^bOne-Way analysis of variance (ANOVA) was carried out to determine the statistical significance.

^cLinear by linear association was carried out to determine the statistical significance.