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Epstein Barr Virus and P53 Gene Expression Correlation with Gastric Adenocarcinoma Patients in Baghdad City

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Abstract: Gastric carcinoma is the most prevalent cancer related deaths worldwide today . The current study was carried out to shed the light on Epstein Barr virus, Tumor suppressor gene p53 associated gastric cancer of some Iraqi patients. According to insitu hybridization technique , this study was determined EBV and p53 gene correlates with some aspects of gastric cancer patients like age, gender , histological grade and stage of the tumor . Fourty six biopsies were obtained from patients with adenocarcinoma who had undergone gastrectomy. The tissue sections were collected during the period between September 2010 until June 2013. Epstein Barr virus was detected insitu hybridization in 18 patients (39%) out of 46. Whereas, the positive results of relation between p53 oncogene and gastric adenocarcinoma patients were detected in 30 (65.2%) out of 46. Out of 46 patients , this study included 32 male and 14 female with mean age 54 ranged between 30-72 years. The histological types included 18 well, 4 moderate and 24 poor differentiated respectively . Most of cases 32 (69.5%) falling in stage I-II and the remaining 14 were in the stage III and IV. The positive results revealed that EBV correlate in highly significant association with each of age, gender, grade and stage of the tumor , also expression of tumor suppressor gene p53 was correlation with all patients aspects in highly significant association.

Key words: gastric cancer, Epstein Barr virus, p53 gene expression, Insitu hybridization.

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Introduction

The one of the most occur cancers worldwide is gastric cancer, it is a major cause of cancer related death , which affects about one million people per year, with less than 30% of five years survival rate.(1,2,3). Gastric cancer incidence and peaks people with seventh decade of life , but rarely occurs before 40 years of age(4). More than 80% of stomach tumor are malignancies, it is refers any cancer arising on any part of stomach. The most common type is adenocarcinoma which comprises 95% of all malignancies (5,6).

In Asian countries gastric cancer remains the most common type of

carcinoma, it is involving both of inherited predisposition and environmental effect, including diet, infection of *Helicobacter pylori* and previous gastric surgery(7,8).

Epstein Barr virus (EBV) is an ubiquitous Herpesvirus , which can infects more than 90% of human worldwide with oncogenic activity and asymptomatic outcome in the most of infected population (9,10). It can be detected the EBV genome in lymphoid and epithelial cell origin of malignancies (11).

The first report of the association between EBV and gastric carcinoma was observed in 1990(12). Many researchers reported that EBV plays an important role in gastric cancer, by presence the virus in almost all tumor cells and viral genome monoclonality (13).

In gastric cancer, several abnormalities at oncogenes and tumor suppressor genes were identified. The most common mutated gene in human cancers is p53 tumor suppressor gene, which mutated more than 67.9% in gastric carcinoma (14,15).

In tumor developing, p53 inactivation is a process of accumulation its genetic abnormalities (15). Inactivation or mutation of p53 suppressor gene make a defect of DNA replication and malignant transformation (16). Two step mechanisms including in an inactivation of p53, one allele completely inactivated by mutation and the other one lost the copy by progress called 17p lost of heterozygosity (LOH). Finally results, complete loss of p53 function (17,18).

The aim of this study was to determine the Insitu hybridization expression for each of Epstein Barr virus and tumor suppressor gene p53 in gastric adenocarcinoma patients and to investigate the relationship between these markers with different clinicopathological aspects like age, gender, tumor grade and stage of all cases.

Materials and Methods

The current study included fourty six gastric adenocarcinoma patients with mean age 54 years ranged between (30-72) years, male-female ratio 2.2:1 with men 32 and 14 women. Whom already undergone surgical operation in Baghdad Medical City between the period of September 2010 until June 2013. No one of those patients had received any anticancer therapy before the surgery. The paraffin embedded tissue blocks were collected randomly from Teaching Laboratories/ Medical City in Baghdad which is already diagnosed of adenocarcinoma of gastric cancer by specialist. Compared with 18 apparently healthy control where there age and sex were matched to patients group.

Epstein Barr virus and tumor suppressor gene p53 were determined in specimens using Insitu hybridization method and performed as recommended in leaflet with kits.

- DNA probe Hybridization/ Detection System: Highly sensitivity Insitu kit. A complete hybridization and immunodetection system were purchased from Maxim Biotech, USA. Cat. No. (IH-60001), (IHD-OOSO).
- DNA probe Hybridization / Detection System in ultra sensitivity Insitu hybridization and immunodetection system were purchased from Maxim Biotech,

USA Cat No. (IH-60004), (IHD-OOBV).
- Mouse anti Human p53 (tumor suppressor protein, oncogene protein) from US Biological code (p1001-32C) for (ISH).

Insitu Hybridization Method

There are three steps of ISH for detection of EBV and P53 gene in gastric carcinoma patients.

- Prehybridization : the tissue sections were cut 4 µm , all of samples were deparaffinized and dewaxed in xylene, series of (100,90,70%)ethanol and D.W. respectively, immersed in pre heated (98 Cº) citrate buffer (pH:6). Tissue deprotinization performed by placing it in proteinase K solution, dehydration were done by immersing the slides in D.W. then (70,90,100%)ethanol.
- Hybridization step done by added EBV/p53 probe into each sections , denature the DNA probe by placed it in oven 98 Cº then removed from oven and incubated at room temperature over night to allow hybridization of probe with target nucleic acid.
- Post hybridization step: using protein block buffer to fall off the coverslip, conjugate were placed onto sections , substrate used , counterstained by Nuclear Fast Red (NFR), dehydration by 90,100% ethanol , xylene and finally mounted with DPX.

Statistical Analysis

The statistical analysis system SAS was used to effect of different factors in study parameters. Chi square test was used to significant compare between percentage in this study (19).

Results

A series of fourty six paraffine embedded tissue blocks of gastric adenocarcinoma patients that included on this study were studied by using insitu hybridization method to detection for Epstein Barr virus and tumor suppressor gene p53.

The current results showed that patients ages ranged between 30-72 years with mean age 54 years. Male to female ratio was 2.2:1 with 32 men and 14 women. Regarding the TNM staging system , most of gastric carcinoma cases (69.5%) 32 out of 46 fall in early stages I_II of disease, and according to the grade of the tumor , the majority of our cases were poorly differentiated type 24(52.1%), whereas, 18(39.1%) well differentiated and 4(8.6%) moderately differentiated type.

-EBV correlated gastric adenocarcinoma patients regarding to their age, gender, tumor grade and histological type. Using ISH technique .

EBV correlate with gastric cancer in 18 (39.1%) out of 46 patients. Eighteen cases were equal or below the age of 50 years, 7 cases of them showed positive EBV expression, whereas, 28 patients were above 50 years of old. Eleven patients were expressed positive EBV results (Figure 1).

In (Figure 2), the results revealed that 14 out of 22 patients were male that showed positive EBV correlation, while, 4 out of 14 cases were female that showed positive results.

Out of 24 cases of poorly differentiated gastric adenocarcinoma ,8 cases were positive EBV correlation , 4 patients of moderately differentiated gastric cancer,

2 of them showed positive EBV association , and out of 18 well differentiated gastric cancer, 8 of them were expressed positive results of EBV,(Figure 3).

According to the correlation between EBV and the stage of gastric carcinoma, and as shown in (Figure 4), out of 32 cases falling in the I and II stages, 12 sample were showed positive expression of EBV, whereas, out of 14 cases falling in stage III and IV, 6 out of them were showed positive expression.

As shown in table 1, there were highly significant association between EBV with all clinicopathological aspects of these patients age ($p = 0.0063$), gender ($p = 0.0025$), tumor grade ($p = 0.0014$) and stage of the tumor ($p = 0.0018$) respectively at $p < 0.01$.

-Tumor suppressor gene p53 correlated with gastric cancer patients according to their clinical aspects.

The results showed p53 correlated with 30 (65.2%) out of 46 patients of gastric cancer.

In the age equal 50 years or below, 11 cases out of 18 showed positive p53 expression, whereas, 19 out of 28 patients above 50 years were expressed positive p53 results, (Figure 5).

In (Figure 6), the 20 male out of 32 were showed positive p53 expression, while, 10 female out of 14 were showed positive p53 expression.

Fifteen out of 24 of poorly differentiated type, 3 out of 4 moderately type, and 12 out of 18 well differentiated gastric adenocarcinoma cases were positive p53 expression as in (Figure 7).

Regarding the association between p53 and the stage of gastric cancer patients , and as seen in (Figure 8), twenty patients out of 32 cases falling in the I and II stages have been shown positive expression of p53 , furthermore, 10 out of 14 cases falling in III-IV stages, have been shown positive expression of p53.

As shown in (table 2), there were highly significant correlation between p53 expression with each of age ($p = 0.0067$), gender ($p = 0.0024$) , grade($p = 0.0128$) and the stage ($p = 0.0024$) of gastric cancer patients respectively at $p< 0.01$.

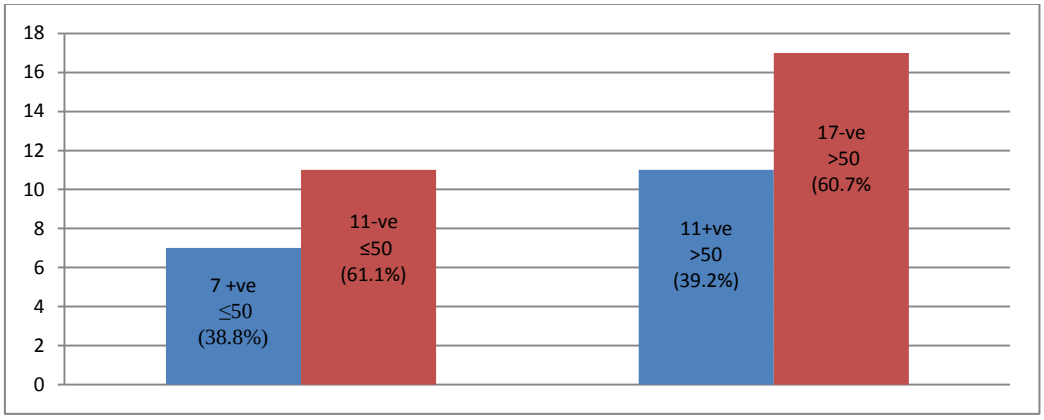

Figure 1 : Distribution of EBV according to the age of gastric cancer patients

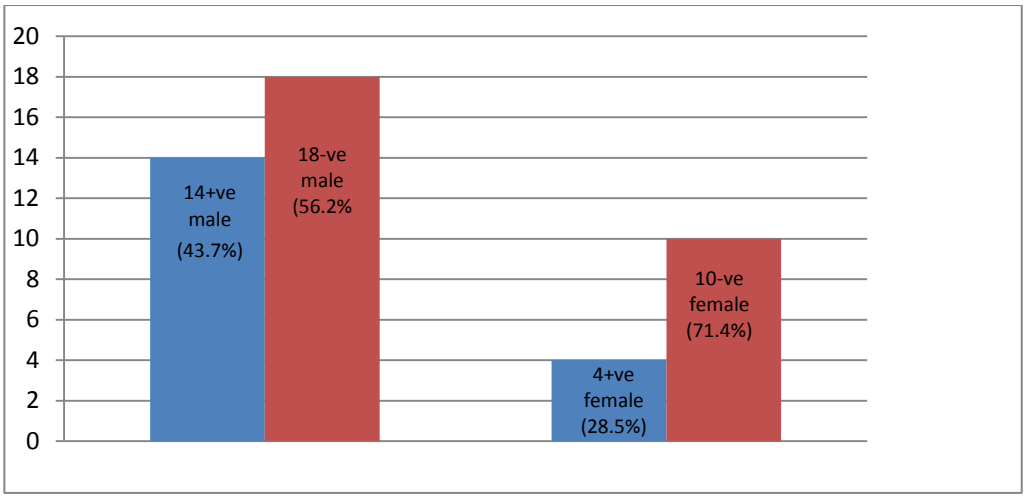

Figure 2: Distribution of EBV according to the gender of gastric patients

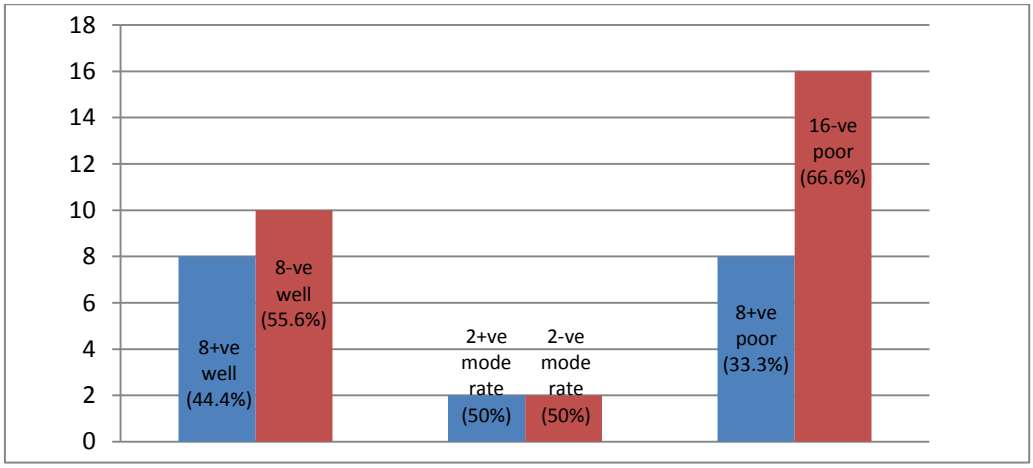

Figure 3: Distribution of EBV according to the tumor grade of gastric cancer cases

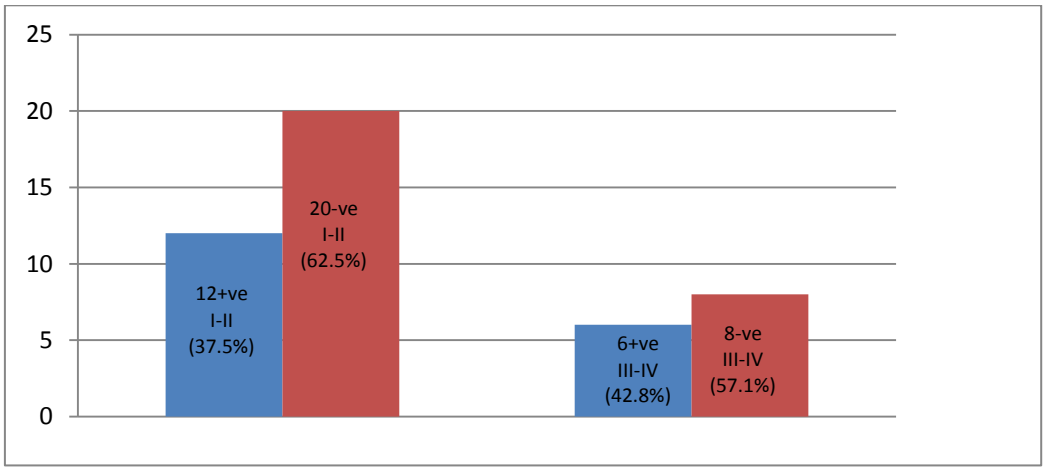

Figure 4: Distribution of EBV according to the stage of the tumor of gastric patients

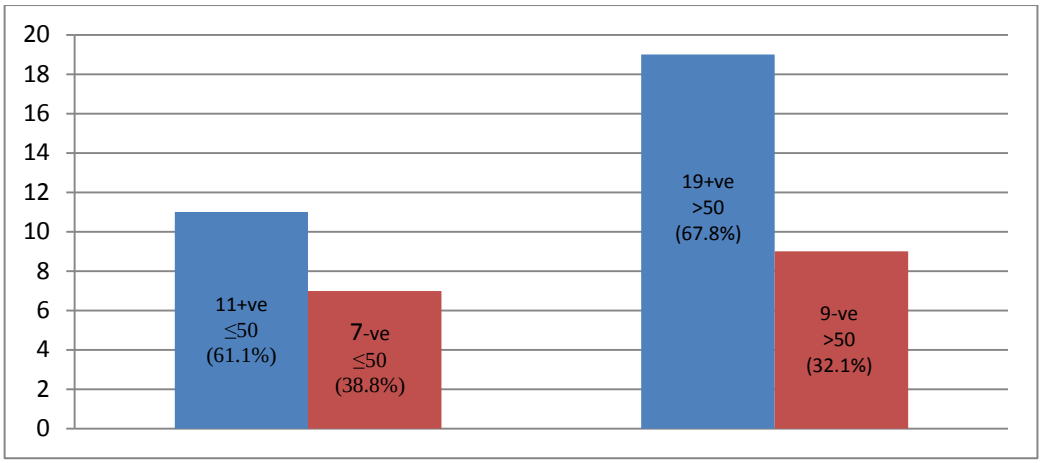

Figure 5: Distribution of P53 expression according to the age of patients

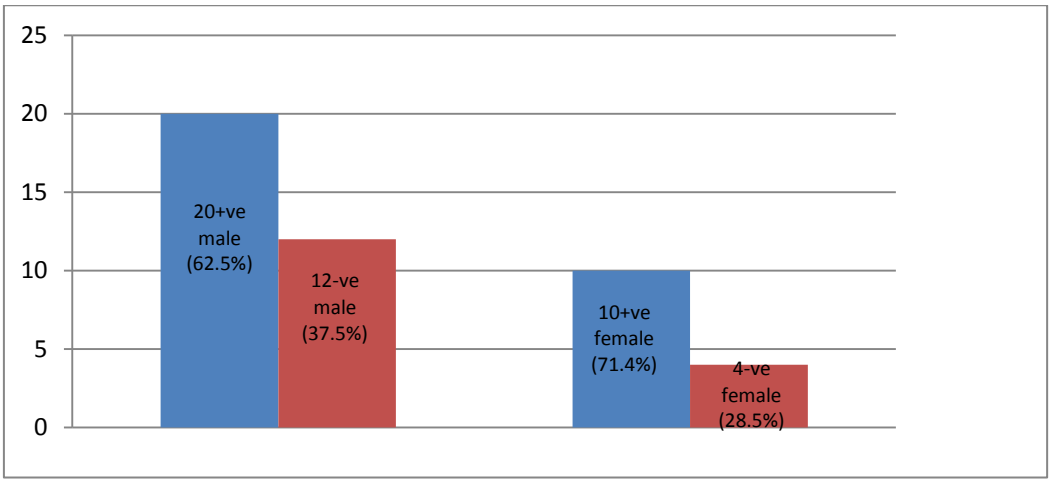

Figure 6: Distribution of p53 gene expression according to the patients gender

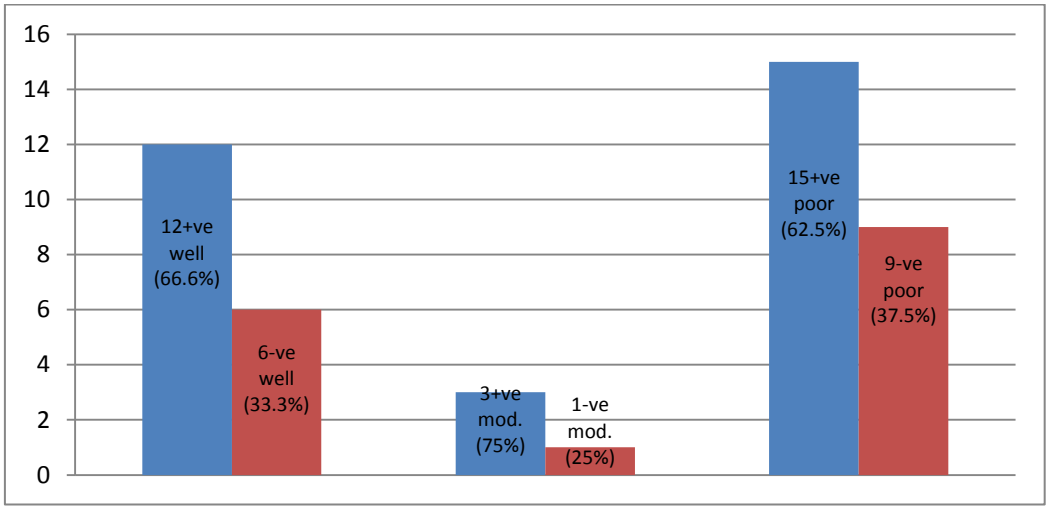

Figure 7: Distribution of p53 gene according to the tumor grade of disease

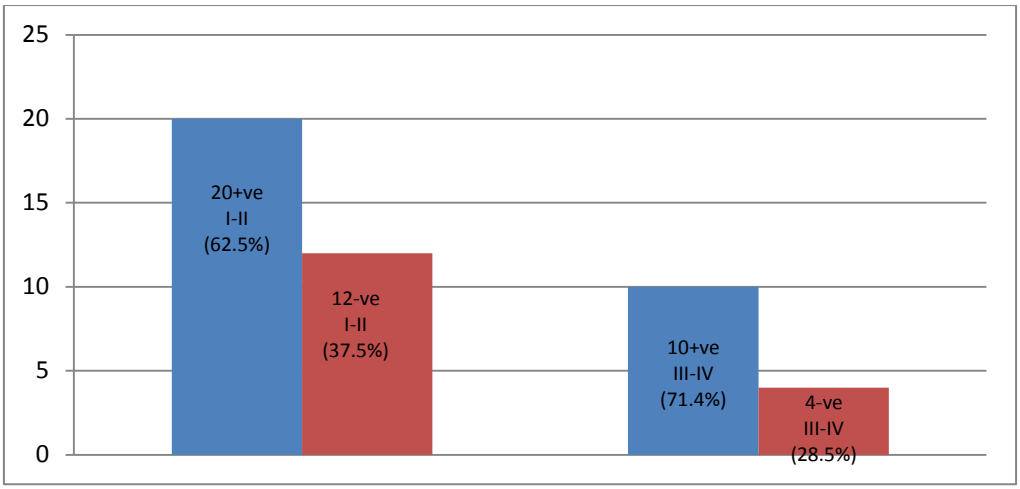

Figure 8: Distribution of p53 gene expression according to the stage of the tumor

Table 1: The distribution of gastric adenocarcinoma patients regarding their age, sex, grade and histological stage in relation with Epstein Barr virus (EBV)

The factor	EBV positive Total No.= 18 (39.1%)	EBV Negative Total No. = 28 (60.8%)	P- value X^2 - value
Age			P = 0.0063
≤ 50	7(38.8%)	11(61.1%)	X^2=9.48 **
> 50	11(39.2%)	17(60.7%)	
Gender			P = 0.0025
Male	14(43.7%)	18(56.2%)	X^2=8.94**
Female	4(28.5%)	10(71.4%)	
Tumor grade			P = 0.0014
Well	8(44.4%)	10(55.6%)	X^2=11.72**
Moderate	2(50%)	2(50%)	
Poor	8(33.3%)	10(66.6%)	
Tumor stage			P = 0.0018
I-II	12(37.5%)	20(62.5%)	X^2=10.33**
III-IV	6(42.8%)	8(57.1%)	

**Highly significant association at p< 0.01

Table 2: The distribution of gastric adenocarcinoma patients regarding their age, sex, grade and histological stage in relation with expression of p53 tumor suppressor gene

The factor	P53 positive Total No.= 30 (65.2%)	P53 Negative Total No. = 16 (34.7%)	P- value X^2 - value
Age			P = 0.0067
≤ 50	11(61.1%)	7(38.8%)	X^2=10.64**
> 50	19(67.8%)	9(32.1%)	
Gender			P = 0.0024
Male	20(62.5%)	12(37.5%)	X^2=12.59**
Female	10(71.4%)	4(28.5%)	
Tumor grade			P = 0.0128
Well	12(66.6%)	6(33.3%)	X^2=9.83**
Moderate	3(75%)	1(25%)	
Poor	15(62.5%)	9(37.5%)	
Tumor stage			P = 0.0024
I-II	20(62.5%)	12(37.5%)	X^2=12.59**
III-IV	10(71.4%)	4(28.5%)	

**Highly significant association at p< 0.01

Discussion

Worldwide , gastric cancer is the one of the most common cancers affected more than one million people per year (1, 3), and still considered as a big problem leading second cause of death from cancer (8,20).

The common age of infected people with gastric cancer is rare before the age of 40 years, increase and peaks in seventh decade of patients life (4).The current results compatible with that, the mean age of studied cases 54 years, ranged between 30- 72 years , most of them (60.8%) in age above 50 years. Also our results in agreement with (21) who reported that majority of their patient age (70%) above 50 years old and the age ranged between 30-80 years.

Expression rate of gender revealed the higher in male 32 compared with female 14, the ratio 2.2:1, which was approach to other reporters studies (21,22,23) whom found male : female ratio 2.3:1 with 28 men and 12 women.

In this study , poor differentiated carcinoma was the major type 52% of cases, weel differentiated 39% and moderately differentiated were 8.6%, furthermore, the cases were highly incidence falling in early stage I-II (69.5%), whereas, in lower incidence at stage III-IV(30.4%), current results disagreed with (21) who reported the moderately differentiated was the major type (62%) followed by poorly differentiated (38%), also in contrast of that as a TNM staging system , which reported that the majority of their cases were falling into stage III of the tumor (92.5%).

Epstein Barr virus plays an important role in gastric cancers, because of the viral presence almost in all of tumor cells (13). It suggested that increasing events of subset of gastric carcinoma is associated with infection by EBV (12). In the present study, and by using ISH technique, EBV positive expression detected in (39%) of gastric cancer patients. It was disagreed with (25) who reported the positive EBV were 6.9% by using ISH.. The study showed there were highly significant association between EBV infection and patients age, and with highly incidence in older than 50 years , this finding results nearly to other previous study (24), who reported the association occur in age from 54 to 78 years. There were highly significant correlation between EBV and the gender of the studied group and most commonly occur in men, this finding approach to other reporters (26,27) whom found the predominance of positive EBV with male gastric patients with advanced age by using the same ISH technique. Also in agreement with (24) who found a statistically significant correlation between EBV and the gender , in contrast with (28) study , who reported nonsignificant association with gender.

Regarding the staging and grading system, there were highly significant correlation between EBV with stage and grade of the tumor and the predominance positive cases falling in early stage and poorly differentiated, this finding compatible with (24) which 56% of their patients falling in stage I-II disease and the remaining 44% in the stage III-IV. But disagreed with others (29) who observed the most of EBV positive cases were in the III-IV stages, also there were no significant relation found between EBV- ISH and the grade (moderately and poorly) differentiated tumor.

Tumor suppressor gene p53 indicated to be the most common mutated in different human cancers including gastric cancer which acts as a dominant oncogene (30). The half life of wild type p53 is short, while the other mutant forms half life is prolonged (31).

Results showed that 65.2% of gastric cancer cases were positive p53 expression, this finding was consist with (32,33) and nearly to the (34) which found that 62%, 66% and 48.2% of gastric carcinoma were positive expression of p53.

According to the age and gender of the present study, there were statistically highly significant correlation between p53 and each of age and gender, this finding disagree with previous studies (21,35) who reported that there were no statistically significant differences in relation between expression of p53 with age and gender of patients.

Regarding the grade and stage of the tumor, statistically , there were highly significant association between p53 expression and each of grade and stage with expressed predominance p53 ISH positive cases in early stage I-II with poorly differentiated type. This finding consist with (34) who found there was statistically significant correlation between p53 expression and tumor grade, in contrast with (21,34,36) whom found no statistically significant relationship between p53 with tumor stage and grade and most of cases falling in stage III. Some Iraqi studies compatible with our results, p53 positive was higher in poor differentiated, then in moderate and well differentiated gastric carcinoma (23).

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