Abstract

Background: Gastric adenocarcinoma (GAC) is a serious disease with dismal outcome. Discovering novel molecular targeted therapies is a recent point of research to improve prognosis. One of the newly discovered targets is the receptor tyrosine kinases (RTKs); it is a member of trans-membrane receptors which had important roles in proliferation and apoptosis. RTKs were found to have different expression patterns in several malignancies. HER2 neu which is a member of HER family is a proto-oncogene that is formed of four receptor tyrosine kinases. Erythropoietin-producing hepatocellular (Eph) molecules are major RTKs members and one of those molecules is EphA2 which has many different functions in cancer as tumor initiation, progression, angiogenesis and spread. We aimed to explore the expression patterns of both HER2 neu and EphA2 in GAC patients using immunohistochemistry, and to correlate their expressions with clinico-pathological factors and prognosis of our patients

Methods: HER2 neu and EphA2 expressions were assessed in sections from forty blocks of parafýn which were diagnosed as GAC. Then we analyzed the correlations between their expressions and disease outcome of GAC patients.

Results: HER2 neu and EphA2 positive expressions in GAC were positively correlated with tumor grade and stage ($p<0.001$ and $p=0.002$ respectively), inadequate response to therapy ($p<0.001$ and $p=0.002$ respectively), increase recurrence rate of GAC ($p=0.002$), and with poor survival ($p<0.001$).

Conclusion: GAC patients with high expressions of both HER2 neu and EphA2 had unfavorable prognosis.

Inclusion criteria: Patients with histologically confirmed GAC.

Exclusion criteria: Concurrent or history of other malignancy.

All patients were subjected to the following: Detailed history taking from patients, full physical-examinations, hematological and biochemical laboratory evaluation (complete blood count (CBC), liver functions and kidney functions tests), CT chest, abdomen and pelvis and bone scan if needed. Upper GI endoscopy was done and three to four biopsies were collected. Total gastrectomy and lymph node dissection was done for operable patients with a minimum of 15 lymph nodes removed. Fluorouracil (5-FU) based chemotherapy regimens were administered according to the tumor stage, radiotherapy is given if needed; response to treatment was evaluated by physical-examination, CT abdomenand pelvis, CT chest. All patients were followed by clinical examination and radiological evaluation every 3-4 months for 2 years.

Immunohistochemical staining

Streptavidine-biotin technique was used for immune histochemical staining with primary monoclonal mouse anti- HER-2/neu Ab-20 (L87+2ERB19) diluted 1/200 at 4°C overnight (Thermo Fisher Scientific, Lab Vision Corporation, Fremont, USA)and primary anti-EphA2 (D4A2) XP® Rabbit mAb at a dilution of 1:200 (Cell Signaling Technology) [15].

Evaluation of immunohistochemical expression of HER2 neu as used in TOGA trial

The staining was membranous and the degree of immunostaining was scored as followed: 0: absent-reactivity or only membranous reactivity in less than ten percent of cancer cells, one: Faint or barely perceptible membranous reactivity in more than or equal to ten percent of cancer cells; cells are reactive only in part of their membrane, two: Weak to moderate complete, basolateral or lateral membranous reactivity in more than or equal ten percent of cancer cells and Three: Strong complete, basolateral or lateral membranous reactivity in more than or equal ten percent of tumor cells [16].

Evaluation of immunohistochemical expression of EphA2

We consider only cytoplasmic staining as positive for EphA2, ten fields for all sections were selected randomly, assessed and graded then we evaluated the extent of stain and gave it scores 0, 1, 2 and 3 (0=0–5%; 1=6–25%; 2=26–50%; 3=more than 50%) and intensity of stain and gave it scores 0, 1, 2 and 3 (0=negative; one=weak intensity; two=moderate intensity; three=strong intensity), and summations of scores of both the intensity and extent of stain gave final scores from 0-6. We used score 3 as a cut off value above which was considered as over expression and below which was considered as low expression [9].

Statistical analysis

Our statistics were by using program of-SPSS 22.0, windows (USA, SPSS Inc., Chicago, IL,) and (Belgium, MedCalc Software bvba 13, Ostend,). Percent of categorical variables were compared using Pearson's Chi-square or Fisher's exact tests when any one of them was appropriate. DFS and OS were assessed using the method of Kaplan-Meier curve and log-rank test. A p-value <0.05 was considered significant.

Results

Patients'-data

GAC patient's data that were included in the study are summarized in Table 1. There were 28 (70%) males and 12 (30%) females with age ranged from (40-80) years (Mean: 59.97 ± 9.20 years), 36 (90%) cases were intestinal type and 4 (10%) cases were diffuse type adenocarcinoma.

Immunohistochemical results (Tables 1-3 and Figures 1 and 2)

A) Positive expression of HER2 neu was detected in 20 out of 40 (50%) cases of adenocarcinoma of the stomach and it was significantly correlated with higher tumor grade, high incidence of L.N metastases and advanced stage of the tumor (p=0.002 and p<0.001 respectively) (Figure 1).

B) Positive expression of EphA2 was detected in 22 out of 40 (55%) cases of adenocarcinoma of the stomach and was significantly positively correlated with grade, L.N metastases and stage of the tumor (p=0.005, p=0.002 and p<0.001 respectively) (Figure 2).

C) The positive expression of both EphA2 and HER2 neu together in stomach adenocarcinoma was detected in 19 out of 40 cases and was significantly positively correlated with grade and stage of the tumor (p=0.002 and p<0.001 respectively) (Table 2).

D) Expressions of both markers were significantly positively correlated with each other (p<0.001).

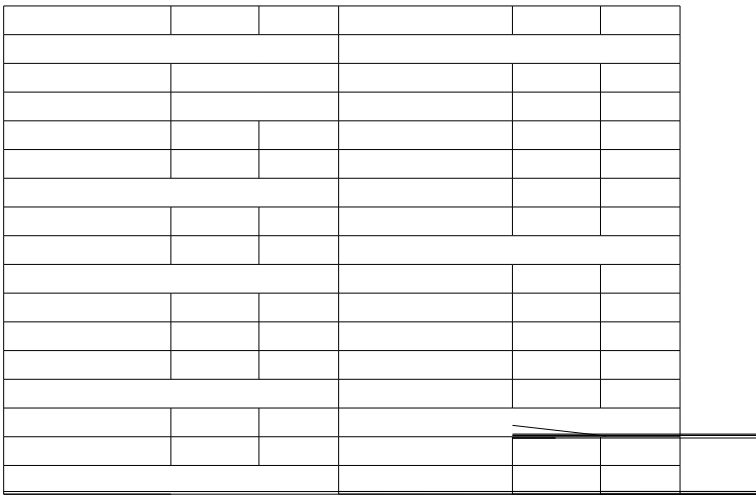

Table 1: Clinicopathological features, immunohistochemical markers and disease outcome of our patients.

E) No significant correlation was found between patient's age or sex, histopathological subtype, initial site, or size of the tumor with markers expression.

Treatment response and survival analysis (Table 3 and Figure 3)

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Patients with advanced disease (stage III) were assessed for response. Of the 29 patients, 21 patients (72.4%) had CR, 2 patients (6.9%) had PR, and 3 patients (10.3%) had SD and PD (Table 4).

Table 3: Correlation between immunohistochemical markers and disease outcome of our patients.

Figure 1: Immunohistochemical staining of HER2 neu in gastric adenocarcinoma (GAC): (A) High membranous expression of poorly differentiated GAC X400 (B) High membranous expression of moderately differentiated GAC X400. (C) Low membranous expression of moderately differentiated GAC X400. (D) Low membranous expression of well differentiated GAC 400. A, B, C and D the original magniýcation was X400.

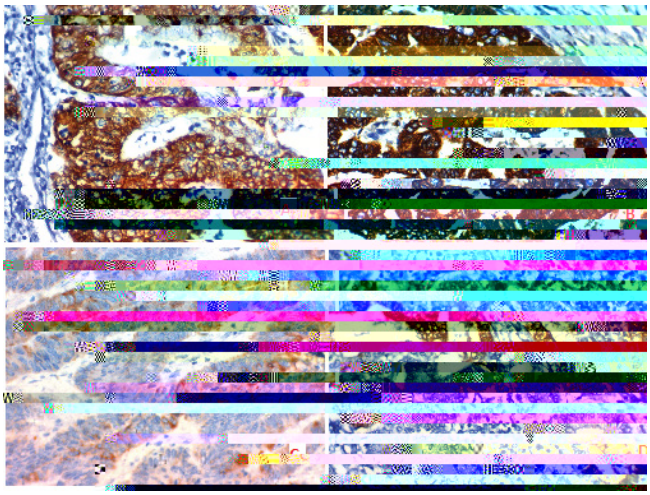

Figure 2: Immunohistochemical staining of EphA2 in gastric adenocarcinoma (GAC) :(A) High expression in the cytoplasm of poorly differentiated GAC X400; (B) High expression in the cytoplasm of moderately differentiated GAC X400; (C) Low expression in the cytoplasm of moderately differentiated GAC X400; (D) Low expression in the cytoplasm of well differentiated GAC X400. A, B, C and D original magniýcation was X400.

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but no significant relation was found between expression pattern of HER2 neu and tumor recurrence.

Survival analysis: After a median follow up of 40 (Range: 6-58) months, Positive expressions of EphA2& HER2 neu were significantly associated with shortened disease free survival (DFS) and overall survival (OS) ($p<0.001$ for both, Table 4, Figure 3).

Discussion

Many researchers had investigated the prognostic role of HER2 expression in cancers of many organs [11]. Previous studies have reported that the frequency of IHC detection of HER2 overexpression in GC varies from 10% to 22.1% [17], while positive expression of HER2 neu was detected in 50% of our cases and this high percentage of positive expression may be due to small patients number or due to inclusion of early as well as advanced stages in our study.

In our research we found that positive expression of HER2 neu was correlated with higher tumor grade, high incidence of L.N metastases and advanced stage of the cancer. Our results were close to Leni et al. [18] who reported that HER2 overexpression was more frequent in advanced GAC with high grade and advanced stage, and that was significantly associated with high disease recurrence and poor prognosis. Our results were consistent with Jia et al. who reported that HER2 over-expression was correlated with increased depth of invasion (P=0.045), lymph-nodes metastasis (P=0.026), and elevated clinical stage (P=0.026) but was not significantly associated with patient age, gender or cancer location [17]. Our results may be explained by that HER2 overexpression leads to increase cellular proliferation and inhibits apoptosis resulting in uncontrolled and excessive growth and spread of cancer.

Other different results were detected by Park et al. and Oh et al. [19,20] who stated that gastric tumor with HER2 neu amplification was only associated with old age and tumor size but it had no relation to prognosis. This discrepancy may be due the use of different immunohistochemical clones, the number of examined cases or the selection criteria that implied further study on a larger scale. Tessa and Raghuveer [21] assessed the expression HER-2 in cervical cancer and proved that it was positively associated with increasing the grade of cancer, presence of lymph node metastases and parametrial spread which was in agree with our results. Our results were also compatible with Park et al. [22] who studied Her2 amplification in colon cancer and reported that it was associated with higher rates of nodal metastasis and decreased patient survival. Hence, HER2 neu overexpression was found to be a prognostic factor for GAC and was negatively correlated with survival rates that were similar to results of Zhang et al. [23] and Park et al. [19] however, Jeung et al. [24] found no significant relation between HER2 neu expression and grade or stage of GAC; such difference that may be related to the nature of studied group and their number.

Also we found that positive expression of EphA2 was positively correlated with tumor grade, L.N metastases and tumor stage ($p=0.005$, $p=0.002$ and $p<0.001$ respectively). The results were similar to Huang et al. [7] and may be explained by that EphA2 stimulates proliferation, migration and spread of GAC cells mainly by increasing the expression of the epithelial mesenchymal transition markers like snail, N-cadherin, b-catenin, stimulating the Wnt/b-catenin pathway and by inhibition of E-cadherin in GAC cells. EPHA2 is overexpressed in a wide range of cancers and is associated with poor prognosis [25]. Many recent studies investigated the RTKs such as EphA2 and reported them as targets for molecular therapy for GAC [26-29], and also proved that EphA2 overexpression was positively correlated with factors that controlled angiogenesis and invasion in cancer cells because EphA2 receptor activation allowed vascular endothelial growth factor (VEGF)-dependent endothelial cell transport, sprouting, survival and expression of metalloproteinase, and these may be the causes of the poor clinical outcome of cancer patients with EphA2 overexpression, moreover the EphA2-EphrinA1 signaling axis regulates many steps that are essential for carcinogenesis and stimulation of downstream

4. Olayioye MA (2001) Update on HER-2 as a target for cancer therapy: Intracellular signaling pathways of ErbB2/HER-2 and family members. Breast Cancer Res 3: 385-389.

5. Menard S, Pupa SM, Campiglio M, Tagliabue E (2003) Biologic and therapeutic role of HER2 in cancer. Oncogene 22: 6570-6578.

6. Janjigian YY, Werner D, Pauligk C, Steinmetz K, Kelsen DP, et al. (2012) Prognosis of metastatic gastric and gastroesophageal junction cancer by HER2 status: A European and USA International collaborative analysis. Ann Oncol 10: 2656-2662.

7. Jorgensen JT, Hersom M (2012) HER2 as a prognostic marker in gastric cancer: A systematic analysis of data from the literature. J Cancer 3: 137-144.

8. Kim JW, Im SA, Kim M, Cha Y, Lee KH (2012) The prognostic signiýcance of HER2 positivity for advanced gastric cancer patients undergoing ýrst-line modiýed FOLFOX-6 regimen. Anticancer Res 32: 1547-1553.

9. Yuan WJ, Ge J, Chen ZK, Wu SB, Shen H, et al. (2009) Over-expression of EphA2 and EphrinA-1 in human gastric adenocarcinoma and its prognostic value for postoperative patients. Dig Dis Sci 54: 2410-2417.

10. Udayakumar D, Zhang G, Ji Z, Njauw CN, Mroz P, et al. (2011) EphA2 is a critical oncogene in melanoma. Oncogene 30: 4921-4929.

11. Tandon M, Vemula SV, Mittal SK (2011) Emerging strategies for EphA2 receptor targeting for cancer therapeutics. Expert Opin Therap Targets 15: 31-51.

12. Huang D, Xiao D, Li G, Ma J, Chen P, et al. (2014) EphA2 promotes epithelial-mesenchymal transition through the Wnt/b-catenin pathway in gastric cancer cells. Oncogene 33: 2737-2747.

13. Washington K (2010) 7th Edition of the AJCC Cancer Staging Manual: Stomach. Ann Surg Oncol 17: 3077-3079.

14. Edge SB, Compton CC (2010) The American Joint Committee on Cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 17: 1471-1474.

15. Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29: 577-580.

16. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, et al. (2010) Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive. The Lancet 376: 687-697.

17. Jia YX, Li TF, Zhang DD, Fan ZM, Fan HJ, et al. (2016) The co-expression and prognostic signiýcance of c-MET, ýbroblast growth factor receptor 2, and human epidermal growth factor receptor 2 in resected gastric cancer: A retrospective study. Onco Targets Ther 9: 5919-5929.

18. Ieni A, Barresi V, Rigoli L, Caruso RA, Tuccari G (2015) HER2 status in premalignant, early, and advanced neoplastic lesions of the stomach. Disease Markers 234851: 1-10.

19. Park DI, Yun JW, Park JH, Oh SJ, Kim HJ, et al. (2006) HER- 2/neu ampliýcation is an independent prognostic factor in gastric cancer. Dig Dis Sci 51: 1371-1379.

20. Oh HS, Eom DW, Kang GH, Ahn YC, Lee SJ, et al. (2014) Prognostic implications of EGFR and HER-2 alteration assessed by immunohistochemistry and silver in situ hybridization in gastric cancer patients following curative resection. Gastric Cancer 17: 402-411.

21. Tessa Joseph, Raghuveer VC (2015) HER-2/neu expression in cervical intraepithelial neoplasia and cervical carcinoma. Int J Biomed Adv Res 6: 47-52.

22. Park DI, Oh SJ, Park SH, Yun JW, Kim HJ, et al. (2004) Clinical signiýcance of HER-2/neu expression in colon cancer. Korean J Gastroenterol 44: 147-152.

23. Zhang XL, Yang YS, Xu DP, QU JH, Guo MZ, et al. (2009) Comparative study on overexpression of HER2/neu and HER3 in gastric cancer. World J Surg 33: 2112-2118.

24. Jeung J, Patel R, Vila L, Wakeýeld D, K ! M