Expression of P21 Protein in Gastric Mucosa of Patients with Gastric Carcinoma and Its Correlation with the Disease Progression

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Abstract: Gastric cancer is the second most common cause of cancer-related death in the world. It exists as two main histological types, diffuse and intestinal, and is thought to result from a combination of environmental factors and accumulation of specific genetic alterations, and consequently mainly affects older patients Esophagogastro-duodenoscopy (EGD) is the diagnostic imaging procedure of choice in the work-up of gastric carcinoma p21 negative regulator of the cell cycle are known as a marker of some malignant diseases. However the clinical importance of p21 has not been clarified also p53 has been studied by many investigators.This study aimed to evaluate expression of P21 proteins in patient with gastric carcinoma and its relation with the histopathological activity, laboratory and clinical parameters and to study its correlation with the disease progression. The present study was conducted on eighty (80) patients attending the out-patient clinics of El-Hussein and El- Sayed Galal University Hospitals during the period (from November 2010 to July 2013). According to the histopathological diagnosis, these cases were subdivided into 6 groups after doing the following (1-Medical history 2-Clinical evaluation 3-Laboratory investigations including (C.B.C, Liver function test, gastric biopsy, histological examination and immunohistochemical examination. The study revealed p21 was detected in normal cases and mean of p21 compared with different gastric lesions showed that gradually decreased from more pathological stage gastritis→metapalsia→dyplasia→adenoma→ and gastric carcinoma .There was a highly significant value of malignant group when compared to control group, gastritis group, metaplasia group and dysplasia group at $p<0.01$. Conclusion: The study revealed that Down-regulation of p21 from normal gastric mucosa to different gastric lesion suggests that p21 expression correlated with disease progression.

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1. Introduction

Gastric cancer is the second most common cause of cancer-related death in the world. It exists as two main histological types, diffuse and intestinal, and is thought to result from a combination of environmental factors and accumulation of specific genetic alterations, and consequently mainly affects older patients.

The steady decline in the incidence and mortality of stomach cancer in most affluent countries has been attributed to changes in dietary pattern, food storage, and control of *H. pylori* infection. The incidence of gastric cancer varies in different parts of the world with highest incidence rates documented in Eastern Asia, Eastern Europe, and South America, while North America and Africa show the lowest recorded rates.

The pathogenesis of gastric cancer involves multiple risk factors including dietary, infectious, occupational, genetic and preneoplastic risk factors, most of which act on the gastric mucosal microenvironment over a prolonged time period. The diagnosis of gastric cancer is often delayed by the lack of early symptoms, with early gastric cancer causing non-specific gastrointestinal complaints, such as dyspepsia, in only 50% of patients. Up to 90% of Western gastric cancer patients first present with advanced carcinomas, which have more serious symptoms such as abdominal pain, bleeding, vomiting, or severe weight loss. Endoscopic screening is considered to be the most sensitive and specific diagnostic test for gastric cancer.

IHC is also widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue.. IHC assists the pathologists in areas of tumor classification, multilineage differentiation, molecular correlates, and infectious etiologies. Moreover, IHC is commonly used to detect markers, which in turn can provide information on the biological behaviour and prognosis of a tumor.

Aim of Work:

This study aimed to evaluate expression of P21 proteins in patient with gastric carcinoma and its relation with the histopathological activity, laboratory and clinical parameters, to study its correlation with the disease progression and to study the role of H pylori in

p21 expression in different gastric lesions.

2. Patients and Methods:

The present study was conducted on eighty (80) patients attending the out-patient clinics of El-Hussein and El- Sayed Galal University Hospitals during the period (from November 2010 to July 2013). The selected patients were (48) males and (32) females, their age ranged from (20-67years) with a mean of 43.5+4.5 years, as well as (10) subjects (normal individuals) serving as a control group. They were (4) males and (6) females, their age ranged from (26-53 years) with a mean of 34±3.5 years. According to the histopathological diagnosis, these cases were subdivided into 6 groups:

- ***Group I: 10 cases as normal control.***
- ***Group II: 20 cases as chronic gastritis.***
- ***Group III: 10 cases as metaplasia.***
- ***Group IV: 10 cases as dysplasia.***
- ***Group V:10 cases as adenoma.***
- ***Group VI: 20 cases as adenocarcinoma.***

The patients were included according to the following criteria:

Inclusion criteria:

- persistent upper gastrointestinal dyspepsia for more than 3 months
- Patients with *H pylori*.

Exclusion criteria:

- Patients receiving non–steroidal antinflammatory drugs.
- Who had taken antibiotics, bismuth or proton pump inhibitors within the past 6 weeks had undergone previous gastric surgery.
- With predominant symptoms of irritable bowel syndrome.
- Children and old age more than 70 years.
- Patients with chronic liver diseases and having esophageal varices.
- Patients refused to be including in our study.

All the studied subjects were subjected to:

1- Complete history taking and clinical assessment.

2- Laboratory evaluation including:

Complete blood picture, Serum bilirubin (conjugated & un conjugated), aminotransferases (ALT&AST) and alkaline phosphatase, Serum albumin, Prothrombin time, Hepatitis markers and Renal function tests:

3- Abdominal ultrasonography.

4- Upper gastrointestinal endoscopy:

Was performed using an Olympus XQ40 endoscope. Thorough endoscopic examination of the oesophagus, stomach and duodenum was performed, abnormalities were recorded and gastric biopsies were obtained from the apparent mucosal lesions.

(A) Histopathological Examination:

The fixed biopsy specimen was processed in ascending grades of ethyl alcohol, xylene and wax at 60°C. Paraffin sections 4µm thick were prepared on 3 aminopropyltriethoxy saline coated slides. Sections were then stained with hematoxylin and eosin for histological evaluation of the severity of gastritis and with Giemsa stain for *H. pylori* detection.

(B): Immunohistochemical staining

3.Results

The results are sown in the following Tables.

Table (1): Comparison between control and gastritis cases regarding p21 expression.

Histopathological groups	N.	P21 Mean ±S.D
Control	10	78.75 ±2.80
Gastritis	20	56.88 ±11.92*

**p<0.05 compared to control group*

This table show that the number of p21 positive mucosal cells decreased in gastritis cases compared to controlled cases with statistical significant difference at ***p*<0.05**

Table (2): Comparison between control and adenoma cases regarding p21 expression.

Histopathological groups	N.	P21 Mean ±S.D
Control	10	78.75 ±2.80
Adenoma	10	28.83±13.36*

**p<0.05 compared to control group*

This table show that that the number of p21 positive mucosal cells decreased in adenoma cases compared to control cases with statistical significant difference at ***p*<0.05.**

Table (3): Comparison between control, metaplasia and dysplasia cases regarding p21 expression.

Histopathological groups	N.	P21 Mean ±S.D
Control	10	78.75 ±2.80
Metaplasia	10	41.67 ±9.264*
Dysplasia	10	32.42±5.68**

*p<0.05 compared to control group; ** p <0.01 compared to control group

This table show that the number of p21 positive mucosal cells decreased in **metaplasia** cases and dysplasia compared to **control** cases with statistical significant difference at p **<0.05** and highly significant p **<0.01** respectively .

Table (4): Comparison between gastritis, metaplasia and dysplasia cases regarding p21 expression.

Histopathological groups	N.	P21 Mean ±S.D
Gastritis	20	56.88 ±11.92
Metaplasia	10	41.67 ±9.264*
Dysplasia	10	32.42±5.68**

* p <0.01 compared to gastritis group; ** p <0.01 compared to gastritis group

This table show that the number of p21 positive mucosal cells increased in **gastritis** cases compared to metaplasia and dysplasia cases with statistical highly significant difference at p <0.01 .

Table (5): Comparison between adenoma and carcinoma cases regarding p21 expression.

Histopathological groups	N.	P21 Mean ±S.D
Adenoma	10	28.83±13.36
Malignant	20	19.34±9.670*

* p <0.01 compared to adenoma group.

This table show that the number of p21 positive mucosal cells increased in **adenoma** cases compared to **malignant** cases with statistical highly significant difference at **p<0.01 .**

Table (6): Results of p21 expression in the studied groups

Histopathological groups	N.	P21 Mean ±S.D
Control	10	78.75 ±2.80
Gastritis	20	50.88 ±11.92*
Metaplasia	10	41.67 ±9.264**
Dysplasia	10	32.42±5.68**^$
Adenoma	10	28.83±13.36**^$#
Malignant	20	19.34±9.670**^$#µ

* p <0.05 compared to control group
** p <0.01 compared to control group
^ p <0.01 compared to gastritis group
$ p <0.01 compared to metaplasia group
# p <0.01 compared to dysplasia group
µ p <0.01 compared to adenoma group

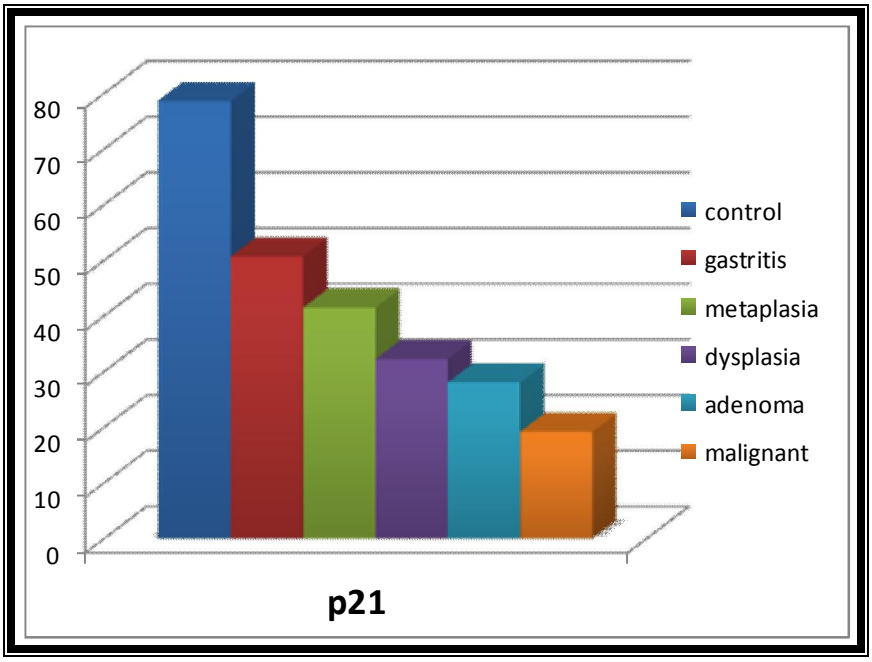

Figure (6): Results of p21 expression in the studied groups

- There was a significant value of gastritis group when compared to control group at p **<0.05.**
- There was a highly significant value of metaplasia group when compared to control group and gastritis group at p **<0.01.**
- There was a highly significant value of dysplasia group when compared to control group and gastritis group at p **<0.01.**
- There was a highly significant value of malignant group when compared to control group, gastritis group, metaplasia group and dysplasia group at p **<0.01.**

Table (7): correlation of h pylori and p21 expression in the studied groups

Hp immun		N.	Mean ±S.D
P21	negative	62	29.77±15.936
	positive	18	39.562±12.88

* $p < 0.05$ compared to negative group (Sig.)

By immunostain examination 22.5% (18/80) of the patients were found to be infected with *H.pylori* as follows: 0 case (0%) from the control group, 9 patients diagnosed as gastritis, 3 patients diagnosed as metaplasia, 2 patients diagnosed as dysplasia and 4 patients diagnosed as adenocarcinoma, while 77.5 %(62/80) were not found to be infected with *H.pylori*.

4. Discussion

Recently several regulators of cell cycle have been identified and have received much attention as prognostic factors in the evaluation of the malignancy potential of tumours, of these regulators p53, p21 negative regulator of the cell cycle are will known as a marker of some malignant diseases. However the clinical importance of p21 has not been clarified also p53 has been studied by many investigators. The expression of p21 gene is regulated by two pathways namely p53 independent and dependant mechanisms (Abbas T, *et al.,* 2008).

However since p21 is induced mainly by a p53 dependant pathway,p21 seems to directly reflect the growth suppressive function of p53 and would be a more reliable marker for the assessment of behavior of cancers than p53, to evaluate p21 as prognostic marker of gastric cancer, we studied its expression in relation to the clinico-pathologic parameters of the disease.

Epithelial cells exit the cell cycle as they move toward the mucosal surface in tne normal mucosa of the stomach. Consequently, these cells are fully differentiated and will be shed to the gastric lumen by renewal mechanisms (Barboza JA *et al.,* 2006).

In this study, p21 positive cells were detected in superficial areas of normal gastric mucosa. These findings support the view that p21 can be induced during differentiation, further-more p21 might be associated with the cellular senescence of non-neoplastic gastric mucosa.P21 functions has been studied by some groups in cultured cell line (Vasiliki Michalaki *et al.,*2012) . In irradiated cells,p21 regulates the cell cycle and arrests the cell at the G1 check-point .p21 positive cells proceeded to DNA repair or apoptosis in cell lines (Besson et al., 2008). Moreover, adirect effect of p21 as an inhibitor of cell proliferation has been demonstrated by its transient expression in tumor cells and in normal fibroblasts. However, p21 expression in gastric carcinoma in vivo has not sufficiently elucidate.

In our study we investigated p21 protein expression in gastric carcinomas. A total of (20) primary gastric carcinoma specimens were immunohistochemically stained for p21 protein expression. Correlations between p21 expression and clinicopathological features were examined. Loss of p21 expression was observed in 12 of 20 tumour tissues (60.4%), and the frequency of p21 loss increased as the stage progressed.

Also In our study, p21 was detected in normal cases and mean of p21 compared with different gastric lesions showed that gradually decreased from more pathological stage gastritis→metapalsia→dyplasia→adenoma→ and gastric carcinoma .There was a highly significant value of malignant group when compared to control group, gastritis group, metaplasia group and dysplasia group at $p<0.01$.

Our result agree with Wataru *et al.* (2005) reported that the expression of p21 was detected in 81 of 343 gastric adenocarcinoma (24%). also agree with Hee Kyung Ahn *et al.* (2011) reported that loss of p2 expression in 103 of 172(60%). so Our study and others suggest that cell cycle deregulation is an important factor in gastric cancer progression.

We compared the clinicopathologic features of patients with p21 –positive and negative primary tumors. Although there was no relationship between p21 expression and age, sex, and other clinical and laboratory. Our result agrees with Fei Liu, (2011). Moreover, the majority of the patients with p21-negative tumors had advanced lesions. And strong association with disease progression was also demonastrated.

In some study Chang *et al.* (2002) and Carbone (2007), decided p21 negative tumor significantly correlated with depth of invasion and lymph node metastasis. These results strongly suggest that the loss of p21 is secondary to disease progression, as cancer cells with high profilerative activity may have a higher potential for metastasis than those with low proliferative activity.

Immunoreactive p21 protein might be the wild, non mutated type, because a recent study failed to detect p21 gene mutations in a large series of various human tumours (Yefei Huang *et al.,* 2013).

Abbas *et al.* (2008) also reported the p21 is thought to play a central role in tumour suppressionExpression of p21 in the primary tumour was frequently lost in patients with either lymph node, liver or peritoneal metastases as compared with patients without metastases. In patients with p21-negative tumours, the risk of recurrence following curative surgery was significantly higher, and the prognosis was significantly poorer than in patients with p21-positive tumours. Loss of p21 expression in primary gastric carcinoma correlates with disease progression. The status of p21 expression may have prognostic value in this disease. primary tumour was frequently lost in patients with either lymph node, liver or peritoneal metastases as compared with patients without metastases. In patients with p21-negative tumours, the risk of recurrence following curative surgery was significantly higher, and the prognosis was significantly poorer than in patients with p21-positive tumours. Loss of p21 expression in primary gastric carcinoma correlates with disease progression. The status of p21 expression may have prognostic value in this disease.

Although further studies on a larger scale will be necessary to elucidate the relevance of p21 expression to the biological behavior of cancers, this study suggests that p21 expression correlated with disease progression and may have prognostic value in gastric carcinoma.

We have examined the relationship between *H. pylori* infection and p21 expression in different stages of disease progression from precursor lesions to gastric carcinoma 22.5% (18/80) of the patients were found to be infected with *H.pylori* as follows: 0 case (0%) from the control group, 9 patients diagnosed as gastritis, 3 patients diagnosed as metaplasia, 2 patients diagnosed as dysplasia and 4 patients diagnosed as adenocarcinoma, while 77.5 %(62/80) were not found to be infected with *H.pylori*.

Our results were similar to that previously reported Yefei Huang *et al.* (2013) *H. pylori* infection may increase expression of ras p21 proteins and induce p53 suppressor gene mutation early in the process of gastric carcinogenesis.

Conclusion

Down-regulation of p21 from normal gastric mucosa to different gastric lesion suggests that p21 expression correlated with disease progression and *H. pylori* infection may decrease expression of p21 proteins early in the process of gastric carcinogenesis.

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