

Biological Prognostic Parameters in Gastric Carcinomas

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Rezumat

Parametri biologici de prognostic în carcinoamele gastrice

Cancerul gastric este a doua cauză de mortalitate prin cancer la nivel mondial. Carcinogeneza gastrică implică o varietate de factori, inclusiv dieta, factori habituali, precum și factorii de mediu. Acest studiu urmărește să coreleze parametrii clinicopatologici ai cazurilor studiate și expresia PCNA și p53 detectată prin tehnici de imunohistochimie. Lotul de studiu a inclus un total de 32 de pacienți la care s-a efectuat gastrectomie pentru cancer gastric. Parametrii de studiu au fost reprezentați de aspecte epidemiologice (vârstă, sex), caracteristici clinice (semne și simptome), rezultate histopatologice (stadializarea pTNM și gradul de diferențiere, tipul histologic, statutul ganglionilor limfatici și prezența invaziei vasculare) și de supraviețuire și rezultatele imunohistochimice (expresia p53 și PCNA) a cazurilor din lotul de studiu. Studiul histopatologic a arătat că, cele mai multe cazuri (26 cazuri) au fost de tip intestinal și șase cazuri tipul difuz. Analiza imunohistochimică a expresiei proteice a p53 a arătat o medie de 20,75% celule pozitive, în timp ce în cazul PCNA media a fost de 47,3%. În termeni de supraviețuire au fost 6 cazuri de deces, la intervale variind între 2 și 189 zile, 5 cazuri au avut prezentări ulterioare în decurs de 12 luni, în timp ce 8 pacienți au fost pierduți din urmărire. La momentul intervenției chirurgicale, 6 pacienți au avut metastaze la distanță, în timp ce alți 6 le-au dezvoltat într-o perioadă de 2-12 luni după intervenția chirurgicală.

Identificarea biomoleculilor care evidențiază tumorile potențial agresiv poate ajuta în abordarea terapeutică după rezecția chirurgicală.

Cuvinte cheie: carcinom gastric, supraviețuire, markeri de prognostic

Abstract

Gastric cancer is the second leading cause of cancer mortality worldwide. (1) Gastric carcinogenesis involves a variety of factors including diet, habitual factors as well as environmental factors. (2,3) This study aimed to correlate clinicopathological parameters of the cases studied and PCNA and p53 expression using immunohistochemistry. The study group included a total of 32 patients that underwent gastrectomy for gastric cancer. The study parameters were represented by epidemiological aspects (age, sex), clinical characteristics (signs and symptoms), histopathological findings (pTNM staging and degree of differentiation, histological classification, lymph nodes status and presence of vascular invasion) and survival, and immunohistochemical analysis (p53 and PCNA expression) of the study group. Histopathological study showed that most of the cases (26 cases) were of the intestinal type and 6 cases of the diffuse type. Immunohistochemical analysis of p53 protein expression showed an average of 20.75% positive cells, while PCNA expression showed an average of 47.3%. In terms of survival there were 6 cases of death at intervals ranged from 2-189 days, 5 cases had subsequent presentations over 12 months, while 8 patients were lost to follow-up. At the time of surgery, 6 patients had distant metastases, while 6 more developed them in a period of 2-12 months after surgery. Identification of biomolecules that highlight potentially aggressive tumors may

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help modulate the therapeutic approach after surgical resection.

Key words: gastric carcinoma, survival, prognostic markers

Introduction

Gastric cancer is the fourth leading cause of cancer mortality worldwide. (1)

Gastric carcinogenesis involves a variety of factors including diet (excessive consumption of salt or nitrosamines or deficitary in vegetable fibres), habitual factors (smoking cigarettes) as well as environmental factors (infection with *Helicobacter Pylori*). (2,3)

The most common classification of gastric carcinoma is Lauren classification that divides gastric carcinoma in 2 categories: intestinal and diffuse type. (4) Intestinal type is preceded by a series of changes in the gastric mucosa as a response to environmental factors or HP infection, following a sequence of events from chronic gastritis, gastric atrophy, intestinal metaplasia, dysplasia, early carcinoma to invasive carcinoma and metastasis. (5)

Gastric cancer has a worse prognosis in patients of Asian origin versus Western countries, possibly due to ethnic differences, moment of diagnosis, extensive surgery or biomolecular characteristics of the lesion. (5,6,7)

Classically, the most important prognostic factors in gastric carcinomas are represented by the depth of the tumor invasion, status of lymph nodes and the presence of distant metastasis, but the evolution of different patients within the same TNM stage suggests that there are other parameters that should be considered in evaluating patients with gastric carcinoma. (8)

A number of biomolecular factors are considered useful in the diagnosis and prognosis of gastric cancer: oncogenes and tumor suppressor genes, growth factors, angiogenesis and proliferation factors, and adhesion molecules.

p53 is a tumor suppressor gene that produces the 53 kDa nucleoprotein, located on the short arm of cz.17. Due to its role in cell cycle control, DNA repair, apoptosis and prevention of gene mutations in cancer, it was also called "the guardian genome". (9) Following cell injury and destruction of DNA, the production of p53 increases, leading to cell cycle arrest in G1/S.(10) It is believed that the environmental factors and bacterial products or the genomic changes that lead to loss of p53 function, represent a critical moment in gastric carcinogenesis. (11)

PCNA is a 34 kDa nucleoprotein expressed in most dividing cells which participates as a DNA polymerase cofactor in the S phase of the cell cycle. (12-15)

Different studies have attempted to determine the prognostic importance of PCNA in gastric carcinomas, but the data in the literature are conflictive: Maeda et al. (16) determined that PCNA expression has prognostic importance

but according to HE et al (17) there is no correlation between the two.

We studied the relationship between the immunohistochemical expression of p53 and PCNA and clinicopathological parameters in 32 cases of gastric cancer.

Material and Methods

The study group included a total of 32 patients that underwent gastrectomy for gastric cancer. The surgical specimens were fixed in 10% buffered formalin immediately after surgery, keeping the ischemia time as low as possible. The usual routine technique was used for paraffin embedding, sectioning and hematoxylin-eosin staining. The sections were evaluated by in optical microscopy.

For the immunohistochemical study, the primary antibodies used were for p53 (clone DO7, Dako dilution 1:100) and for PCNA (clone PC-10 Dako dilution 1:100). The second antibody was biotinylated anti-mouse immunoglobulin and diaminobenzidine tetrahydrochloride (DAB) was used as a chromogen. All sections were counter-stained with hematoxylin.

The study parameters were represented by epidemiological aspects (age, sex), clinical characteristics (signs and symptoms), histopathological findings (pTNM staging and degree of differentiation, histological classification, lymph nodes status and presence of vascular invasion) and survival, and immunohistochemical analysis (p53 and PCNA expression) of the study group. Survival was evaluated by the Kaplan – Maier log-rank test.

For the histopathological classification of the cases both Lauren (4) and WHO classifications were used (18).

The positivity for p53 and PCNA was evaluated on the basis of the nuclear reactivity of at least one tumor cell and graded as follows: low (<10%), moderate (10-50%) and high (> 50%).

Results

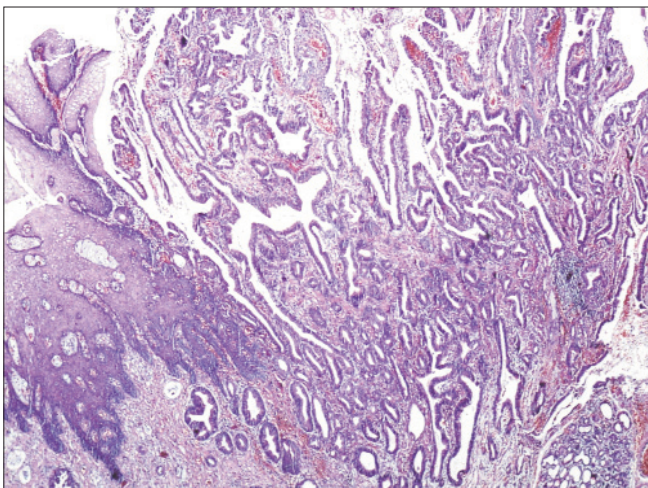
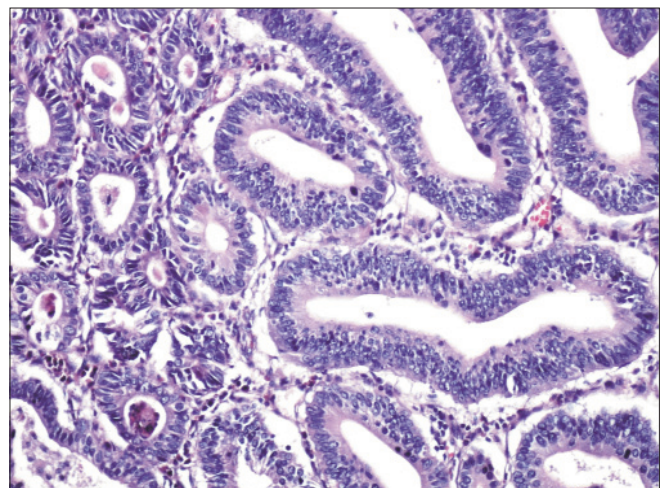
The study group included 23 men and 9 women, with a M/F ratio of 2.55, aged between 39 and 82 years, with a mean age of 67.56 years. Most of the patients (16 cases) underwent subtotal gastrectomy. Total gastrectomy was performed in 6 cases.

Most of the cases were in advanced stages (26 cases), while only 6 cases were diagnosed as early gastric cancer. According to the TNM classification cases were divided in: stage I (6 cases), stage II (2 cases), stage III (14 cases) and stage IV (5 cases). (Table 1)

Histopathologically, most of the cases (26 cases) were classified according to Lauren classification as intestinal type gastric carcinoma (Fig. 1, 2), while only 6 cases were diffuse gastric carcinoma (Fig. 3). According to the degree of differentiation, cases were divided in well differentiated (6 cases), moderately differentiated (14 cases), poorly differentiated (9 cases) and undifferentiated (3 cases) (Fig. 4). There was lymph nodes invasion in 23 cases and vascular invasion in 11 cases.

Table 1. Clinicopathological parameters of the cases studied and immunohistochemical expression of p53 and PCNA

	p53-negative	p53-low	p53-moderate	p53-high	PCNA-low	PCNA-moderate	PCNA-high
Sex							
male	10	4	5	4	4	15	4
female	2	3	3	1	1	6	2
Age (years)							
<50	1	1	0	0	1	0	1
50-59	0	2	2	1	1	4	0
60-69	5	1	0	1	3	3	1
>70	6	3	6	3	0	13	5
Differentiation							
1	2	2	2	0	1	5	0
2	8	2	2	2	2	7	5
3	1	3	3	2	2	6	1
4	1	0	1	1	0	3	0
Stage							
I	1	1	4	0	2	4	0
II	0	1	1	0	0	2	0
III	9	3	2	4	2	10	6
IV	1	2	1	2	0	1	5
Lauren classification							
intestinal	11	6	6	3	5	16	5
diffuse	1	1	2	2	0	5	1
Lymph nodes invasion							
yes	10	4	4	5	2	16	5
no	2	3	4	0	3	5	1
Vascular invasion							
yes	5	2	2	2	0	8	3
no	7	5	6	3	5	13	3
Size (cm)							
<4.5	6	4	4	1	3	9	3
>4.5	6	3	3	5	2	12	3
Survival							
death	3	1	0	2	2	4	0
<6 months	5	1	1	1	2	5	1
6-12 months	5	1	4	0	3	5	2
>1 year	1	1	1	0	2	1	0

**Figure 1.** Gastric carcinoma-intestinal type localised at eso-gastric junction-H&E staining obx4**Figure 2.** Gastric adenocarcinoma-well differentiated-H&E staining obx10

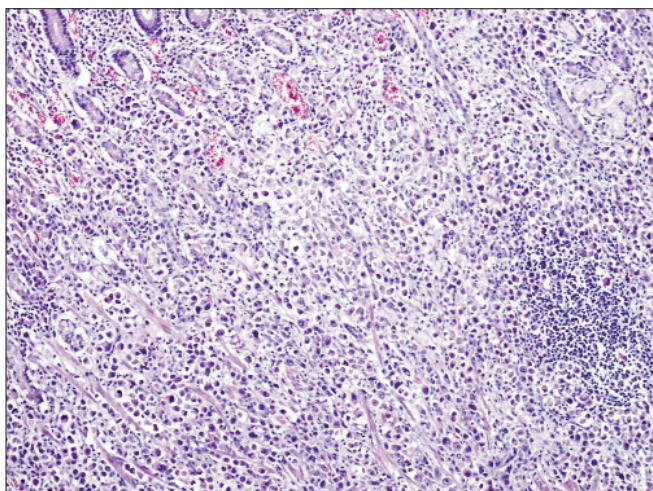


Figure 3. Gastric carcinoma-diffuse type-H&E staining obx4

The size of the tumors varied between 1.5 cm and 6 cm, with a mean of 4.5 cm. When we used the mean value as a reference point, cases arranged almost perfectly around the set value (17 cases >4.5 cm, 15 cases <4.5 cm).

The nuclei of p53 positive cells were stained brown and were present in both tumoral areas and normal gastric mucosa. We only took into consideration the nuclear positivity in tumoral cells. (Fig. 5, 6)

Immunohistochemical analysis revealed different percentage of nuclear positivity for p53 in 20 cases (Table 2).

p53 expression in the resected tumoral tissue sections showed that the average positive cells were 20.75%. (Fig. 7)

P53 expression correlated with advanced age, male gender and histological subtype, but could not be used as an independent marker of prognostic.

The mean PCNA index was 47.3%. (Fig. 8) Only 6 cases showed PCNA over 50%, most of the cases ranging between 10 and 50 % (21 cases) (Table 3). High (Fig. 9, 10) and moderate positivity for PCNA was present in most cases with lymph node metastasis (80.76%) and in all cases with vascular invasion (Fig. 11).

Expression of p53 and PCNA was higher in patients with age (Fig. 12, 13) and advanced stages of disease. The average p53 expression was significantly higher in tumours than in normal tissues.

In terms of survival there were 6 cases of death at intervals ranged from 2-189 days, 5 cases had subsequent presentations over 12 months, while 8 patients were lost to follow-up (Fig. 14), longer survival after surgery being in the group with well and moderately differentiated tumours (Fig. 15). A case relapsed in gastric stump, another presented postoperative eventration, and there was one case of subphrenic abscess and pancreatic fistula and one case of distal subocclusive syndrome.

At the time of surgery, 6 patients had distant metastases: lung, liver, intestinal and peritoneal and in a period of 2-12 months after surgery 6 patients were diagnosed with distant metastasis: lung (3), liver (2) and peritoneal (1).

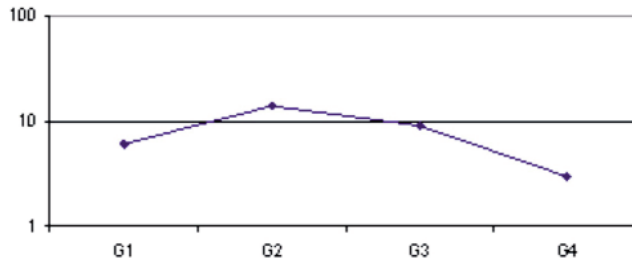


Figure 4. Distribution of cases according to the degree of differentiation

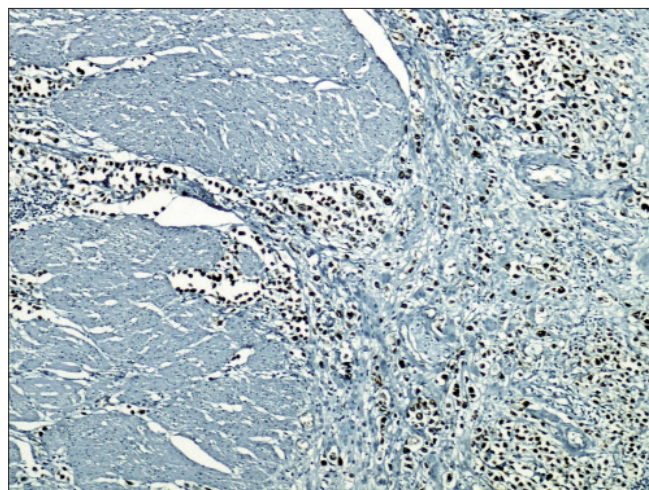


Figure 5. Gastric carcinoma-diffuse type-moderate immunostaining (40%) for p53 obx10

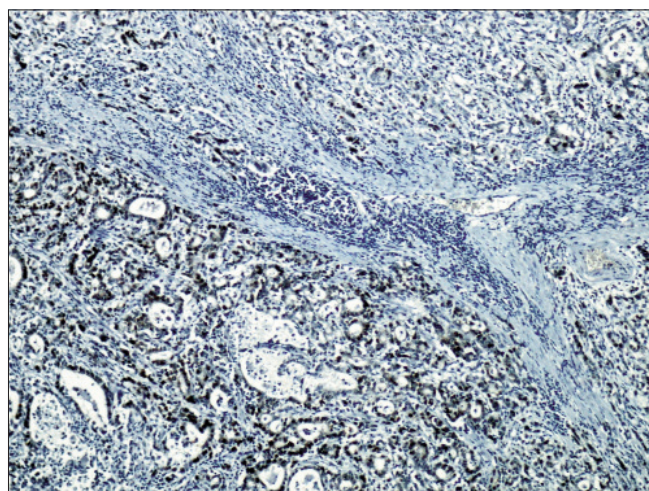


Figure 6. Gastric carcinoma intestinal type-high immunostaining(70%) for p53 obx10

Table 2. p53 expression

Expression of p53	Absent	Low (<10%)	Moderate (10-50%)	High (> 50%)
No cases	12	7	8	5

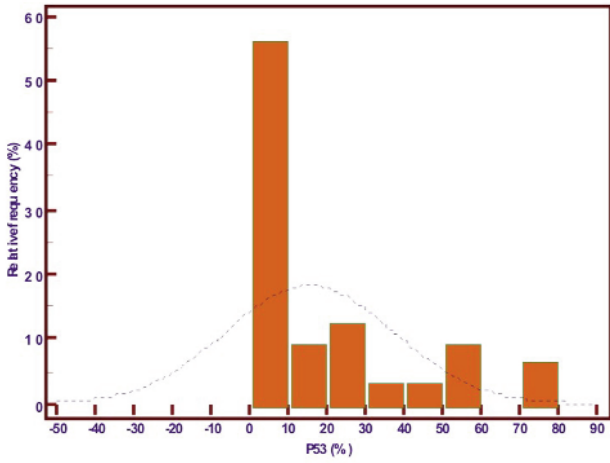


Figure 7. Relative frequency of p53 immunostaining (%)

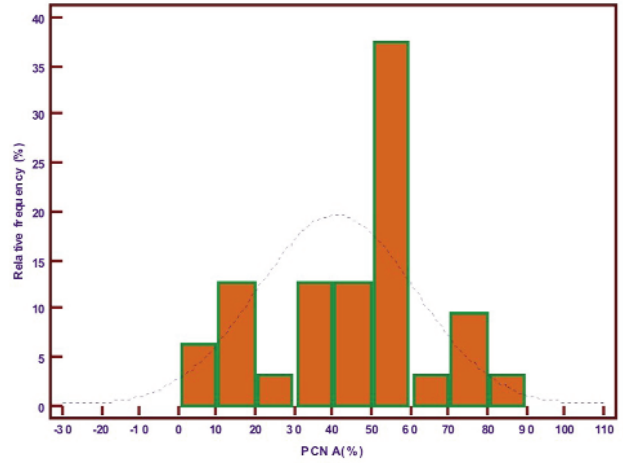


Figure 8. Relative frequency of PCNA immunostaining (%)

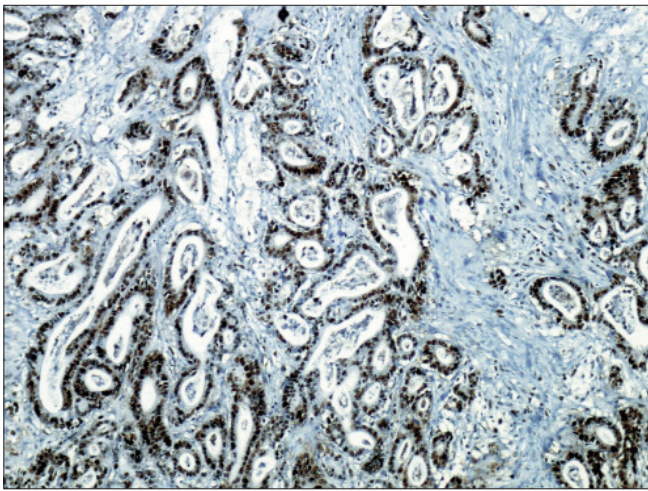


Figure 9. Gastric adenocarcinoma-high immunostaining for PCNA (90%) obx10

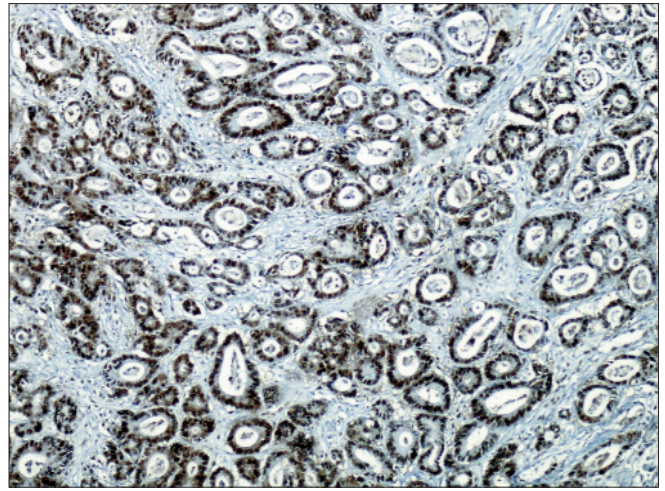


Figure 10. Gastric adenocarcinoma-high immunostaining for PCNA (>85%) obx10

Discussions

P53 gene mutations were identified in several tumoral subtypes, such as colorectal, breast, esophagus, endometrium and gastric carcinoma (19-23). P53 mutations are described in the early stages of development of gastric cancer and they can be identified in the normal mucosa. P53 positivity in gastric cancer is reported between 0-77% (10), the intestinal type being more frequently positive than the diffuse type (24). The wide variation of percentage of positivity could be due to the different types of antibodies used as well as the interobserver

Table 3. PCNA expression

Expression of PCNA	Low (< 10%)	Moderate (10-50%)	High (> 50%)
No cases	5	21	6

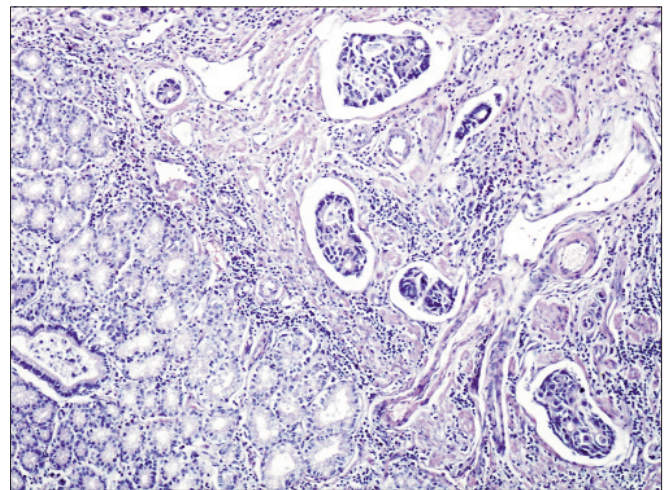


Figure 11. Gastric carcinoma, tumoral embolus-H&E staining ob x 4

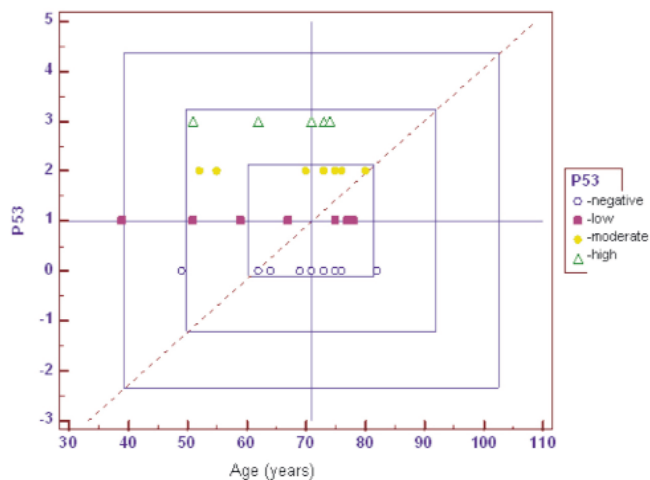


Figure 12. Age- p53 immunostaining correlations

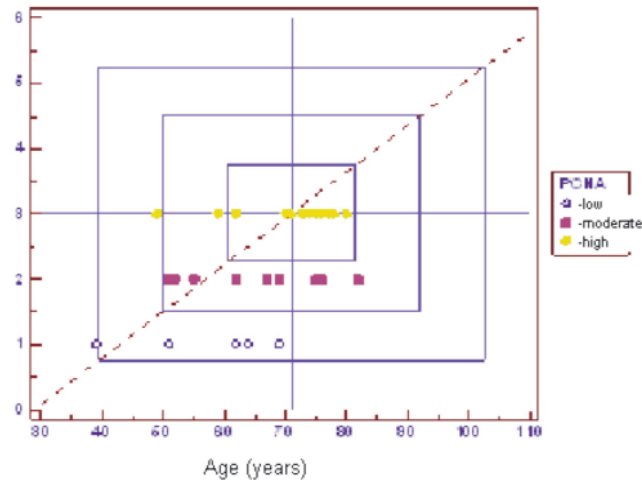


Figure 13. Age- PCNA immunostaining correlations

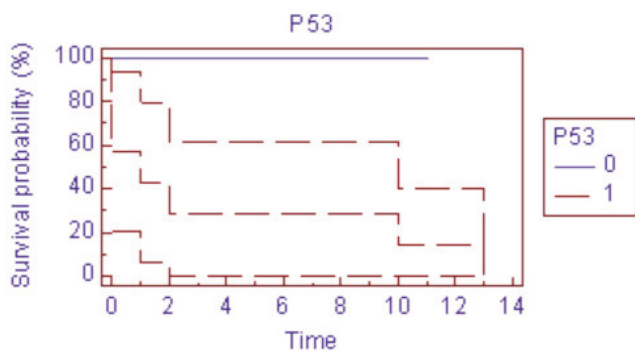


Figure 14. Survival probability for patients with p53 positive carcinomas

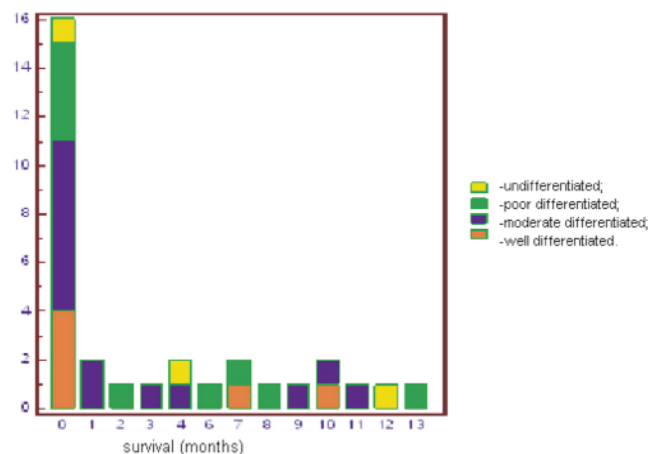


Figure 15. Correlations between survival and differentiation

variability in interpretation. (25) We identified p53 expression in tumoral cells in 15.62% of cases. The expression of p53 in normal mucosa was not quantified in this study.

Some authors have shown that p53 expression is associated with tumors larger than 5 cm and therefore with higher TNM stage (26) or the location in the stomach, therefore carcinomas located in the upper portion, which generally are more aggressive, show increased positivity for p53. (27,28) Therefore, p53 expression may be useful to identify a group of patients with aggressive tumors who may benefit from neoadjuvant chemotherapy (29,30).

Tumors with size greater than 4.5 cm showed more frequent high positivity for p53. In the subgroups of negative or highly positive for p53 most cases presented with lymph node metastasis, while in the other subgroups cases were distributed evenly. The theory that tumors that are either negative or strongly positive are more likely to metastasize comparing with tumors with intermediate levels of p53 expression that have a lowest risk of metastasis has also been reported by Shiao, 2000 (31).

Murakami et al. demonstrated p53 mutations in gastritis associated with HP infection, indicating HP's direct role in p53 carcinogenesis. (32) The gradual increase of p53 positivity in the gastric mucosa has been observed from chronic gastritis and in intestinal metaplasia (33,34), the positivity becoming more evident with the lesion progression from intestinal metaplasia, dysplasia, early carcinoma to invasive carcinoma. The highest positivity was observed in metastatic lesions. (35)

In our study p53 was positive in 20 cases of gastric cancer, showing generally intense and moderate nuclear staining present only in tumor cells, in 77.7% of all females and 56.52 % of male patients.

Rugge et al., 2000 described a lower incidence of p53 mutations in patients under 40. There was a single case from a patient aged 39 in this age category with no expression of p53. (36) There are reports in the literature showing a higher incidence of p53 alterations in intestinal type of gastric cancer compared with diffuse type. We did not find similar results in our study, but it could be biased by the lower number of cases studied. (37)

The study of proliferating activity harbours important prognostic information. Patients in our study with more advanced disease showed higher degree of positivity for PCNA, which is according to the data in the literature in which patients with invasion deeper than the muscle layer had significantly higher PCNA positivity than that in patients with only mucosal or submucosal invasion. In our study, PCNA showed higher expression in diffuse type gastric cancer. (38) There was also a correlation between overexpression of PCNA and lymph node metastasis, suggesting a close correlation of this protein with higher grade of tumour malignancy, multivariate analysis indicating that PCNA labelling index is an independent significant factor for lymph node metastasis. (39)

Conclusions

The study of biomolecular pathways of carcinogenesis and of immunohistochemical markers with prognostic significance may be useful in patients with gastric carcinomas with multimodal integrated approach by correlation between surgical treatment, histopathological diagnosis and oncological therapy.

Thus the identification of biomolecules that highlights potentially aggressive tumors may help modulate the therapeutic approach after surgical resection.

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