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Original Research Paper

Pathology

RELATIONSHIP BETWEEN SERUM CEA AND HER- 2 RECEPTOR EXPRESSION IN GASTRIC CARCINOMA

Mithun Kumar Roy	MS (Applied Laboratory Sciences), Bangladesh University of Health Sciences (BUHS).Medical Technologist, Sir Salimullah Medical College, Mitford, Dhaka-1100.
Salma Akhte	Assistant Professor & Head Dept. of Pathology, Universal Medical College. Dhaka - 1215
Muhammad Saiedullah	Assistant Professor and Head, Dept. of Physiology and Molecular Biology, Bangladesh University of Health Sciences (BUHS), Dhaka.
Mohammad Abul Mohsin	Professor & Advisor, Dept. of Applied Laboratory Sciences Bangladesh University of Health Sciences (BUHS), Dhaka.
Mohammad Moniruzzaman*	Assistant Professor, Dept. of Immunology, Bangladesh University of Health Sciences (BUHS), Dhaka-1216. *Corresponding Author
Abdul Khaleque Akond	Professor of Pathology, Tairunnessa Memorial Medical College, Board Bazar, Gazipur-1704

ABSTRACT

BACKGROUND: Gastric cancer (GC) is considered as one of the leading causes of cancer associated mortality. Carcinoembryonic antigen (CEA) and Human epidermal growth factor receptor- 2 (HER-2) gene expressions are routinely used as diagnostic and prognostic tools in GC patients. However, the relationship between serum CEA and tissue HER-2 gene expression is unknown.

AIMS AND OBJECTIVES: The aim of this study was to evaluate the agreement between serum CEA level and HER-2/ neu gene expression in gastric cancer in Bangladeshi population.

MATERIALS AND METHODS: In this cross-sectional observational study, total 75 suspected subjects were included: 50 subjects with GC and 25 subjects without GC according to inclusion-exclusion criteria. Blood samples were collected in plain tube, serum was separated and preserved at -20 C for further analysis. Biopsy specimens were collected in 10% phosphate buffered formalin and later on paraffin embedded. HER-2 expression studied by Immunohistochemistry (IHC) using Herceptest kit.Serum CEA was determined by Enzyme Linked Immunosorbent Assay (ELISA). Histopathological analysis was carried out by HE stain and tumor gradation done. Data were expressed as mean ± SD, number (percent).Results of CEA, HER-2 were ranked as negative or positive.

RESULTS: Among the cases, well differentiated adenocarcinomas are the most prevalent (48%)
followed by moderately (38%) and poorly differentiated adenocarcinoma (12%) and chronic gastritis (84%) was common among the controls. All the control subjects were negative for HER-2 expression and serum CEA. Among the cases, 54% were positive for HER-2 expression and 46% were positive for serum CEA. CEA and HER-2 collectively classify 68% of the subjects correctly.
Spearman's rank correlation coefficient (ρ) between serum CEA and HER-2 expression was 0.127 (p=0.3787) among the cases and inter-rater agreement (κ) between CEA and HER-2 among the total patients was 0.342. The area under the receiver operating characteristic curve (AUC) was 0.730 (95% CI: 0.615 to 0.825) for CEA and 0.770 (95% CI: 0.658 to 0.859) against Immunohistochemical techniques (IHT) classification. The sensitivity and specificity were 46% and 100% for CEA and 54% and 100% for HER-2. Pairwise comparison between CEA and HER-2 classification against GC showed that the difference between areas was 0.0400 (95% CI: -0.0522 to 0.1320) and it was not significant (p=0.395).

CONCLUSION: It may be concluded that a) both serum CEA and tissue HER-2 expression have poor sensitivity regarding diagnostic importance in subjects with confirmed Histopathological diagnosis of gastric cancer.However, HER-2 expression has little higher sensitivity (HER-2 54% vs. CEA 46%) as compared to serum CEA level b) weak correlation or to fair concordance exists between serum CEA and HER-2 expression and c) collective use of CEA level and HER-2 expression may provide better diagnostic outcome.

KEYWORDS :

INTRODUCTION

Gastric Cancer (GC) is one of the most common tumors and remains the second leading cause of cancer mortality in the world[1]. The high incidence of GC and its consequence mortality rate severely threaten human health[2]. According to the most recent estimates GC accounts for 8% of the total cancer cases and for 10% of the total cancer related deaths[3].

GC is characterized by a clear geographical distribution, with over 70% of the cases occurring in developing countries[4]. Several risk factors for GC, including *Helicobacter pylori* (*H. pylori*) infection, genetic alterations, and chromosomal instability, have been reported[5]. About 90% of GC are classified as adenocarcinomas, while the remaining 10% is represented by Non-Hodgkin lymphomas, leiomyosarcomas, squamous cell carcinomas, and undifferentiated carcinomas[4].

The majority of tumor markers are effective prognostic tools that are used to identify groups of patients at risk of relapse or metastasis or to monitor cancer survivors following treatment[6].HER-2 overexpression found to 7–34% of gastric and gastroesophageal junction (GEJ) adenocarcinomas using different scoring methods or assay[7,8]. In cancers, HER-2 acts as an oncogene, mainly because high level amplification of the gene induces protein overexpression in the cellular membrane and subsequent acquisition of advantageous properties for a malignant cell[9].The Carcinoembryonic antigen (CEA) is a cell-surface-anchored protein involved in cell-cell adhesions. CEA serves as a functional receptor for colon cancer such as ligands for E-selectin and L-selectin, which may be critical for the metastatic spreading of colon cancer cell[10,11]. For the initial diagnosis of cancer, serum CEA was reported to be positive in 11.8%-37%[12-16] and a significant elevation of serum CEA was found in presence of distant metastasis[17]. Though CEA and HER-

2 gene expressions are routinely used as diagnostic and prognostic tools in GC patients, the agreement between serum CEA and tissue HER-2 gene expression is unknown.

MATERIALS AND METHODS

This cross-sectional observational study was done in the Department of Pathology, Bangladesh University of Health Sciences (BUHS) and Department of Medicine, Sir Salimullah Medical College, Mitford, Dhaka during the period of January 2015 – April 2016.Study subjects were admitted patients with endoscopic biopsy and positive for gastric cancer in the Dept. of Medicine, Sir Salimullah Medical College and BUHS Hospitals. Patients were consecutively enrolled according to inclusion and exclusion criteria. Total 75 adults with clinically suspected gastric malignancy were included.Gastric biopsy specimens from suspected patients were collected in 10% buffered formalin and paraffin embedded technique. Blood samples were collected in plain tube, serum was separated and preserved at -20 C for further analysis.

HER-2 status was evaluated by IHC (Herceptest kit (k5204, Dako /cytomation, Denmark) in paraffin embedded stomach tissue and considered as standard. The IHC scoring system[18-21] was based on intensity of reactivity, whether complete or incomplete and the percentage of reactive cells.

Table 1: Patterns of score.

Score	Staining pattern	HER-2 expression
0	No staining is observed or membrane staining is observed is less than 10% of the tumor cells	Negative
1+	A faint /barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane	Negative
2+	A weak-to moderate complete membrane staining is observed in more than 10% of the tumor cells	Equivocal
3+	Tumor cell clusters with strong complete membrane staining is observed in more than 30% (formerly 10%)of the tumor cells	Positive

Measurement of serum CEA concentrations: Serum CEA was measured by ELISA (Immulite -1000)with a detection range of 0-500 ng/ml. Measurements were performed strictly according to the manufacturer's instructions and quality control was ensured.

Histopathological Examination:Representative blocks were taken from stomach biopsies and placed in 10% formalin. Histopathological analysis with routine Hematoxylin and Eosin (H & E) stain was done for tumor grading (well, moderately, poorly differentiated or undifferentiated) and histopathological types (Lauren classification – intestinal, diffuse)[22].

Statistical analysis:

MedCalc® 11.4 used for statistical analyses, p value<0.05 was considered as statistical significance of any appropriate statistical tools. Agreement between serum CEA and HER-2 expression in GC was determined by Spearman's rank correlation, Inter-rater agreement (κ) and area under the ROC curve. Qualitative variables between case and control were compared by Fisher's exact test.

RESULTS:

In this cross-sectional study, total 75 subjects were included according to inclusion-exclusion criteria. Among them, 42 were male and 33 were female. The mean age of the total subjects was 54±13 years (range: 25 to 90 years). Characteristics and food habits, family history of gastric carcinoma and past history of gastric lesion of the study subjects were presented in Table 2. The two groups were matched for age (p=0.2690) and sex (p=1.0). No significant differences were observed among eating habit or other habits recorded. Furthermore, no significant difference was observed for family history of gastric carcinoma (OR: 6.165, p=0.1625) or past history of gastric lesion (OR: 4.571, p=0.0910) between case and control.

Table 2.Characteristics of the study subjects.

Variables	Case (n=50)	Control (n=25)	p vale
Age (Years)	55±14	52±12	0.2690†
Sex (Male/Female) [N]	28/22	14/11	1.0*
Dry fish (yes/no) [%]	100%/0%	96%/4%	-
Dry meat (yes/no)[%]	34%/66%	48%/52%	0.3156*
Smoker[%]	46%/54%	44%/56%	1.0*
Alcohol[%]	12%/88%	0%/100%	0.1699*
Family history of gastric carcinoma	10%/90%	0%/100%	0.1625*
Past history of gastric lesion	96%/4%	84%/16%	0.0910*

Results were expressed as mean ± SD, number and percent as appropriate.†, Student unpaired t-test; *,Fisher's exact test performed as applicable.

Histopathological study revealed that for 50 cases with gastric carcinoma 24 (48%) were well, 19(38%) moderately and 6(12%) poorly differentiated.

HER-2 status of the study subjects: HER-2 gene expression was observed by IHT and revealed that all subjects (n=25) in the control groups were negative for HER-2. Among the cases, 23 subjects(46%) were negative for HER-2 and 27 subjects(54%) were positive for HER-2(Table-3).

Table 3. HER-2 expression status of the study subjects case (n=50) and controls (n=25).

HER-2 status	Case	Control	OR	p value
	Number (%)	Number (%)		
Positive	27 (54)	0 (0)	(3.441to1035) 59.68	<0.001
Negative	23 (46)	25 (100)		

Chi-squared test

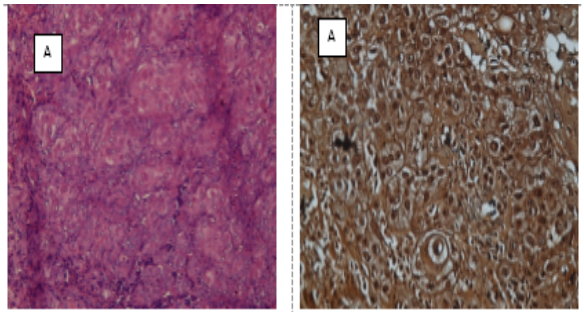
Serum CEA levels: Serum CEA levels (81.8±117.8ng/ml in case vs2.9±0.7 ng/ml in control) differed significantly (p<0.001) between case and controls. Twenty three (46%) of cases had serum CEA levels >5ng/ml. However, all the controls (100%) as well as 27 (54%) cases had serum CEA concentrations ≤5ng/ml (Table4).

Table 4. Serum CEA status of the study subjects case (n=50) and controls(n=25).

	Case	Control	Significance
	Number (%)	Number (%)	
*Serum CEA	81.8±117.8	2.9±0.7	p<0.001
Positive	23 (46)	0 (0)	
Negative	27 (54)	25(100)	

*Compared by student''t' test

CEA negative or positive was considered using 5ng/ml as cut off value for the subjects.



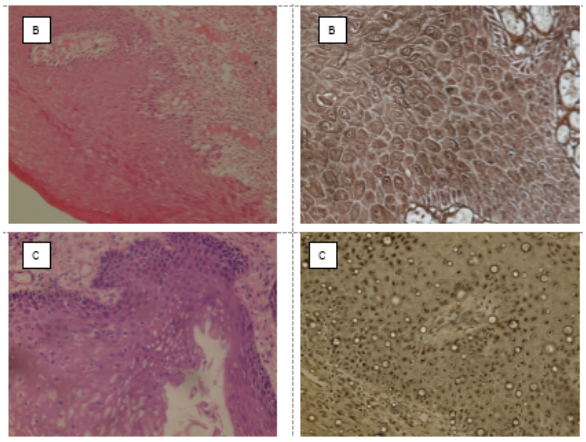


Fig 1: Histopathological slides (H and E stain, Left side) and HER-2 stain (Right side) for well differentiated adenocarcinoma (A), moderately differentiated adenocarcinoma (B), poorly differentiated adenocarcinoma.

Correlation or concordance between CEA and HER-2: The Spearman's rank correlation coefficient (ρ) of CEA with HER-2 was 0.127 (p=0.3787) within the cases. Inter-rater agreements (κ) between CEA and HER-2 are 0.126. In the total subjects, κ = 0.342. Both methods correctly classify 53 subjects (Negative = 39 and positive = 14) and discordance was observed for 22 subjects.

Comparison of CEA and HER-2 classification against GC: The area under the receiver operating characteristic curve (AUC) was 0.730 (95% CI: 0.615 to 0.825) for CEA and 0.770 (95% CI: 0.658 to 0.859) against IHT classification. The sensitivity and specificity were 46% and 100% for CEA and 54% and 100% for HER-2. Pairwise comparison between CEA and HER-2 classification against GC showed that the difference between areas was 0.0400 (95% CI: -0.0522 to 0.1320) and it was not significant (p=0.395) (Fig 2).

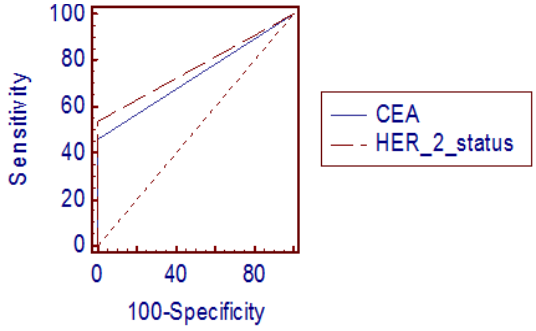


Fig 2: Comparison of AUC between CEA and HER-2.

DISCUSSION

Welling with carcinoma is a challenge for surgeons and oncologists. Early detection and correct assessment is of utmost importance in regard to outcome at treatment. In this study, serum CEA and tissue HER-2 expression were determined from specimens obtained from 75 subjects (50 cases and 25 controls based on histopathology) to explore the relationship between serum CEA and HER-2 expression in reference to histopathological findings.

Among the cases, majority of the subjects had well differentiated adenocarcinoma (48%) followed by moderately (38%) and poorly differentiated adenocarcinoma (12%) whereas in the control, most of the study subjects had chronic gastritis (84%) according to tissue histopathology.

Serum CEA is currently regarded as nonspecific classical marker of cancer used for early stage diagnosis gastric cancer whereas HER-2 expression by small biopsy is readily used for prognostic purposes. In this study, all subjects in the control group were negative for HER-2 expression and serum CEA. Among the cases, 54% were positive for HER-2 expression and 46% for serum CEA indicated that determination of HER-2 expression from small biopsy sample or measurement of serum CEA alone may produce high proportions of false negative results due to heterogeneity of HER-2 expression by gastric tumor[23] or use of nonspecific cancer markers (e.g., serum CEA). Furthermore, we observed a lower sensitivity against IHC for serum CEA compared to HER-2 expression which was further confirmed by comparison of area under the ROC curve in the total subjects. The area under the ROC curve against reference method showed no significant difference between serum CEA and HER-2 classification with little higher sensitivity for HER-2 (CEA 46% vs. HER-2 54%) at the cut-off value. This finding is consistent with the findings of Yano et al (2006)[24].

Spearman's rank correlation coefficient (ρ) between serum CEA and HER-2 expression and Inter-rater agreement (κ) revealed poor correlation or concordance between CEA and HER-2 expression among cases. In total subjects, fair agreement exists between serum CEA and tissue HER-2 expression (κ = 0.342).

Use of CEA and HER-2 collectively may provide better diagnostic importance in preoperative stage of confirmed gastric cancer since combination of two classify 68% subjects correctly among the cases. The measurement of CEA as diagnostic purpose is debated. Some studies[13,25] demonstrated that levels of serum CEA in preoperative stage predicts prognosis of gastric cancer while others have contradicted [16,26]. Since CEA can facilitate tumor metastasis and HER-2 is a key driver of tumorigenesis and the recommended therapeutic target, collective monitoring of CEA and HER-2 can improve the out-come in the management of gastric cancer patients.

CONCLUSION:

Both serum CEA and tissue HER-2 expression have poor sensitivity regarding diagnostic importance in subjects with confirmed histopathological diagnosis of gastric cancer. However, HER-2 expression has little higher sensitivity (HER-2 54% vs. CEA 46%) as compared to serum CEA level. Weak correlation or poor to fair concordance exists between serum CEA and HER-2 expression. Collective use of serum CEA and HER-2 expression in preoperative stage may improve its diagnostic purposes.

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