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ORIGINAL ARTICLE

Immunohistochemical Assessment of HER3 Expression in Pancreatic Cancer

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ABSTRACT

Pancreatic cancer (PC) is one of the most lethal tumor malignancies, which has a poor prognosis and can be the cause of death despite tumor removal. New molecular investigations for therapeutic purposes have shown the expression of HER3 in pancreatic adenocarcinoma. Family receptors HER3 play an effective role in malignancies such as lung and colon cancer. The aim of this study is to investigate the expression of HER3 in pancreatic cancer samples and the relationship between the expression of the receptor HER3 and the clinical complications of patients. Samples were taken from 75 patients with pancreatic cancer and subjected to immunohistochemical staining to evaluate the expression. Based on the intensity of staining, scores were assigned to each sample for HER3 receptor. Scores of +2 or +3 were defined as positive staining. The obtained data were analyzed using SPSS 16 software and Chi-square tests. $P<0.05$ was considered statistically significant. The results showed the expression of HER3 in normal pancreatic tissue. The expression decreased from the early to late stage ($p=0.05$). Multivariate survival analysis showed that only stage had independent prognostic value ($p=0.015$). HER3 expressed in normal pancreatic tissue, but the expression is lost in pancreatic cancer. According to the study, it seems that targeted treatment in pancreatic cancer has no relationship with expression.

Keywords: EGFR; HER3; Pancreatic adenocarcinoma; Prognosis

INTRODUCTION

Pancreatic cancer is one of the malignant tumors that causes the most deaths in the world due to its late clinical manifestations[1]. Adenocarcinoma of the pancreas has a poor long-term survival. Late clinical manifestations of this disease lead to advanced tumor and metastases, that's why few patients are eligible for surgery. The only treatment that increases the patient's chance of survival is pancreaticoduodenectomy. Extensive studies have been conducted to identify effective and helpful treatment strategies for pancreatic cancer. Until now, therapeutic goals such as chemotherapy have not been very effective[2,3]. New investigations of the effect of different factors such as growth factor receptors against molecular targets in the treatment of several types of cancer have shown promising results such as effects on tumor growth, apoptosis, and metastasis. Anti-human epidermal growth factor receptor (HER or ErbB) agents seem to have promising results in several types of cancer, including gastrointestinal malignancies[4,5]. Among important membranous growth factor receptors, HER-1 as epidermal growth factor receptor (EGFR), HER-2 as c-erbB-2 and HER-3 (c-erbB-3) and HER-4 (cerbB-4) which contain an extracellular ligand binding domain, a transmembrane region and an intracellular one with protein tyrosine kinase activity[6,7]. Human epidermal growth factor receptor HER3 (ErbB3) is a cell membrane-associated protein encoded by the ERBB3 gene. This receptor is known as a therapeutic factor in cancer treatment. In addition to HER2 and HER3, both receptors belong to a family of epidermal growth factor receptors (EGFR, HER) tyrosine kinase, and after the dimerization of the receptor, it is activated, which plays an effective role in regulating the cellular signaling pathway[8,9]. Studies have shown that HER3 has a favorable interaction with its structural homologue. Heterodimerization between HER2 and HER3 causes the subsequent signaling cascades of PI3K/AKT and Ras/Raf/MAPK, thus the existence of HER3 as an allosteric activator is required to maintain HER2-

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mediated signaling, and aberrant HER2-HER3 signaling is strongly associated with carcinogenesis and tumor cell proliferation [10–12]. Studies have shown that overexpression of HER3 protein is significantly effective in the pathogenesis of tumors, which can have adverse clinical consequences. Few studies have been done on the expression of HER3 in pancreatic cancer, which has shown this factor as a prognostic factor. Therefore, the purpose of this study is to investigate the immunohistochemical expression in pancreatic cancer samples and to investigate the relationship between the expression and the clinical pathology of pancreatic cancer patients.

Objectives

Due to the fact that few studies have been done to investigate the relationship between the factor HER3 and pancreatic cancer, the results of this research deal with the expression of HER3 factor and the prognostication of pancreatic cancer.

MATERIALS AND METHODS

To investigate, tumor samples were collected from pancreatic cancer patients undergoing treatment, and clinical and histopathological characteristics such as age, gender, stage, and grading were evaluated and analyzed. Data were collected from electronic record review, and all study procedures were conducted in accordance with the Declaration on Human Research. This study was conducted on tumor samples collected from patients with pancreatic cancer and undergoing treatment in Ali Ibn Abi Talib Hospital, Zahedan city. Inclusion criteria was Age$\geq$18 years, having measurable waste $\geq$2 cm with CT or MRI. The function of the organs is normal and there is no history of chemotherapy and Exclusion criteria was having peripheral neuropathy, brain metastasis, pregnancy, infection, alcohol or drug addiction. The study was approved as a research project in the Faculty of Medicine of Azad University of Zahedan and has a code of ethics. Patients' information such as age, gender, and place of residence were extracted from their medical records. Diagnostic samples were confirmed by the pathologist, and samples with incomplete information in the file, insufficient tissue for examination, or having necrosis and inflammation were excluded from the study.

Immunohistochemistry

For immunohistochemistry, the tissue blocks with the highest tumor percentage were selected for hematoxylin and eosin (H&E) staining and further analyzed by the pathologist. The expression of HER3 (Monoclonal Mouse Anti-Human) was evaluated with an immunohistochemistry technique on 5-μm-thick tissue sections obtained from paraffin-embedded specimens fixed in 10% (v/v) neutral buffered formalin. The sections were deparaffinized and hydrated by passing through xylene and a graded series of ethanol, followed by washing in distilled water. For HER3, peroxide blocking was performed with 3% H_2O_2 at room temperature for 10 minutes. The monoclonal mouse antibody anti-human HER3 was used, and samples were incubated overnight at 4°C. Incubation with the secondary antibody was performed for 30 minutes, followed by application of diaminobenzidine chromogen for 5 minutes. Subsequently, the slides were counterstained with Meyer's hematoxylin for 1 minute, dehydrated in a graded series of alcohol, treated with xylene, and cover slipped. The negative control for the validation of staining was sections incubated with secondary alone without primary antibody. A positive control was included in each immunohistochemistry run. The slides were scored by a pathologist blinded to the clinicopathological data. Stained slices were evaluated semiquantitatively as negative (no staining or staining in less than 10% of cancer cells) or 1+, 2+ or 3+. Staining in less than 10% of tumor cells (score 0), weak staining in 10% of tumor cells (score 1+), moderate staining in 10% or more tumor cells (score 2+) and strong staining in 10% or more cells Tumors (score 3) are considered. Scores 0, +1 and +2 are considered for negative protein expression and +3 score for positive protein expression.

Statistical analysis

The chi-square test was used to compare frequency distributions. Multivariate survival analysis was done using the Cox regression method. P-values below 0.05 were considered significant. Statistical analysis was carried out using SPSS software.

RESULTS

Characteristics of the patients

Patients' characteristics are summarized in Table 1. Pancreatic cancer samples were collected from 75 patients, 43 males and 32 females. Median age was 62 years (range 33-76). Five patients presented with stage IV disease at diagnosis and did not undergo surgery, while the remaining 40 patients underwent pancreatic cancer resection. Among those with the surgically removed tumors, 32 patients received a R0 resection, while in 8 patients, margins were microscopically involved (R1). Tumor grading showed 3 well-differentiated tumors (G1), 13 moderately differentiated (G2) and 7 poorly differentiated tumors (G3). Most of the patients presented with stage II disease – specifically, 10 with stage IIA and 22 with stage IIB disease while 2 patients had stage I and 3 patients stage III disease. HER3 staining was negative in 18 tumors and 1+ in 9 (9.9%). HER3 overexpression was demonstrated in 18 tumors (19.7%). Specifically, 13 samples had 2+ staining (14.3%) and 5 samples 3+ staining (5.4%).

Immunohistochemistry

Table 2 summarizes the expression of HER3 that showed mainly and occasionally membrane staining. HER3 staining was negative in 18 cases, 1+ in 9, 2+ in 13 and 3+ in 5 tumors. Strong expression of HER3 was thus seen in 18 tumor samples.

Clinicopathologic correlations of HER3 expression

There were no statistically significant differences between expression of HER3 and other clinicopathologic features. In univariate survival analysis, only (early vs late) stage was significant, and none of the other clinicopathologic features had prognostic value. In multivariate survival analysis including stage, grade, and HER3 expression, only stage had independent prognostic value (p=0.015) and no other features were selected.

Table 1: Patient characteristics (N = 45)

Patients	No. (%)
Sex	
Male	26 (65)
Female	19 (35)
Age, years (mean)	62
Range	32-74
TNM Stage	
Stage 1 (T1-2, N0, M0)	2 (2)
Stage 2A (T3, N0, M0)	10 (23)
Stage 2B (T1-3, N1, M0)	22 (50)
Stage 3 (T4, N0-1, M0)	3 (5)
Stage 4 (any T, any N, M1)	8 (20)
Grading	
G1	3 (3)
G2	13 (30)
G3	7 (18)
Gx	22 (49)
Surgery	
R0 Resection	32 (75)
R1 Resection	8 (11)
R2 Resection	0 (0)
No Resection	5 (14)

Table 2: HER3 expression in 45 cases of pancreatic cancer

	N	%
Negative	18	19.7
1+	9	9.9
2+	13	14.3
3+	5	5.4

DISCUSSION

The present study was performed to evaluate HER3 expression in Pancreatic cancer. The results obtained based on the total score indicated the positive HER3 expression in 19.7% of PC samples. HER3 is one of the four members of epidermal growth factor receptors as ERBB, which is activated by connecting to Neuregulin-1 and Neuregulin-2 ligands. Since HER3 lacks intrinsic kinase activity, induction of signal occurs through formation of Heterodimers with EGFR, HER2 and HER4[13]. Most studies examining the HER3 expression in PC[14,15], in which some significant results were obtained with respect to increased expression and lymph node metastases, prognosis and invasion[16,17]. It was recently found that HER3 plays an important role in response to radiotherapy of head and neck carcinomas and blocking its activity along with radiotherapy may be beneficial for the treatment of human tumors[18]. Scharpenseel H et al.[19] studied the expression of HER3 in pancreatic cancer and showed that expression was observed in all samples, and the staining intensity of tumor cells was more intense in invasive areas. The results of another study showed that systemic conditions such as some diseases have an effect on HER3 expression and can indicate cell proliferation and inhibition of apoptosis[20]. In a study by Krop I et al.[21] EGFR expression was positive in 60% of pc samples. In a study conducted by Yu HA et al., the intensity of expression HER3 as the first membrane family of receptor tyrosine kinase was related to tumor growth of pancreatic cells[22]. Considering that studies have shown that EGFR expression is related to inflammation, therefore, inflammation was identified as a factor to reduce the expression of this receptor in pancreatic cancer. Our study results showed HER-3 expression in 19.7% of tumors. In line with the results of our study Seshacharyulu P et al. showed a significant increase in HER-3 expression in pancreatic cancer compared to control tissue[23]. In the present study, the results showed a significant decrease in HER-3 expression and the advanced stage of pancreatic cancer, which is a new result. In line with our study, the results of the study by Capone E et al. showed very low expression of HER-3 in advanced stage ovarian cancer, suggesting that loss of HER-3 expression may be associated with pancreatic tumor stage progression[24]. In the investigation conducted by Yonesaka K et al. expression of EGFR was significantly higher in pc samples[25]. These results were in contrast with the results of our study. Also, studies showed that the staining factor based on intensity is not a suitable criterion, because the intensity of staining may be reported differently by different pathologists. Also, studies have shown that the high expression of the factor has a direct relationship with causes such as intrinsic growth potential, ability to multiply independently of stimulation, aggressive growth, and high tumor recurrence. It seems that the lack of more studies in the field of expression has led to less knowledge of pancreatic cancer and the causes and

identification of its precursors.

CONCLUSIONS

Pancreatic cancer is one of the cancers that has a poor prognosis, and more studies are needed for its treatment. This study showed that HER3 highly expressed in the normal tissue of the pancreas, while the expression is lost in the tumor tissue, even the expression decreases from the initial to the final stage. Therefore, it seems that the clinical use of HER-targeted therapy in pancreatic cancer and HER expression is not supported.

ABBREVIATIONS

- HER: Human epidermal growth factor receptor
- IHC: Immunohistochemistry
- EGFR: Epidermal growth factor receptor
- HER3: Human epidermal growth factor receptor 3

DISCLOSURES

- **Funding/Support:** medical university of Islamic Azad University of Zahedan, Zahedan, IR Iran.
- **Conflict of interest:** The authors declare they have no conflict of interest regarding the publication of this paper.

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Authors' Contributions: Study concept and design: H.H. Analysis and interpretation of data: A.S. Drafting of the manuscript: H.H. Statistical analysis: SH.SH.

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