The Immunohistochemical Assessment of Muc5ac in Patients with Gastric Carcinoma (Gc) in Iraq

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Abstract

The expression of MUC5AC has been associated with the loss of the differentiation, TNM system, and nodal metastasis, in many cancers including gastric carcinoma (GC).Objective: To evaluate whether the MUC5AC could be used as a predictor in patients with GC and to assess the correlation between the expression of MUC5AC& the clinicpathological parameters as age, sex, histopathological subtypes, grade and stage of the tumor. This is a retrospective study conducted on 60 randomly selected patients (30 normal vs 30 GC), at the Pathology Department of the Gastroenterology and Hepatology Teaching Hospital&some private laboratories. They were collected and diagnosed during the period 2014-2018. Histological sections were stained with H&E and IHC stained for MUC5AC. The Statistical analysis was done using SPSS system, and the difference was regarded as significant. Expression of MUC5AC was significantly Decrease (p<0.05) in GC group compared with control group.The mean age of the patients was 48.39 years, expression of the MUC5AC was shown in 66.6%of the GC cases, in this study, there was a significant correlation between MUC5AC positivity and lymph node involvement, there was non-significant correlation between expression of MUC5AC and age, sex, histopathological subtypes, grade and stage of GC. Results showed that decrease and increase of expression of the MUC5AC was a closely associated with GC and infected lymph node respectively.These results suggested that MUC5AC can be utilized as an ancillary marker for diagnosis the infected lymph node and malignant transformation of GC but it seemed to have no distinct role in predicting grade and stage outcomes in patients with GC.

Keywords: gastric carcinoma, MUC5AC, Immunohistochemical expression.

التقييم المناعي الكيميائي النسجي لـ MUC5AC في مرضى سرطان المعدة في العراق

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الخلاصة

يرتبط التعبير عن MUC5AC مع فقدان التمايز، نظام التصنيف المرحليTNM ، وانبثاث العقد اللمفاوية، في العديد من السرطانات البشرية بضمنا سرطان المعدة .(GC) تهدف الدراسة الحالية الى تقييم ما إذا كان يمكن استخدام MUC5AC كمنبئ في مرضى سرطان المعدة،ولتقييم العلاقة بين تعبير MUC5AC والمؤشرات السريرية المرضية كالعمر وجنس المرضى، الأنواع النسجية للورم،درجة تمايز الورم ومرحلة الورم.

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تم إجراء هذه الدراسة بأثر رجعي على 60 مريضاً تم اختيارهم عشوائياً (30 لمجموعة السيطرة مقابل 30 لمجموعة الاصابة(GC ، تم جمعها من مستشفى أمراض الجهاز الهضمي والكبد وبعض المختبرات الخاصة للفترة (2014–2018). من كل قالب برافين تم تلوين شريحتين احدهما بـH&E ، والثانية بـMUC5AC ، وقد تم التحليل الإحصائي باستخدام نظامSPSS ، واعتبر الاختلاف معنويا عند 0.05 .P<النتائج: اظهر التعبير عن MUC5AC انخفاض معنوي في مجموعة الاصابة GC مقارنة بمجموعة السيطرة. وكان متوسط العمر 48.39 سنة. ظهر التعبير عن MUC5AC في 66.6٪ في مجموعة الاصابة بسرطان المعدة، وقد كان هناك ارتباط معنوي بين التعبير الايجابي للـ MUC5AC واصابة العقدة الليمفاوية، في حين لم يكن هناك ارتباط معنوي بين التعبير عن MUC5AC وعمر ، جنس المرضى، الانواع النسجية للورم، درجة تمايز الورم، ومرحلة الورم. الاستنتاج: وجد أن الانخفاض والزيادة في مستوى التعبير عنMUC5AC ، كان مرتبطًا مع التحول السرطاني واصابة العقد الليمفاوية على التوالي، هذه النتائج تقترح أنه يمكن استخدام MUC5AC كعلامة مساعدة لتشخيص اصابة العقدة الليمفاوية والتحول السرطاني لسرطان المعدة ولكن يبدو أنه ليس لها دور مميز في التنبؤ بنتائج تمايز ومرحلة تطور سرطان المعدة.

Introduction

Gastric Carcinoma (GC) is 5th of the most common malignant gastrointestinal tumors, and remains the third most common reason of lethal in the worldwide [1]. The interaction of both environmental and genetic factors contributes to the etiology and pathogenesis of these aggressive cancers; mainly bacterial infection by Helicobacter pylori. The incidence and mortality of the GC show vary geographically and being high in East Asia (China) [2]. In Iraq, the GC is one of the commonest ten cancer it ranks the 7th in both sexes [3]. Over the past few years, the histopathological classification of the GC has been largely done according to Lauren's criteria, in which intestinal subtype and diffuse subtype adenocarcinoma are the two major histopathological subtypes, plus mixed subtypes [4]. MUC5AC protein, a gastric-type mucin of the cardia and corpus of the stomach, is expressed in normal gastric epithelium which is a well-established gastric marker gene [5], and is often used for the clinical assessment and prognosis of the GC [6], but the association between IHC expression of MUC5AC protein and malignant potential of the GC is still controversial [7]. Objective: To evaluate whether the MUC5AC could be used as a predictor in patients with GC and to assess the correlation between the expression of MUC5AC& the clinicpathological parameters as age, sex, histopathological subtypes, grade and stage of the tumor.

Materials and Methods:

This retrospective study was conducted on 60 patients. Thirty samples of HGC (gastroectomy specimen) were collected at five years period (from 2014 to 2018) diagnosed at Gastroenterology and Hepatology Teaching Hospital (20 males and 10 females). The control group included (30) gastric normal tissue (GN) were collected from private Laboratories in Baghdad city (13 males and 17 females) The information about the patient (age, sex, tumor grade, histopathological subtype, depth invasion, lymph node involvement and stage of tumor) was recorded after review of all available hematoxylin-eosin slides from the archive of Department of Pathology of the above hospitals. For each patient and control group included in this study, immunohistochemical staining was performed according to the manufacturer's guidelines serial sections from paraffin embedded block were taken and it had been sectioned into 4µm thickness, put on positively charched slides. Immunohistochemical was carried out with the MUC5AC monoclonal antibody (abcam, UK; optimal dilution, 1:200). Sections were Deparaffinized in xylene and rehydrated in graded ethanol alcohol series, endogenous peroxidase inactivation of clinical tissues was performed for MUC5AC hydrated heating in 50 mm EDTA buffer solution (pH 7.0) at 95c using a water bath (Gallen kump, England) for 30 min for antigen retrieval. The primary anti-MUC5AC, the antibody was applied for one hour at room temperature. After washing in PBS two times, the secondary antibody (Dako, Denmark) was applied for 30 min at room temperature, and then incubated with antibody conjugated to HRP (Dako, Denmark) for 10 min at room temperature was then done. Next, the slides were counterstained with Mayer's hematoxylin for 5min and dehydrated in alcohol prior to mounting. IHC reaction is considered positive when cytoplasmic dark brown is for MUC5AC protein. Positive control sample for MUC5AC protein was prepared from the HGC tissue and stained by used MUC5AC antibody (Figure-1).
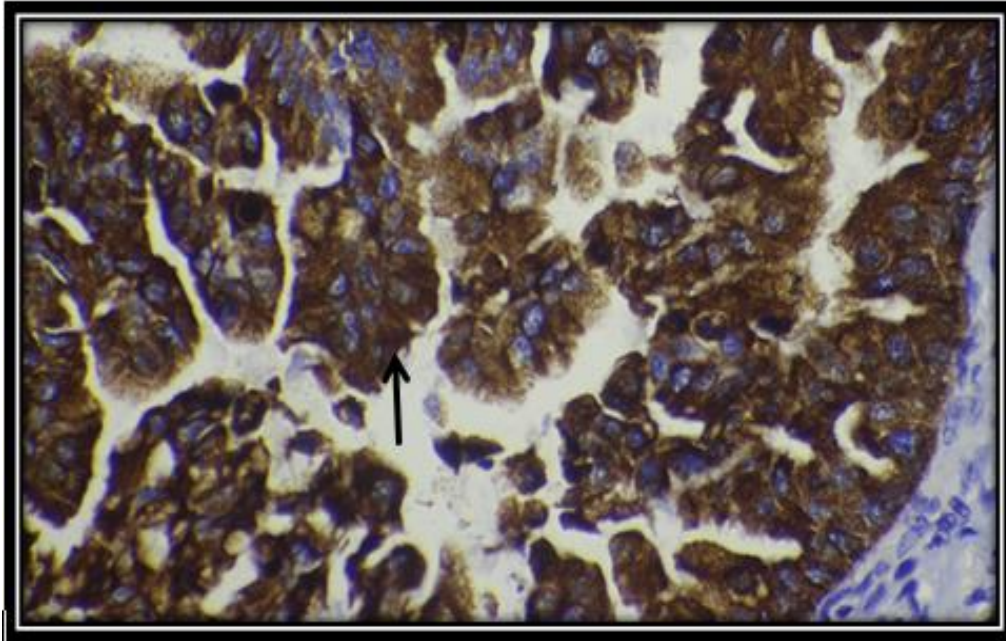
Figure 1-Section in intestinal subtype of GC (positive control; brown color of cytoplasm; black arrow) (IHC, 40X)

Negative control sample for MUC5AC protein was obtained from the same tissue and stained similar to test samples except without adding the primary antibody (Figure-2).
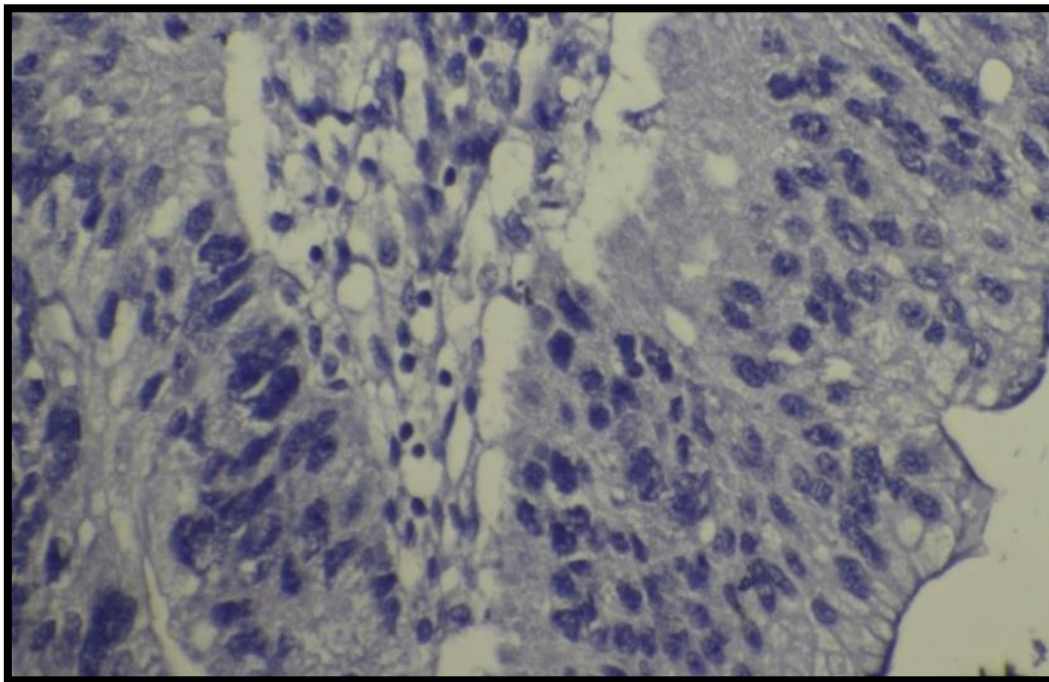
Figure 2-Section in intestinal subtype of GC (negative control; no colored malignant cells) (IHC, 40X).

The results of the IHC assessment of MUC5AC protein according to scoring guidelines proposed by Kageyama-Yahara *et al*. [8] Score 0: 0%, Score 1: 1-9%, Score 2: 10-50%, Score 3: $\geq$ 51% tumor cells expressing MUC5AC protein were positive.   Expression MUC5AC protein as analyzed statistically in relation to Clinic pathological parameters of GC were evaluated by Fisher's exact probability test, Student t-test. Values were considered statistically significant when $p<0.05$.
**Results**

A total of 60 cases were studied, the control group included thirty of GN cases, the patients' age ranged of between 33-48 years old, and the mean age was 39.27±4.266 years. Thirteen of the patients were males while the other seventeen were females. The carcinoma group included thirty patient of GC, the patients' age ranged between 18-76 years old, and the mean age was 48.39 years. Twenty of the patients were males while the other ten were females. The diffuse subtype of GC was fourteen (46.6%), while intestinal and mixed subtypes represented the twenties (40%) and four cases (13.3%) respectively. In nineteen cases (63.3%) the tumor was moderately differentiated, the rest ten cases (33.3%), were poorly differentiated. However, the well-differentiated one was found in one case (3.3%) only in this work. We divided the cases into two groups a Based to the depth of invasion twenty-eight of cases (93.3%) were with serosal invasion While two cases (6.6%) were without serosal invasion. Twenty-five cases (83.3%) showed lymph node involvement. Only five cases (16.6%) showed no lymph node involvement. According to the TNM staging system, fourteen cases (46.6%) were in the early stage of carcinoma (IIA &IIB) and sixteen cases (53.3%) were in advanced stage of carcinoma (IIIA, IIIB &IV).

IHC expression of the MUC5AC protein:

IHC expression of the MUC5AC protein was significantly higher in control group than in carcinoma group (93.3% versus 66.6%). The difference in the distribution of MUC5AC protein expression among studied groups is significant (P<0.0001), Table-1, Figures-(3, 4 & 5).

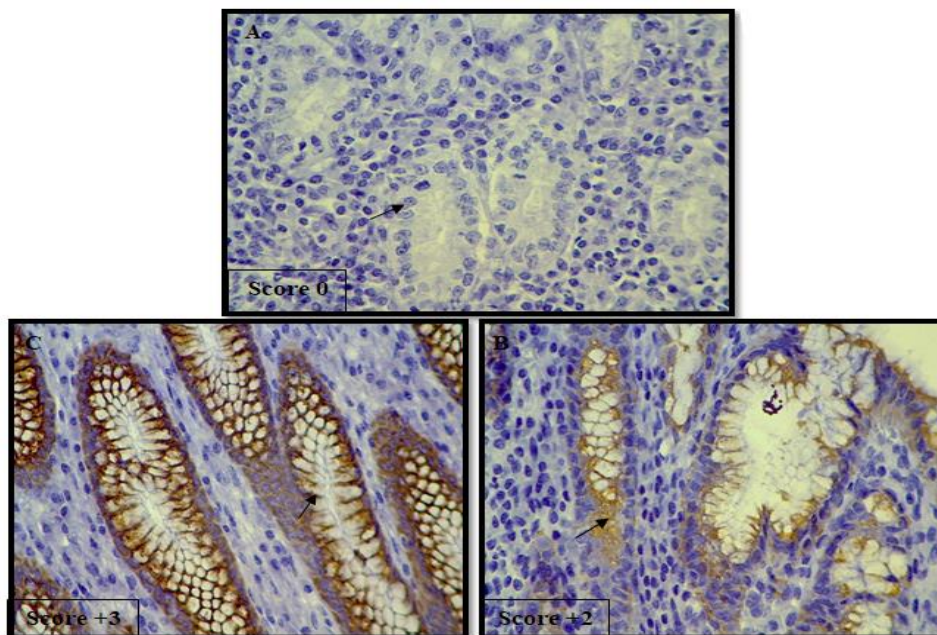**Table 1-**Frequency distribution of IHC expression of MUC5AC protein in control and carcinoma groups

| studied group	MUC5AC2 expression score				expression
	0	+1	+2	+3	
Control cases	2(6.6%)	0(0%)	2(6.6%)	26(86.6%)	28(93.3%)
HGC cases	10(33.3%)	5(16.6%)	7(23.3%)	8(26.6%)	20(66.6%)
Total	12(20%)	5(8.3%)	9(15%)	34(56.6%)	48(80%)
p-value	**P= 0.0001**				
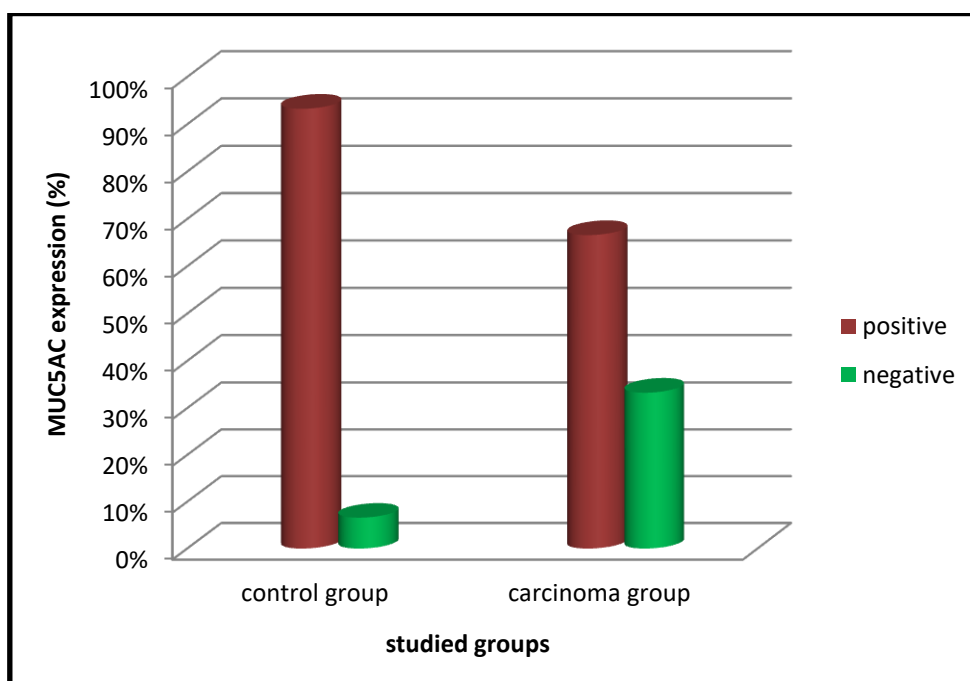
Figure 3-IHC expression of MUC5AC protein in control and carcinoma groups

Figure 4-section in the normal stomach tissue, showing: A: negative expression of MUC5AC (Score o), B, C: positive expression of MUC5AC as brown stained cytoplasm (Score +2 &+3 respectively; the black Arrow) (IHC, 40X)
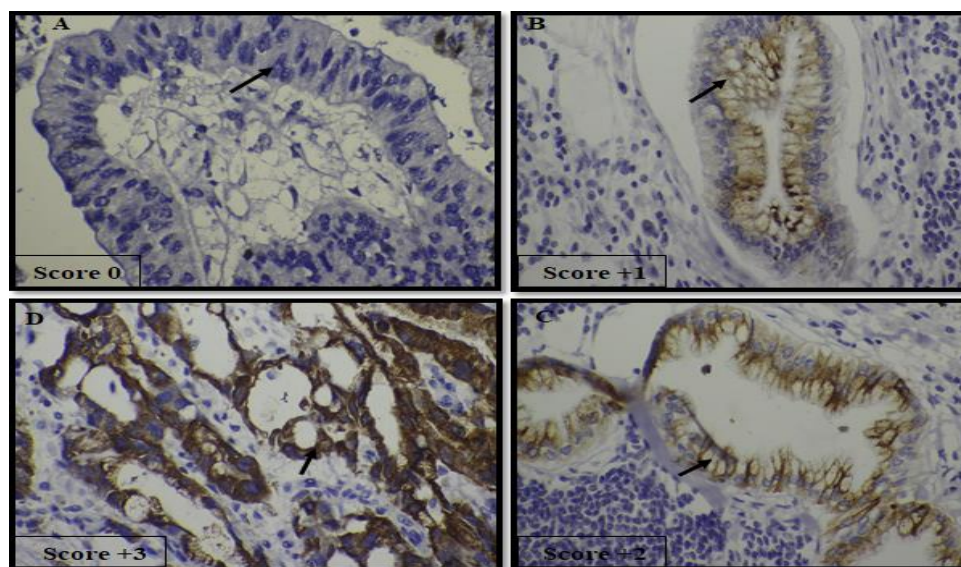
Figure 5-Section of the well to moderate differentiated intestinal subtype of GC showing, A: negative expression of MUC5AC (Score o), B, C &D: positive expression of MUC5AC as brown stained cytoplasm (Score +1, +2 &+3 respectively; the black Arrow) (IHC, 40X)

Correlation of IHC expression of MUC5AC protein used with different clinicopathological parameters in GC are summarized in Table- 2.Out of twenty males, fourteen (46.6%) males and out of ten females, six (20%) females were shown MUC5AC positivity. The highest percentage of the IHC expression of MUC5AC protein was 40% in the age of ≥48 and the lowest percentage was 26.6% in the patient at age <48 years, however, the p-value did not reach the statistical significant correlation between the MUC5AC protein and age and gender of the studied cases As shown in Table-2. The histopathological subtype of the tumor showed that percentage of IHC positively expression for MUC5AC protein was nine (30%) in the intestinal subtype of GC, and seven (23.3%) in the diffuse subtype of GC. While in the mixed subtype of GC it was four (13.3%). There was no statistically significant difference between the MUC5AC protein positivity & all the histopathological subtype. As shown in Table-2. Fourteen (46.6%) cases with moderately differentiated grade were shown positivity

expression for MUC5AC protein, and five cases (16.6%) were shown immunoreactivity for MUC5AC with poorly differentiated, while only one case (3.3%) was shown MUC5AC protein positivity expression, this positivity had statically not significant with the grading in this work as shown in Table- 2. According to the serosal invasion of the tumor, there were nineteen cases (63.3%) positive for MUC5AC out of twenty-eight of serosal invasion cases, while only one case (3.3%) out of two without serosal invasion cases were MUC5AC positive, There was no statistically significant correlation seen between MUC5AC and the depth invasion of tumor As shown in Table-2. In consideration to the lymph node involvement, out of twenty-five cases of lymph node involvement, sixteen (53.3%) cases of them showed positive expression for MUC5AC, while out of five cases of no lymph node involvement, four cases of them (13.3%) showed positive expression for MUC5AC. These results were statistically significant, as shown in Table- 2. Regarding the relationship between GC cases and stage of tumor, out of fourteen cases of the GC falling in early stages (II) eight (63.3%) cases of them showed positive expression for MUC5AC, while twelve cases falling in advanced stage showed positive expression for MUC5AC. these results were no significant correlation with TNM stage, as shown in Table-2

Table 2-Correlation of IHC expression of MUC5AC protein with different clinicopathological parameters in GC

Clinic pathological characteristic		MUC5AC protein expression		p-value
Age	>48	positive	8(26.6%)	P= 0.47$^{N.S}$
		negative	6(20%)	
	≤48	positive	12(40%)	
		negative	4(13.3%)	
Gander	Male	positive	14(46.6%)	P=0.79$^{N.S}$
		negative	6(20%)	
	female	positive	6(20%)	
		negative	4(13.3%)	
Subtype of tumor	intestinal	positive	9(30%)	P=0.22$^{N.S}$
		negative	3(10%)	
	diffuse	positive	7(23.3%)	
		negative	7(23.3%)	
	mixed	positive	4(13.3%)	
		negative	0(0%)	
Tumor grade	Well differentiated	positive	1(%3.3)	P= 0.54$^{N.S.}$
		negative	0(0%)	
	Moderate differentiated	positive	14(46.6%)	
		negative	5(16.6%)	
	poorly differentiated	positive	5(16.6%)	
		negative	5(16.6%)	
Tumor invasion	pT2	positive	1(%3.3)	
		negative	1(%3.3)	
	pT3&T4	positive	19(63.3%)	

		negative	9(30%)	P= 0.79[N.S.]
Involvement of LN	N0	positive	4(13.3%)	**P=0.02**
		negative	1(3.3%)	
	N 1,2,&3	positive	16(53.3%)	
		negative	9(30%)	
TNM stage	II	positive	8(26.6%)	P=0.59[N.S.]
		negative	6(20%)	
	III&IV	positive	12(40%)	
		negative	4(13.3%)	

Discussion

GC is one of the carcinoma types with the highest incidence and related mortality. GC is the fifth most common cancer in the world, and it is the third leading cause of cancer death in the world, [9]. There is a large geographic difference in the distribution of GC worldwide [1]. In our study, the mean age was 48.9 years as shown by many other Iraqi studies [10]. Also which is comparable with other studies where they found the peak incidence was in the fifth to sixth decades [11]. In this study 66.6% patients were male and 33.3% patients were female with M: F ratio 2:1, these findings are in agreement with other Iraqi and abroad studies [12, 13]. In the present study, the diffuse subtype was the most common histopathological subtype of the GC, this is similar to other Iraqi and abroad findings [13, 14] while Badary *et al*. [15] found that the intestinal subtype was higher than other subtypes of the HGC. This discordance could be attributed to environmental, racial and geographical differences, in addition to sample size difference. In the current study noticed that Most of the cases of GC were moderately differentiated which is in accordance with other Iraqi studies and those from other countries [16, 17], but some studies have found discordant results to the current study, for example Gharsall *et al*. [18] and He *et al*. [19] that found poorly differentiated was the most common grade. According to TNM staging system, the majority of carcinoma cases were of stage III&IV These findings agree with that obtained by other authors [20, 21]. This presentation (in stag III&IV) partly resulted from the lack of screening programmers for GC. Regarding the IHC expression of MUC5AC, IHC staining was performed on thirty pairs of normal and GC tissue samples to analyze the difference in IHG expression of the MUC5AC. The proportion of IHC expression score was higher in the normal tissues than in the GC tissues (normal vs cancer 93.3% vs. 66.6%; p<0.0001). This result is comparable to the other literatures which stated that the IHC expression of the MUC5AC was strong in the foveolar epithelium of the normal stomach than in GC [22]. In the present study, no significant correlation was found between IHC expression of MUC5AC protein and, age, gender, which is in accordance with results obtained by other authors [23]. And the current one although Kim *et al*. [24], who found a significant correlation between MUC5AC positivity and gender of the patients. In the current study no significant correlation was found between the IHC expression of MUC5AC and histopathologial subtype, this is concordant with the finding obtained by Leteurtre *et al.* [25] and Lazăr *et al*. [26], our results were different from those of Gürbüz *et al*. [27] and Pinto-De-Sousa *et al*.[28], where they found statistical correlation between the IHC expression of MUC5AC and histopathological subtype of tumor. Significant correlation was found between the MUC5AC expression and lymph node involvement. Regarding other parameters of HGC including grade, serosal invasion and TNM system of tumor, the current work revealed no significant correlation with MUC5AC expression this is similar to the findings of other authors [28, 29] the different results were found from other studies done by Kim *et al*. [7] who found significant correlation between MUC5AC and depth on invasion, grade, lymph node metastasis stage of tumor. Such discordant results may be due to many factors, including differences in antibody types, fixation, detection methods, and numbers of patients included, different staging systems or small groups in different stages have been used, even variations in statistical analyses in addition to racial and geographical factors.

Conclusion

We recorded that decrease and increase of IHC expression of the HGC was a closely associated factor with GC and lymph node involvement respectively. These results suggested that MUC5AC can be utilized as ancillary marker for diagnosis the infected lymph node and malignant transformation of

HGC but it seemed to have no distinct role in predicting grade and stage outcomes in patients with GC.

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