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Retrospective Study

Protective effects of female reproductive factors on gastric signet-ring cell carcinoma

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Abstract

BACKGROUND

The overall incidence of gastric cancer is higher in males than females worldwide. However, gastric signet-ring cell carcinoma (GSRC) is more frequently observed in younger female patients.

AIM

To analyze clinicopathological differences between sex groups to reveal sex disparities in GSRC, because of the limited evidence regarding association between sex-specific differences and survival of GSRC patients.

METHODS

We reviewed medical records for 1,431 patients who got treatment for GSRC at Cancer Hospital, Chinese Academy of Medical Sciences from January 2011 to December 2018 and surveyed reproductive factors. Clinicopathological characteristics were compared between females and males. Cox multivariable model was used to compare the mortality risks of GSRC among men, premenopausal women, and postmenopausal women.

RESULTS

Of 1431 patients, 935 patients were male and 496 patients were female (181 menstrual female and 315 menopausal female). The five-year overall survival was observed for male, menstrual female group and menopausal female group (65.6% *vs* 76.5% *vs* 65%, $P < 0.01$, separately). The menstrual female was found as a protective factor (HR = 0.58, 95%CI: 0.42, 0.82).

CONCLUSION

The mortality risks of premenopausal female patients are much lower than male. The contribution of this study is to provide the evidence of protective effects from female reproductive factors in GSRC patients. It would be interesting to assess the effects of female reproductive factors to investigate a new treatment for a group of selected GSRC patients.

Key Words: Gastric carcinoma; Signet-ring cell; Female reproductive factor; Menopause

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Core Tip: The overall incidence of gastric cancer is higher in males than females worldwide. However, gastric signet-ring cell carcinoma (GSRC) is more frequently observed in younger female patients. Limited studies focuse on sex-specific differences in GSRC. This study proposed clinicopathological differences between sex groups to reveal sex disparities in GSRC and confirmed that female reproductive factors provided the protective effects and the mortality risk of menstrual female patients was much lower. The investigation of mechanism on female reproductive factors may provide new insight of treatment for GSRC.

INTRODUCTION

Gastric cancer (GC) is the 5th most common adenocarcinoma and ranks the 3rd in mortality all over the world[1]. In China, estimated 390000 people die of GC annually, which accounts for more than 50% of the global death cases and imposes a severe health burden[1-3]. Commonly, the age-standardized incidence rates of GC have shown a male predominance with the male-to-female rate of more than 2:1 in most populations around the world[1,4]. The difference, however, cannot be entirely attributed to the different prevalence of established major risk factors, such as tobacco smoking[5] and *Helicobacter pylori* infection[6] between two sexes. A study reported that incidence of intestinal GC after menopause increased with time, and the incidence after menopause 10 years was comparable to that of males[7]. An umbrella review included 616630 women of 6 observational studies showed menopausal hormone therapy was associated with decreased risks of GC[8]. The female reproductive factors and sex hormones may have protective effects on gastric adenocarcinoma.

Gastric signet-ring cell carcinoma (GSRC) is a distinct type of GC, and its incidence has been steadily increasing in Asia, Europe, and the United States, accounting for over 30% of the new gastric adenocarcinoma cases[9]. GSRC belongs to the diffused, undifferentiated, and poorly differentiated types in the Laurén classification, Nakamura's classification, and Japanese Gastric Cancer Association, respectively[10-13]. GSRC in early and advanced stages is more frequently observed in younger female patients than gastric adenocarcinomas[14]. A contradiction with the protective effect of female reproductive factors on gastric adenocarcinoma seems to exist. There is also evidence that female reproductive factors induced diffuse-type GC through estrogen activities[7].

Moreover, various reproductive factors have provided contradictory results in relation to the risk of GCs. The protective effects of female reproductive factors imply the potential role of sex hormones in carcinogenesis. Importantly, the explanation for the predominance of GSRC in young females might provide significant clues to the etiology of the tumors and pave the way for research on innovative preventive and

therapeutic treatments. The effect of female reproductive factors on GSRC tumorigenesis and tumor development remains unclear despite the advances in the understanding of its epidemiology, and clinicopathology. The purpose of this study was to estimate the effects of female reproductive factors on the prognosis and combined modality treatment of GSRC.

MATERIALS AND METHODS

Study population and follow-up duration

Our study involved 1431 participants who were histologically confirmed with GC with signet-ring cells and underwent curative resections from January 2011 to December 2018 at the Cancer Hospital, Chinese Academy of Medical Sciences, China. Subtotal gastrectomy was performed for distal GCs, whereas total gastrectomy was conducted for proximal third GCs. Patients with definitive signs of distant organ or peritoneal seeding metastases did not undergo gastrectomy and were referred to evaluation for chemotherapy instead. Based on the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines, standard D2 Lymphadenectomy was achieved in patients with curative intentions[15]. All the surgical specimens were confirmed separately and independently by at least two experienced pathologists. Disagreements in this step were solved by discussion, especially on the proportion of signet-ring cells. The follow-up data were prospectively collected and regularly updated by surgeons every six months after the surgery. The overall survival was defined from the date of gastrectomy to the date of death or the end of the follow-up period (April 30, 2020).

Measurements

The demographic characteristics included age and body mass index (BMI). We considered age as an ordinal variable (< 50, 50−60, and ≥ 60 years) to approximate tertiles. We defined BMI based on the standard cutoff points established by the Work Group on Obesity in China (WGOC) in categories of underweight and normal: ≤ 24 kg/m^2, overweight and obese: > 24.0 kg/m^2[16]. Health-related lifestyle indicators,

including alcohol drinking (yes: any alcoholic beverage consumption in the last 12 mo, or no: no alcohol consumption in the last 12 mo) and smoking (yes: Any regular tobacco consumption, or no: never smoked), were excluded from the analysis based on the variable selection outcome of best-subset selection approach and the uneven distribution. We categorized the proportion of signet-ring cells as ≤ 10%, 10%–50%, 50%–90%, and > 90%, but we used the proportion of signet-ring cells as a dichotomous variable (≤ 50% and > 50%) in the adjustments for confounding variables and to ensure that each level had a sufficient number of observations. Clinicopathological characteristics, such as BMI, the proportion of signet-ring cells, T stage, N stage, adjuvant chemotherapy, neoadjuvant chemotherapy, nerve invasion, and lymphatic vessel invasion were subjected to analysis since they were closely associated with the survival of GC patients with signet-ring cell carcinoma based on *a priori* knowledge.

Statistical analysis

The continuous variables were expressed as mean ± SD, while categorical variables were expressed as number of observations and percentages (%). Differences in the potential covariates between the sex groups were assessed by Wilcoxon-Mann-Whitney test for continuous variables and the chi-square test for categorical variables. The proportional hazards assumption was estimated, and the Cox multivariable model was used to calculate the hazard ratios (HRs) and the 95% confidence intervals (CIs) for the association between the sex factors and the survival of GC patients with signet-ring cell carcinoma. The model was adjusted for following potential covariates: BMI, the proportion of signet-ring cells, T stage, N stage, adjuvant chemotherapy, neoadjuvant chemotherapy, nerve invasion, and lymphatic vessel invasion. Subgroup analyses were conducted by (1) BMI; (2) Signet-ring cells proportion; (3) Adjuvant chemotherapy; (4) Neoadjuvant chemotherapy; (5) Nerve invasion; and (6) Lymphatic vessel invasion to explore if the impact of the sex difference was stronger in certain groups. Interaction terms between exposures and these covariates were added into the multivariable model, and Wald tests were employed to examine if the interaction terms were

statistically significant. The survival curves were estimated by the Kaplan-Meier method. In the present analysis, two-sided *P*-values < 0.05 were considered to indicate statistically significant differences. All statistical analyses (and figures created) were performed with Stata 15.0 (StataCorp LLC: College Station, TX, United States).

RESULTS

Clinicopathological features of GSRC in male and female patients

Table 1 displays the distributions of the demographic and potential risk factors by sex difference. Of the 1431 GC patients with signet-ring cell carcinoma, 935 (65.3%) were male, and 496 (34.7%) were female. Overall, over one-third of the participants were aged ≥ 60 (42.0%) years, with a mean age of 56.3 (SD: 11.3) years. There were no significant differences between the sex groups in terms of histological differentiation, N stage, and adjuvant or neoadjuvant chemotherapy (*P*s > 0.05). The female subjects were more likely to be younger, non-smoker, non-drinker, diffused Lauren type, T1 stage, metastasis, without nerve invasion, without lymphovascular invasion, at middle and lower tumor location, have lower BMI, higher signet-ring cells proportion, and more lymph nodes removed (*P*s < 0.05).

The data of the Cox proportional hazards regression model are presented in Table 2. Overall, the menstrual female subjects had a significantly lower risk of mortality (HR = 0.58, 95%CI: 0.42, 0.82) than male participants in the multivariable model. We did not observe this protective effect in menopausal females (HR = 0.91, 95%CI: 0.72, 1.14).

Subgroup analysis in the different sex groups with GSRC

Other variables associated with the overall survival included BMI, T stage, N stage, adjuvant or neoadjuvant chemotherapy, and lymphovascular invasion. The results of the subgroup analyses (Table 3) showed that the impact of menstruation was more significant in the female participants with lower levels of signet-ring cells (HR = 0.56, 95%CI: 0.38, 0.82, *P*-interaction = 0.038), nerve invasion (yes *vs* no, HR = 0.60, 95%CI: 0.40, 0.89, *P*-interaction = 0.029), lymphovascular invasion (yes *vs* no, HR = 0.49, 95%CI:

0.30, 0.80, *P*-interaction < 0.001), or without adjuvant chemotherapy (HR = 0.38, 95%CI: 0.22, 0.65, *P*-interaction < 0.001) and neoadjuvant chemotherapy (HR = 0.53, 95%CI: 0.36, 0.77, *P*-interaction < 0.001). There was a reversal effect across the strata of BMI for menopausal females (crossover interactions).

Comparison of the overall survival in GSRC between the sex groups

The survival curves in Figure 1 depict the survival probability based on the sex difference. Menstrual female patients had a significantly better overall survival than the male and menopausal female groups ($P < 0.01$). As can be seen in Figure 2, the survival analysis showed better prognosis of menstrual female patients in the non-adjuvant chemotherapy group ($P < 0.01$) and the inadequate survival advantages of menstrual female patients in the adjuvant chemotherapy group ($P = 0.73$). In addition, we conducted comparisons of the overall survivals between the sex groups of GSRC patients with different levels of signet-ring cells (Figure 3). The advantages of menstrual female patients in survival existed as compared to the male and menopausal female groups, however this advantage is not significant in the GSRC group with > 50% signet-ring cells (Supplementary Figure 1).

DISCUSSION

Although the incidence of GC has been decreasing, this disease remains the third cause of cancer mortality worldwide and in China. The proportion of GSRC in all GC cases has been increasing recently, especially at the young and female populations. There are obvious differences on morbidity of GSRC between sexes. To find a new treatment and improve the overall survival of GSRC, this study has clinical significance to investigate the disparities of reproductive factors between male and female GSRC patients.

In this study, we focused on influence of the sex-specific differences on the prognosis of GSRC. Our results showed that there is a stronger positive association with overall survival in menstrual female patients with GSRC, compared to in male or menopause female patients. The findings of the present large patient-based

retrospective study may provide valuable insights into pathways for reducing GSRC mortality in the future.

The multivariate analysis results in our study showed that being a menstrual female was a protective factor (HR = 0.58, 95%CI: 0.42, 0.82) against GSRC. A similar effect was previously reported in various types of GCs[17,18]. In a study with 758 patients, Kim *et al*[7] proposed that female reproductive hormones might be a potentially protective factor against intestinal-type GC, and the incidence of intestinal-type GC after the menopause increased and became comparable to that in men. A Japanese study revealed that the risk of GC was lower in menstrual females (HR = 0.33, 95%CI: 0.23, 0.49). Protective effects were observed in differentiated histological types (HR = 0.25, 95%CI: 0.11, 0.55) and undifferentiated histological types (HR = 0.39, 95%CI: 0.23, 0.63)[19]. The aforementioned consistent results evidence the effects of female reproductive factors on the reduction of the risks of different GCs. In a large Chinese prospective study with female subjects, the risks of GC increased with age of menopausal women (HR = 0.80 per 5-year increase in the menopausal age, 95%CI: 0.66, 0.97)[20]. A similar finding was also obtained in a cohort study including one million women whose risks of GC in the menopausal age were considerably higher than those in the menstrual age (RR = 1.46, 95%CI: 1.07, 2.00 and RR = 1.59, 95%CI: 1.15, 2.20, respectively) from United Kingdom[21]. Our results indicate that the effect of female reproductive factors on GSRC development is steady and robust.

Here, we hypothesize that the sex hormone or sex hormone receptor, such as estrogen and the estrogen receptor (ER), are the key inducers of the protective effects of female reproductive factors against GSRC. The influence of estrogen was studied by other researchers, but the results were controversial[22]. Estrogens regulate the tissue growth, differentiation, and function, which is mediated by ER-α and ER-β. The oncologic importance of estrogen and ER in carcinomas occurring in the breast and ovaries has been well investigated but not in GCs. The expression of ER in the stomach is a basic point to explore the relationship between sex hormones and survival of GC. Some scientists evidenced the expression of ER in gastric tissues, which provided the

fundaments of the probable functions of estrogen in this respect[23-25]. Hess *et al*[26] established that the estrogen receptor and female sex hormone were expressed in the male reproductive tract and may have certain functions. The protective effect of estrogen was discovered also in men who received hormone therapy for prostate cancer; notably, the results showed a decrease risk of GC[27].

As known, ER has several receptors, including ERa, ERb, and ERg. The biological actions of estrogen are mediated through two specific ERs, ERa and ERb, which belong to the nuclear receptor superfamily[28]. Zhao *et al*[25] and Matsuyama *et al*[29] reported that both ERa and ERb were expressed in poorly differentiated adenocarcinoma specifically in gastric signet-ring cell adenocarcinomas with characteristics of sex hormone dependency. Different effects of these two ER types were found in various studies. For example, Kameda *et al*[27] proposed that ERa expressed in diffuse-type GC promoted the proliferation of ERa-positive GC cells. The suggested mechanism was that the activation of the ERa pathway stimulated cancer cell proliferation by activating the hedgehog pathway in a ligand-dependent but dose-independent manner *via* Shh induction of diffuse-type GC. ERb is homologous to ERa, particularly in the DNA-binding domain, but it is structurally and functionally differenta. ERb manifested strong cytoplasmic staining with anti-ERb antibody in addition to the stained nuclei[29]. This result provides evidence for the potential of GSRC treatment targets. Recently, using genomic analysis, Kang *et al*[29] identified estrogen-related receptor gamma (ERγ) as a potential tumor suppressor in GC. The molecular mechanisms of its action suggest that the activation of ERγ by the antagonizing Wnt signaling through DY131 could suppress GC cell growth and tumorigenesis. Studies on tumorigenesis regulated by estrogens or estrogen receptors are rare but will be urgently needed in the future.

Since chemoresistance is a distinct feature of GSRC, we noticed that the adjuvant chemotherapy was a protective factor in multivariate analysis. Then, we performed further subgroup analysis on adjuvant chemotherapy effects. Interestingly, protective effects exerted by female reproductive factors were observed only in the non-adjuvant chemotherapy group (HR = 0.38, 95%CI: 0.22, 0.56) and the non-neoadjuvant

chemotherapy group (HR = 0.53, 95%CI: 0.36, 0.77). However, female reproductive factors lost their advantages and did not improve the survival after chemotherapy. It is possible that these conditions of poor survival and chemoresistance are mediated by female reproductive factors, such as ERa and ERβ, with unknown mechanism. Recently, Wang *et al*[30] proposed that the loss of ERβ in GSRC cells might increase the potential for malignant invasion into the deep tissues easier through the mTOR signaling pathway. Therefore, ERβ might inhibit the malignancy of GSRC and can thus become a potential target in its adjuvant treatment. The investigation of Wang did not discuss the connection between ERβ and chemotherapy. We noticed that the prognostic trends are similar in all three studied groups. However, in the subgroup analysis of adjuvant chemotherapy, the mean survival time in the menstrual female group (41.4 mo) was the shortest as compared to those of the male (47.6 mo) and the menopausal female (45.1 mo) groups. We considered that the probable reason was the proportions of signet-ring cells in GCs. However, no association was identified between the signet-ring cell proportions and influence of female reproductive factors on the prognosis of subjects who received chemotherapy. The results of recent studies confirm the difficulties to understand the possible mechanism between sex hormones and the chemoresistance in GSRC. However, more research on this topic needs to be undertaken to clearly elucidate the association between ER and chemotherapy outcomes.

Furthermore, here, we investigated the effects of female reproductive factors on the prognosis of the GSRC with various proportions of signet-ring cells. Being a menstrual female was a protective factor (HR = 0.56, 95%CI: 0.38, 0.82) and was associated with a better overall survival than that of menopausal female and male with GSRC cells < 50%. However, menstrual females lost their GSRC-associated advantages over menopausal female at a proportion of the signet-ring cells > 50%. We supposed that ERβ downregulation or ectopic expression in the cytoplasm could lead to a worse prognosis of GSRC with a higher proportion of signet-ring cells. Nevertheless, the probable mechanism remains unclear. Future studies on the topic are therefore highly recommended.

Strengths and limitations

This is the first large population study focused on the relationship between female reproductive factors and GSRC with an 8-year follow-up duration. Our results highlight the significant effect of female reproductive factors on GSRC prognosis. The clinical importance of our present findings is critical as they have outlined the prognostic role of female reproductive factors in GSRC. Therefore, further strong evidence is provided which will increase the GSRC treatment effectiveness. In this study, we have also discussed the effect of female reproductive factors on the survival in multidimensional aspect with relatively robust statistics such as multivariable Cox proportional hazards model and different subgroups, which could considerably diminish the impact of confounders and explore the potential effect in a specific group.

Despite the aforementioned strengths of our study, several limitations have to be acknowledged. First, the data concerning the expression levels of the estrogen and progesterone receptors are unavailable in our study. Thus, we cannot conduct further analysis on the effects of estrogen and progesterone receptors on GSRC prognosis and other molecular mechanism. Further investigations are needed to clarify the biological role of estrogen or ER in the carcinogenesis and chemoresistance of GSRC. Second, future studies with a more specific measure of signet-ring cells in GC and more comprehensive long-term outcomes (*e.g.*, the recurrence or chemoresistance, could offer more information) will be needed to verify the conclusion in this study.

CONCLUSION

In conclusion, sex disparities, especially female reproductive factors might be protective against GSRC and serve as a significant prognostic factor in GSRC patients. Further studies including more comprehensive measures will be essential to elucidate the mechanism of female reproductive factors on GSRC.

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