Epidermal Growth Factor Receptor (egfr) Immunohistochemical Expression in Gastric Carcinoma

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ABSTRACT:

BACKGROUND :

Gastric cancer is the the fourth most common cancer and the second leading cause of cancer-related deaths. Gastric cancer is a major cause of cancer morbidity and mortality worldwide.

OBJECTIVE:

To estimate the immunohistochemical expression of epidermal growth factor receptor(EGFR) in the gastric cancer in relation to other parameters like grade and stage.

METHODS:

Formaline fixed ,paraffin-embedded blocks from 51 patients (29 male and 22 female) with gastric carcinoma were included in this study. Ten biopsies of normal gastric tissue were selected as a control group. Envision (DAKO) technique was applied to study the immunohistochem- ical expression of EGFR in paraffin embedded sections of gastric cancer.

RESULTS:

Positive immunohistochemical expression of EGFR was seen in 41.2% of cases as both membranous and cytoplasmic brown staining while there was negative staining in the normal control group ($p<0.05$). EGFR immunoexpression was correlated with the histological type(more in the intestinal variant than the diffuse type) ($p<0.0.5$).

CONCLUSION:

These findings provides further evidence for the role of EGFR in the tumorgenensis of gastric cancer. However, EGFR could not be well correlated with stage of tumor and hence may be poor prognostic parameters of the state of malignancy.

KEY WORD:EGFR,gastric carcinoma**.**

INTRODUCTION:

The importance of gastric cancer comes from the fact that early diagnosis of the disease ensures long survival and it's a leading cause of death due to cancer in several countries[(1)].

In middle East there is wide geographical variation in the incidence of gastric cancer[(2)]. In Iraq, gastric cancer cases are 7 times lower than Iran(4.5 versus $26.1/10^5$ respectively) but it is still a highly killing type of gastrointestinal cancer [(3)] .

Gastric cancer usually develops through a cascade of well-defined and recognizable precursors (inflammation –metaplasia– dysplasia– carcinoma sequence)[(4)].

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The epidermal growth factor receptors (EGFRs) family belongs to the receptor tyrosine kinase (RTK) superfamily and are important signaling proteins in both physiological and cancer conditions, for example cell-cycle progression, proliferation, survival and invasion [(5)]. EGFR is a member of the EGFR family of tyrosine kinase receptor proteins and is a molecular target in a variety of cancers, including colorectal cancer and non-small cell lung cancer (NSCLC)[(6)]. EGFR has a significant predictive ability for estimating overall survival in gastric cancer(GC)[(7)].

This study was designed to illustrate the immunohistochemical over-expression of EGFR in gastric cancer in relation to grade and stage of the tumor in a sample of Iraqi patients who were referred to Baghdad teaching hospital.

PATIENTS AND METHODS:

Retrospective study was applied,starting from November 2013 to November 2014, 51 patients (29 male and 22 female) had gastric carcinoma and 20 cases of non cancerous lesions as a control group were subjected to the present immunohistochemical evaluation of EGFR.The biopsies of the cancerous cases were taken by gastrectomy(23 cases) and endoscopy(28 cases). All cases were referred to Baghdad teaching hospital and GIT hospital in Baghdad. All cases, were examined independently by two histopathologists.The cases were applied then to the immunohistochemical study using Anti-EGFR monoclonal antibody, a DAKO Monoclonal Mouse Anti-Human *EGFR*-pY 1197 antibody with a phosphorylation site specific ,clone DAK-H1-1197 an isotype : IgG2a,Kappa. The biopsies were taken from different anatomical locations of stomach. The main histological types studied were the intestinal and the diffuse signet cell types. The malignant samples were staged according to TNM staging system[8].The mean age of patients was 51.5 years(both for the carcinoma and control groups). The criterion for a positive immune reaction of *EGFR* in the malignant cells was a brown membranous and cytoplasmic staining. A four scaled scoring system was applied to assess the intensity of immune staining; score 0(negative), score 1(weak), score 2(moderate) and lastly score 3(strong) staining[9]. Three scaled grading system was used in this study ; grade 1(well differentiated),grade 2(moderately differentiated) and grade 3(poorly differentiated)[10].

Statistical analysis

The results were statically evaluated with the help of SSPS software using the Chi-square test ($p < 0.05$).

RESULTS:

All the gastric tissue from the control group showed *negative* EGFR immunostaining (Table 1&figure 1).The positive results for EGFR immunehist- ochemical staining appear as a brown membranous and cytoplasmic color(figures 2,3&4).The cancer cases showed positivity in 21 cases(41.2%) while the negative cases were 30 cases(58.8%) out of the remaining 51 cases included in this study ($p<0.05$) (table 1).

Table 1:Immunohistochemical expression og EGFR in both normal & cancerous gastric cases.

Pathological parameters	EGFR positive	EGFR negative	Total	P value
Control	0(0%)	20(100%)	20(28.2%)	
Malignant	21(41.2%)	30(58.85)	51(71.8%)	< 0.05

As seen in table 2,the overexpression ofEGFR was reported in 15 cases(44.1%) in intestinal type and 6 cases(37.5%) in diffuse/signet type. There was a significant difference between the two histological types($p<0.05$). *EGFR* was shown to be positive in 57.1%,45.5% and 36.4% of grade 1,2,and 3 respectively. These results illustrates that there was no significance difference among the different grades in relation to *EGFR* immunostaining($p>0.05$).EGFR overexpression was shown in 3 cases(50%) ,3cases(37.5%), 3cases(50%)and 1 case(50%)of stage I,II,III and IV respectively. The results were close among different stages of gastric carcinoma with no significant difference,($p>0.05$).

Table 2:Imunohistochemical expression of EGFR in gastric carcinoma in relation to grade , histological type and stage.

Pathological parameters	EGFR positive	EGFR negative	Total	P value
Histological type				
Intestinal	15(44.1%)	19(55.9%)	34(66.7%)	<0.05
Diffuse	6(37.5%)	11(62.5%)	17(33.3%)	
Grading				
Grade 1	4(57.1%)	3(42.9%)	7(13.7%)	
Grade 2	5(45.5%)	6(55.5%)	11(21.6%)	>0.05
Grade 3	12(36.4%)	21(63.6%)	33(31.4%)	
TNM staging				
I	3(50%)	3(50%)	6(27.3%)	
II	3(37.5%)	5(62.5%)	8(36.3%)	>0.05
III	3(50%)	3(50%)	6(27.3%)	
IV	1(50%)	1(50%)	2(9.1%)	

Table 3 shows that small differences exist among the grades of gastric cancer in relation to staining scores (p>0.05).There was no significant difference(p>0.05) in the relation of cancer staging and the intensity scores of EGFR immunostaining

Table 3: Association of EGFR staining scoring with grade and stage.

Intensity	Score 0	Score 1	Score 2	Score 3	Total	P value
Grade						
I	3	0	3	1	7(13.7%)	>0.05
II	6	1	2	2	11(21.6%)	
III	21	8	3	1	33(64.7%)	
Stage						
I	3	1	1	1	6(28.6%)	>0.05
II	5	1	0	1	7(33.3%)	
III	3	1	1	1	6(28.6%)	
IV	1	0	1	0	2(9.5%)	

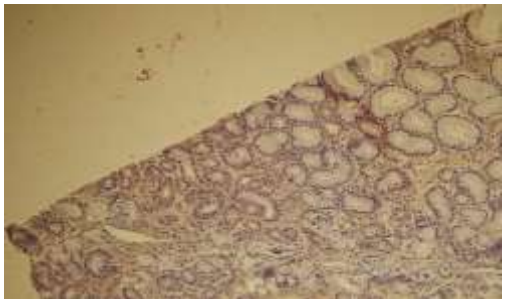

Figure1:Normal gastric mucosa showing negative immuno- histochemical expression for EGFR (4X).

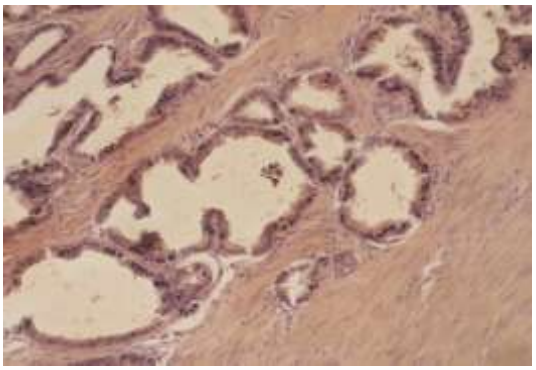

Figure 2: Well differentiated adenocarcinoma (intestinal type) shows positive EGFR immunohistochemical expression(10X).

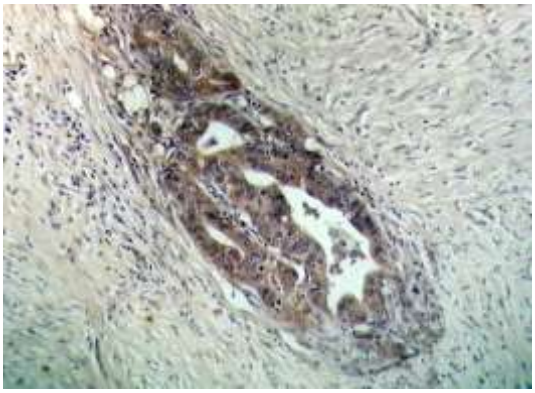

Figure 3: positive EGFR immunostaining in moderately differentiated gastric adenocarcinoma(10X).

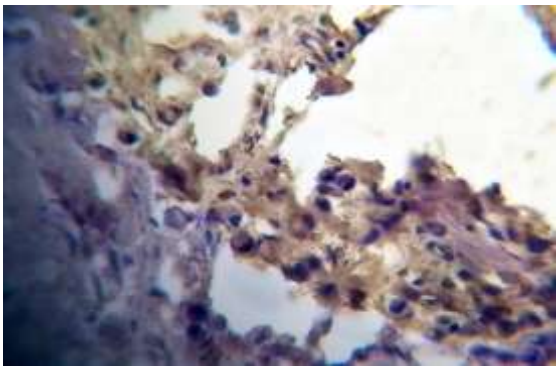

Figure 4: Positive EGFR cytoplasmic staining, score +2 inDiffuse/signet gastric carcinoma(40X).

DISCUSSION:

EGFR(HER-1) is protein tyrosine kinase ,correlated with prognosis and response to therapy in a variety of human cancers, rendering EGFR may be promising prognostic and therapeutic targets in canine and human gastric epithelial neoplasm[11.12] .

In the present study ,the positivity for *EGFR* in gastric cancer was 41.2%(21 out of 51 cases) while in the control group it scored nil, with a significant difference from the control group($P<0.05$). This may indicate a possible role of this factor in the development of tumor with similar results obtained in other researches [12-17]. The researches were so variable about the percentage of EGFR immunohistochemical expression in gastric cancer, some were lower and others higher than that of our study.

In relation to the positive *EGFR* immunostaining , the intestinal histological variant showed more immunostaining than the diffuse/signet type. There was a significant difference between the two groups ($p<0.05$). Several studies showed similar results[18-20].

It was noticed from this work that a gradual increase in number of the positive cases with the increase in the grading of gastric cancer. In spite of that there was no significant difference ($p>0.05$).This fact was insisted by other researchers[10,21].Same for the relation with the cancer stage was seen with no big difference($p>0.05$).This may clear that *EGFR* works independently from the stage[12,19,22].

The variations of the results between the different studies might be due to different methodical setups and to the type of the kit employed for EGFR immunohistochemical detection and the number of cases involved in the study [123]. Other factors include EGFR gene amplification or mutation, transcriptional abnormalities or autocrine stimulation by enhanced expression of the ligands EGF and TGF alpha and the type of scoring system used to interpretate EGFR overexpression [124].

CONCLUSION:

The role of EGFR in the carcinogenesis of gastric cancer was significantly higher than in the normal gastric tissue and EGFR immunostaining was significant more in the intestinal variant than the diffuse/signet histological variant.

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