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ESTROGEN RECEPTOR BETA (ERß) IN GASTRIC CANCER – A SYSTEMATIC REVIEW AND META-ANALYSIS

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Abstract – *Introduction:* Gastric cancer represents a major public health challenge worldwide due to its significant morbidity and mortality rates. Among the factors associated with the development of gastric cancer, estrogen receptor beta (ERβ) has emerged as a potential therapeutic target. The aim of the study was to investigate the relationship between ERβ expression and gastric cancer.

Patients and Methods: English medical literature searches were conducted for ERβ expression in patients with gastric cancer *vs*. healthy controls. Searches were performed up to August 31, 2023, using MEDLINE, PubMed, Embase, and Google Scholar. Meta-analysis was performed using Comprehensive Meta-Analysis Software (Version 4, Biostat Inc., Englewood, NJ, USA). Pooled odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. Heterogeneity was evaluated using the Cochran Q test, and it was considered present if the Q test *p*-value was less than 0.10. I^2 statistic was used to measure the proportion of inconsistency in individual studies, with $I^2 > 50\%$ representing heterogeneity. We also calculated a potential publication bias.

Results: Six studies representing 11 sub-studies were selected according to the inclusion criteria. The odds ratio of ERβ expression in fixed effect analysis was 0.347, 95% CI 0.270-0.445, 65.3% lower in gastric cancer than in normal mucosa. Heterogeneity and inconsistency were low, and no publication bias was demonstrated.

Conclusions: This meta-analysis showed that ERβ expression is lower in gastric cancer biopsy specimens than in healthy controls, which may have a significant effect on the survival of the patients.

Keywords: Gastric cancer, ERβ, Systematic review, Meta-analysis, Gene expression.

Abbreviations: ERβ = estrogen receptor beta, OR = odds ratio, CI = confidence interval.

INTRODUCTION

Estrogen is a steroid hormone that plays a crucial role in the development, differentiation, and growth of various tissues, such as the male and female reproductive tract, the gastrointestinal tract, the mammary gland, and the skeletal, nervous, and immune systems. Most of the effects of estrogen are mediated by binding to estrogen receptors (ERs) and activating downstream signaling pathways by binding to DNA and modulating the expression of target genes. ERs are divided into estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) and were first described in 1985 and 1996 respectively[1-4]. ERβ is a member of the nuclear receptor superfamily expressed in various cancers, including colorectal, gastric, esophageal, and pancreatic cancers (GI cancers). The expression of ERβ in these cancers is found to be associated with the progres-

sion of the disease and the response to treatment[5-7]. This effect is supported by different pieces of evidence that indicate that ERβ has a role in driving cells into the apoptotic cycle[8], as well as in the metabolism of cancer cells[9]. Gastric cancer represents a significant worldwide health concern, especially in eastern Asia, as it ranks as the fifth most common cancer in the world, and it is one of the leading causes of cancer-related death, contributing 5.6% of the total number of new cancer cases diagnosed in 2020 and 7.7% of the number of cancer-related deaths[10,11]. More than 95% of gastric cancers are adenocarcinomas, which are typically classified based on anatomic location (cardia/proximal or non-cardia/distal) and histologic type (diffuse or intestinal)[12]. There is evidence of sex differences in the incidence of gastric cancer. Different epidemiological studies confirmed the male predominance in gastric cancer regardless of race and ethnicity. Although the underlying reasons are not yet clear, many studies suggest the protective effect of exposure to estrogen in a way that may decrease the risk of gastric cancer[13,14]. Evidence demonstrates that ERβ may be responsible for the progression of gastric cancer[15]. The expression of ERβ in gastric cancer is decreased compared to normal gastric mucosa. Ge et al[16] found that low expression of ERβ in gastric cancer is associated with advanced tumor stage and lymph node metastasis. Ryu et al[17] showed that the ERβ positive group of gastric cancer patients had a better 3-year disease-free survival and overall survival rate compared with the negative group in survival analysis. Frycz et al[18] revealed abnormal expression of ERβ in cancerous tissues and indicated that it may be associated with specific clinicopathological features in patients with gastric cancer. Due to its differential expression in healthy and cancerous tissues, ER-β may be a potential diagnostic or therapeutic target in gastric cancer. In this meta-analysis, we looked at studies that investigated the relationship between ERβ protein expression and gastric cancer, comparing the lesion with normal adjacent mucosa.

MATERIALS AND METHODS

Identification of Studies and Data Extraction

To identify studies and extract data in this meta-analysis, the MEDLINE, PubMed, Embase, and Google Scholar databases were searched until 31.8.2023. We looked for human studies written in English, using the following search text and/or Medical Topic Heading (MeSH) terms: *gastric cancer OR gastric adenocarcinoma [All Fields] AND "ERβ" [MeSH Terms].* In addition, a manual search of all review articles, published editorials, and retrieved original studies, was made. Hand searches included articles bibliography. This meta-analysis was performed according to the PRISMA extension statement for interventions.

Selection Criteria – Primary Endpoints

We defined the inclusion and exclusion criteria before starting the study investigation. Thus, appropriate studies were included in the meta-analysis provided that the following criteria were met: **a.** published as complete articles with data that can be extracted; **b.** written in English, and **c.** comparing gastric cancer tissue with normal controls. Studies that did not meet these criteria were excluded. The resolution of OR and 95% CI was defined as the primary endpoint. Case-control studies comparing ERβ expression in biopsy specimens of gastric cancer compared to healthy controls were included. We selected studies that used standard immunohistochemistry or Western blot with antibodies against ERβ and PCR for RNA and only those that expressed results by the percentage of moderate and/or strong positive staining. Thus, studies where results were expressed with mean± SD of staining scores were excluded since meta-analysis could not be performed.

Heterogeneity, Sensitivity, and Publication Bias

The heterogeneity of the studies was calculated using the Cochran Q test and I^2 inconsistency index, and it was considered to be present if the Q test *p*-value was less than 0.10. The higher the

I^2, the greater the heterogeneity. The values of 25%, 50%, and 75% indicate low, moderate, and high heterogeneity, respectively. The sensitivity testing was conducted by removing individual studies from the overall result. The publication bias was analyzed using a funnel plot complemented by Begg-Mazumdar and Egger statistics. We constructed comparison-adjusted funnel plots and checked their symmetry to assess whether small-scale trials influence the efficacy results.

Statistical Analysis

Meta-analysis was performed using Comprehensive Meta-Analysis Software (Version 4, Biostat Inc., Englewood, NJ, USA). Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using the fixed effects model to compare mucin expression in individual studies.

RESULTS

A Systematic Review of the Selected Studies[19-24]

Qin et al[19] investigated the expression of estrogen receptors in gastric mucosal biopsies obtained by endoscopy from patients with gastric cancer and functional dyspepsia. Expression of ERβ was measured by immunohistochemistry and Real-Time Polymerase Chain Reaction. Gan et al[20] looked at gastric biopsy specimens from a large Chinese cohort, that included patients who underwent gastrectomy for histopathological confirmed gastric carcinoma. The expression profile of Er*β* was examined in both gastric tumors and normal tissues. Xu et al[21] performed immunohistochemical staining for ERβ in paired tissues of gastric cancer and corresponding normal tissues obtained from continuous patients who underwent curative resection of gastric cancer. Takano et al[22] evaluated the relation between carcinogenesis, tumor progression and expression of ERβ mRNAs in tumor tissues and corresponding normal gastric tissues obtained from patients who underwent surgical resection of gastric carcinoma. Matsuyama et al[23] found positive immunohistochemical staining patterns of ERβ in signet ring cell carcinomas obtained from ten stomach cancer patients who had undergone a surgical operation. Wang et al[24] studied a cohort that comprised 39 patients (20 males and 19 females) with histologically confirmed primary gastric adenocarcinomas treated with surgery. Clinical paired samples of cancerous and non-cancerous tissues from patients with gastric adenocarcinoma were quantitatively analyzed for both ERs mRNA and protein levels.

Results of the Meta-Analysis

Our literature search revealed 32 studies that explored ERβ expression in gastric cancer biopsy specimens (Figure 1). We excluded 6 studies not on human beings and not in full text, and 20 studies that were not published in English, that were duplications, editorials, review articles, or whose results could not be calculated. From the results of these papers, we could not retrieve the relative strength of each study as we could for studies where results were expressed as a percentage of moderate or strong staining. We were left with six studies (11 data sets or sub-studies) that fulfilled the inclusion criteria. These studies were in human beings that measured ERβ staining intensity using immunohistochemistry and/or western blot (WB) with antibodies against ER-β protein and/or RT-PCR amplification for ER-β mRNA levels. The studies compared the staining score between gastric cancer and controls, were published up to 31.8.2023, and were conducted in two countries: four from China and two from Japan[19-24].

Altogether there were 1,355 gastric cancer patients and 1,346 controls; of these, 983 (72.50%%) and 1,166 (86.60%) had positive staining for ERβ, respectively.

All 11 data sets have higher ERβ expression in healthy controls than in gastric cancer. The OR of ERβ expression in fixed effect analysis (the mean effect size) was 0.347, 95% CI: 0.270-0.445, $p<0.0001$, 37.5% lower in patients with gastric cancer (Figure 2).

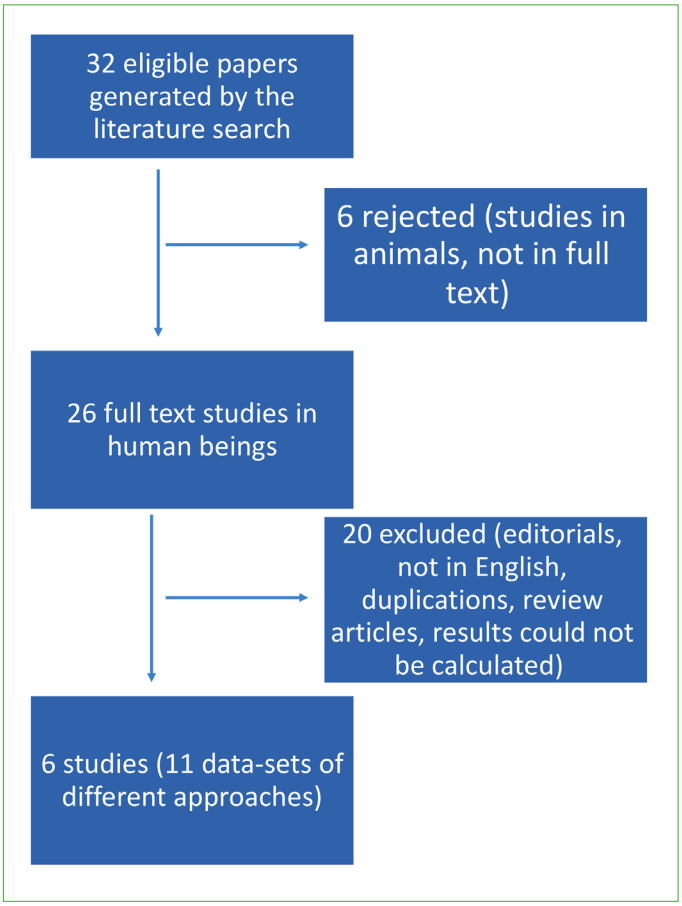

Figure 1. Flow chart of studies included in the meta-analysis.

The Z-value tests the null hypothesis that the mean effect size is 1.0. The Z-value is -8.324 with $p<0.0001$. Using a criterion alpha of 0.050, we cannot reject this null hypothesis. The relevant funnel plot (Figure 3) appeared symmetric, reflecting the lack of effects from small studies in the meta-analysis, and denies a significant publication bias.

The Q-statistic provides a test of the null hypothesis that all studies in the analysis share a common effect size. If all studies shared the same true effect size, the expected value of Q would equal

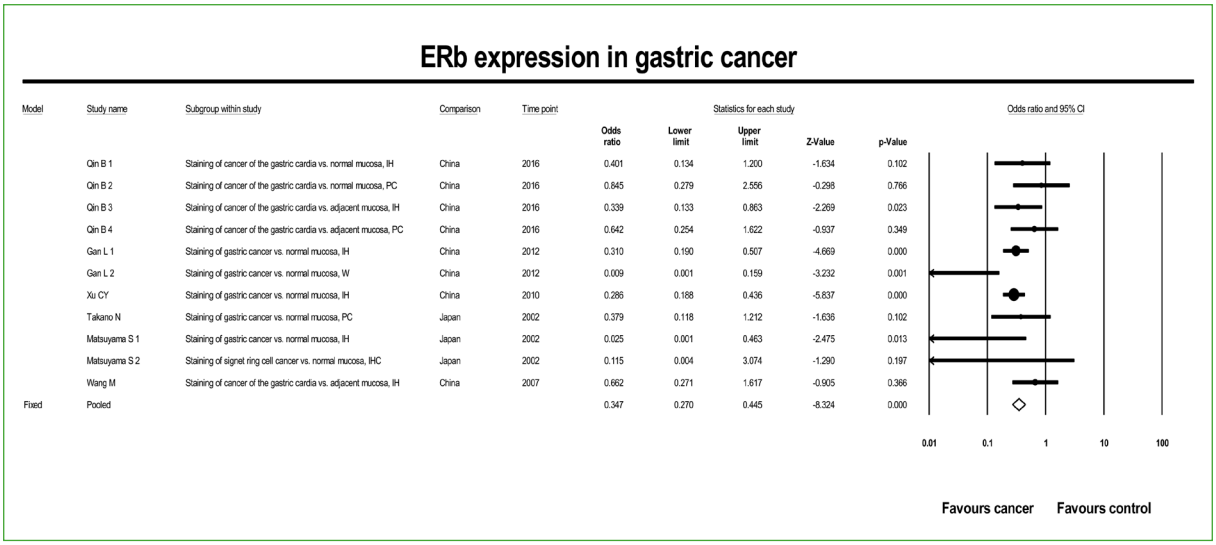

Figure 2. Forest plot illustrating OR and 95% CI for ERβ expression in gastric cancer *vs.* healthy controls.

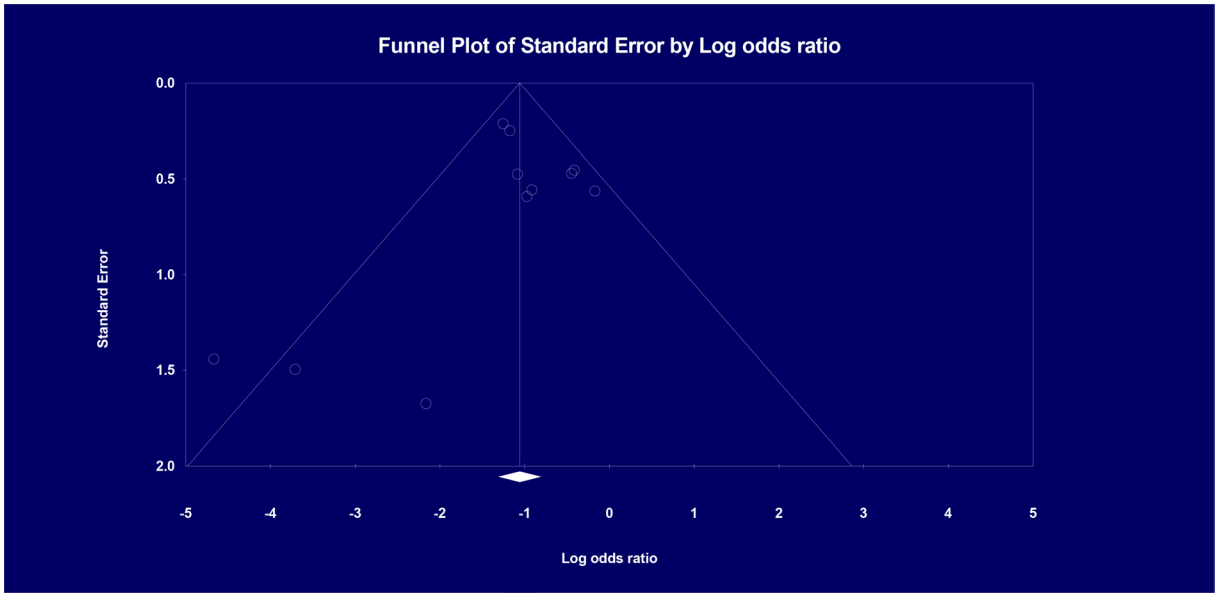

Figure 3. Funnel plot for publication bias.

the degrees of freedom (the number of studies minus 1). The Q-value is 17.102 with 10 degrees of freedom and p=0.072. Using a criterion alpha of 0.100, we can reject the null hypothesis that the true effect size is the same in all these studies. Thus, we have low heterogeneity.

The I^2 statistic is 41.528%, which tells us that some 41.528% of the variance in observed effects reflects variance in true effects rather than sampling error. Inconsistency yielded insignificant overall results, meaning that the comparative effect sizes that were obtained by direct and indirect comparisons are consistent. The distribution of the true effect is shown in Figure 4. The mean effect size is 0.32 with 95% CI of 0.25-0.53, and falls in the prediction interval 0.14-0.96, the true effect size in 95% of all comparable populations. We measured sensitivity by excluding individual studies and recalculating the overall meta-analysis outcome. This process was repeated for each of the studies. Deviations from the primary results were not significant with ORs varying between 0.328 95% CI 0.253-0.426 and 0.385 95% CI 0.283-0.525 compared with the primary score of OR at 0.347 95% CI 0.270-0.445.

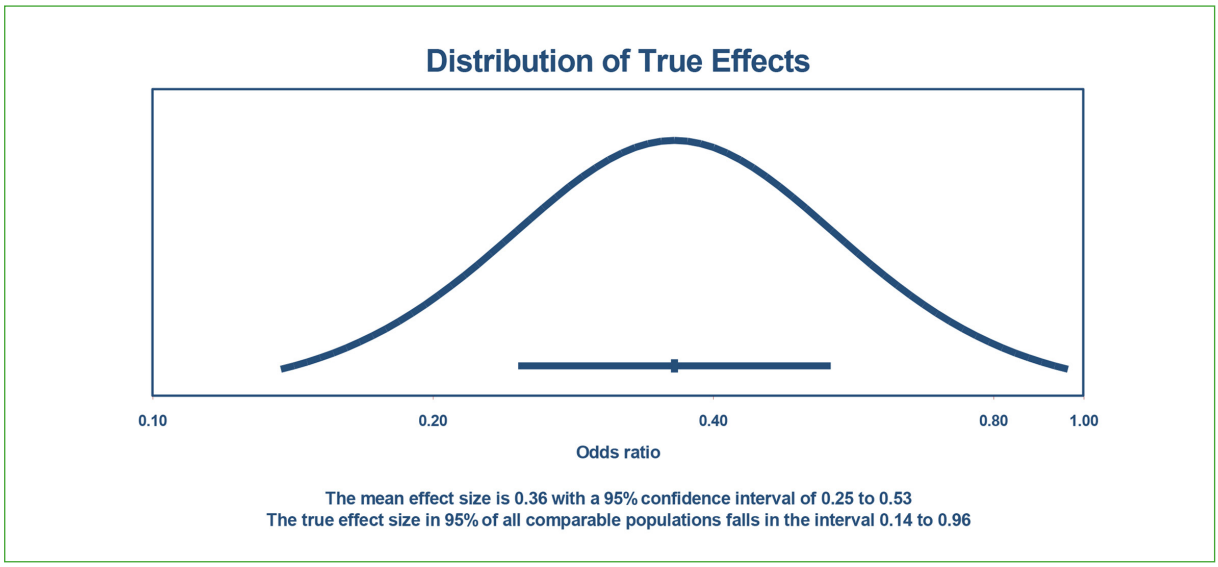

Figure 4. Distribution of true effect.

DISCUSSION

Epidemiologic studies indicate a global male predominance of gastric cancer, suggesting a possible role of sex hormones[25]. ERβ is known for its involvement, whether promoting or inhibiting proliferation, in the development and progression of hormone-sensitive cancers by binding to DNA and modulating the expression of target genes. Several studies[19-24] have reported differential expression of ERβ in healthy mucosa compared to gastric cancer tissue. Altogether, this may contribute to the idea of 'the guardian' role attributed to ERβ in gastric mucosa, as it is speculated to have antitumor activity[26,27]. In this meta-analysis, we confirmed the observation of decreased ERβ expression in gastric cancer and found an OR of 0.347 (95% CI: 0.270-0.445). This means that in gastric cancer tissue, the expression of ERβ decreased significantly, almost 70%. Several potential limitations should be considered in this meta-analysis: (1) Only English language literature was included. (2) Some individual data could not be obtained from articles; we extracted only explicit quantitative data per individual, group, or subgroup of patients. (3) We included studies that used different detection methods and cut-off values, which may affect heterogeneity of results. (4) The studies we included were carried out in Japan and China, necessitating the elimination of any potential geographical bias and the extension of investigations to different demographics. Our results support the hypothesis of ERβ being a tumor suppressor in the gastric mucosa. Reduction of ERβ expression in the mucosa might weaken or shut off downstream pathways that maintain healthy mucosa and prevent the development of malignancy. Since all data sets that were found indicate a decrease in ERβ levels among gastric cancer patients, it can serve as a potential prognostic indicator for identifying early-stage or malignant transformations. In addition, its activating may be a potential therapeutic route. Further research is necessary to investigate the efficiency of hormone replacement therapy and its effect on preventing malignancy development.

CONCLUSIONS

This meta-analysis showed that ERβ expression is lower in gastric cancer biopsy specimens than in healthy controls, which may have a significant effect on the survival of the patients.

Conflict of Interest

The authors have declared no conflicts of interest.

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None to declare.

Authors' Contributions

Both authors performed the research and wrote the article.

Data Availability

The data are accessible upon request.

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