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Ki-67 Expression in Gastric Cancer and Correlation with Clinico-Pathological Characteristics

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***Abstract*- Background:** The characteristics of the cellular kinetic reflect the aggressiveness of the tumors and even their prognosis, many studies proving the correlation between the increased proliferation activity and a poor prognosis in a variety of neoplasms. Aim: The analysis of immunohistochemical Ki-67 expression in 55 patients with gastric cancer, the correlation with clinico-pathological factors and patients prognosis.

Patient and Methods: Our study included 55 cases of gastric adenocarcinomas, we analyzed the immunohistochemical expression of the marking index Ki-67 antigen using the MIB1 monoclonal antibody, following the correlations with various clinicopathological factors (gender and age of patients, location, macroscopic type, histological type, degree of tumor differentiation and the TNM staging). For a proper grouping of the results, we classified gastric carcinomas into two categories: adenocarcinomas with high Ki-67 score (≥20%) and adenocarcinomas with low Ki-67 score (<20%).

Results: We observed a close correlation between the TNM stage and the Ki-67 score. The results of our study do not reveal any correlation between the Lauren's Classification of gastric adenocarcinomas, degree of differenciation, the lymphonodular invasion, the depth of tumor invasion and the Ki-67 score ($p>0.05$).

Conclusion: In our study, high scores of Ki-67 are found in advanced TNM stages. Consequently, Ki-67 may be useful in identifying a group of patients with aggressive tumors.

***Index Terms*-** Ki-67, gastric cancer, clinico-pathological characteristics

I. Introduction

The immunoexpression of Ki-67 has become a useful tool to determine the potential of tumor proliferartion. Its high expression was considered as an indicator of poor prognosis in several types of cancers, including gastric cancer [3 ; 16 ; 1 ; 2]. The expression of Ki67 protein is closely related to cellular cycle. This antigen appears in the G1 phase of the cellular cycle, S , G2 and M but not in the G0 phase. Therefore, the anti-Ki67 antibody allows the immunohistochemical determination of the fraction of tissue growth [9 ; 4]

Despite the prognostic and predictive value of Ki67 revealed by some studies and a recent meta -analysis, data from the literature are contradictory, and the use of this parameter is not validated for this indication [10].

The use of Ki-67 as prognostic and predictive marker of some cancers has been widely studied. Recent data suggest that the rate of K-i67 than 10% -14% defines a high-risk group in terms of prognosis [11].

Nishimura et al suggest that the rate of K-i67 before neoadjuvant chemotherapy is a strong predictor of efficacy of the therapy. Following multivariate analysis, they found that the response rate was significantly associated with Ki-67. Indeed, high levels of Ki-67 showed complete response and patients with higher cell proliferation (Ki-67 > 20%) may be better candidates for neoadjuvant chemotherapy. After neoadjuvant chemotherapy, lower values of Ki-67 indicates a better prognosis [12 ; 13].

II. Research Elaborations

Tumor tissue sample collection started in January, 2009 and was completed in December, 2012. The tissue samples obtained from the Anatomic Pathology archives were distributed among 7 Laboratories of Anatomy-Pathology throughout Casablanca nnd Rabat. The study involved 55 cases, all of Moroccan patients who had undergone either biopsy or total /partial gastrectomy for adenocarcinoma of the stomach.

The following patient characteristics were retrieved: type of surgery, age at diagnosis, gender, tumor type, tumor grade, depth of invasion, number of lymph nodes resected and number of lymph nodes with metastases. Each resection specimen underwent gross sectioning and histological examination and reviewed by a pathologist to confirm the presence of tumor and identify areas of tissue for analysis.

The Ki-67 status was assessed using the mouse anti-human anti-body Ki-67 (MIB1). A known positive control section was included in each run to ensure proper staining. The tumor cells were considered Ki-67 positive in the presence of brown nuclear staining of intestinal or diffuse type. Grading was based on the percentage of stained tumor cells. Ki-67 was considered as high expression when positive cells were more than 20% and as low expression when positive cells were 20% or less. A favorable prognostic category for Ki-67 is considered to be less than to 20% of tumor cells staining positively.

Age, gender, degree of differentiation, histological type of cancer and TNM stage were individually compared with Ki-67

expression using the Chi^2 correlation test and Fisher's exact test. Tumor invasion, numbers of lymph nodes with metastasis were compared between high Ki-67 and low Ki-67 groups using the Fisher exact test.

III. Results

The group consisted of 55 patients (37 males and 18 females) with ages between 26 and 78 years (median age = 57 ± 15,18 years). The correlation between the proliferation index (Ki-67) status and the main clinico-pathological features of gastric cancer investigated are presented in Table 1.

Even though all lesions were positive, we noted great intratumoral heterogeneity in the distribution of the Ki-67 score. Tumoral cells were considered positive in the presence of nuclear coloration in brown of a tubular or diffuse type. Cells in mitosis associated the nuclear and cytoplasmic stain.

In our study, we remarked various Ki-67 scores. For a proper grouping of the results, we classified gastric adenocarcinomas into two categories: adenocarcinomas with high Ki-67 (≥20%) and adenocarcinomas with low Ki-67 (<20%). We noticed an increased frequency of high Ki-67 adenocarcinomas in elderly patients without statistical significance.

We noted a significantly greater frequency of adenocarcinomas with high Ki-67 score in male patients (68%) without a statistical significance. A low Ki-67 score was observed among the elderly patients (44%) in comparison with patients younger than 60 years (56%) without any statistical significance.

The histological forms associated to high Ki-67 values are represented by the intestinal type (52% of cases) and diffuse type (48% of cases). Well differentiated adenocarcinomas (G1) presented Ki-67 value of 36%. Among the moderately differentiated carcinomas, 20% presented high Ki-67 values. In a significantly greater percentage (44%), poorly differentiated carcinomas were characterized through intense proliferative activities, with high Ki-67 scores. In a significantly greater percentage (44%), poorly differentiated carcinomas were characterized through intense proliferative activities, with high Ki-67 scores.

The results of our study do not reveal any correlation between the Lauren's Classification of gastric adenocarcinomas, the lympho-nodular invasion, degree of tumor differentiation, the depth of tumor invasion and the Ki-67 score ($p>0.05$).

High scores of Ki67 were observed in 68% of tumors with a size greater than or equal to 5 cm without any statistical significance. We also noted high scores for Ki-67 in 44% of pT3 adenocarcinomas, 36% of pT4 adenocarcinomas, low scores for Ki-67 were observed in 4% of pT1 adenocarcinomas and 16% of pT2 adenocarcinomas. Also, the level of lympho-nodular invasion is not correlated with the Ki-67 value. For adenocarcinomas with loco-regional lymph node invasion (N +), we found a relatively high score of Ki-67 in 22 cases. In addition, a high rate of 76% was significantly (p-value <0.05) attributed to advanced TNM stages (III / IV). Concerning distant metastases, the number of cases presenting synchronous distance metastases in our study is very low and does not enable us to make any conclusion.

Thus, the results of our study show no correlation between the Lauren classification, loco-regional lymph node invasion, the degree of tumor differentiation, depth of tumor invasion and Ki-67 score (p-value> 0.05).

In addition, in our study, the tumor proliferation index calculated (76%) indicates the existence of a relationship between Ki-67 and advanced TNM stages (III and IV) (p-value <0.05).

IV. Discussion

The prognosis of patients with gastric cancer can be influenced by the alteration of oncogenes or tumoral suppressor genes, determining alterations of the kinetics of cell proliferation. The characteristics of cellular kinetics reflect the aggressiveness of tumors and even their prognosis, a series of studies demonstrating the correlation between the marked proliferative activity and an unfavorable prognosis in a variety of neoplasias.
Ki-67 monoclonal antibodies detect a nuclear antigen expressed exclusively at the level of cells in the proliferation phase (phases G1, S, G2 and mitoses), but not in the G0 phase. Therefore, Ki-67 antibodies allow for the imunohistochemical determination of the tissular growth fraction [4].

Correa postulated that gastric cancer develops through a complex sequence of events from normal mucosa to superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, and finally to intestinal type gastric carcinoma. In these premalignant stages, the apoptotic activity of the cells is lower than their proliferation rate, and this difference grows along the multi-step gastric carcinogenesis [5 ; 6].

For a better systematizing of the results we classified gastric adenocarcinomas into two great categories: adenocarcinomas with high Ki-67 score (≥20%) – 30 cases (54.54%); adenocarcinomas with low Ki-67 score (<20%) –25 cases (45.45%).

We noted a significantly greater frequency of adenocarcinomas with high Ki-67 score in male patients (68%) without a statistical significance. We also noticed a lower Ki-67 score among the elderly patients (44%) than in patients younger than 60 years (56%) without any statistical significance. This observation is different from the data obtained by Lazar D et al., who observed a significant interaction between Ki-67 score and age, a Ki-67 >40% being associated with an unfavorable prognosis in patients over 68 years of age [17].

High values of Ki-67 were observed in 64% of intestinal-type adenocarcinomas and 36% of diffuse-type adenocarcinomas. However, no correlation was observed between the proliferation of neoplastic cells and the histological type of tumors, according to the Lauren classification, which is in accordance with other studies [8].

Well differentiated adenocarcinomas (G1) presented Ki-67 value of 36%. Among the moderately differentiated carcinomas, 20% presented high Ki-67 values. In a significantly greater percentage (44%), poorly differentiated carcinomas were characterized through intense proliferative activities, with high Ki-67 scores.

We did not note a correlation between the level of tumor invasion and the Ki-67 value. We noted high scores for Ki-67 in 44% of pT3 adenocarcinomas, 36% of pT4 adenocarcinomas.

Furthermore, loco-regional lymph node invasion is associated with the overexpression of high Ki-67. In adenocarcinomas with loco-regional lymph node invasion, overexpression of Ki-67 was observed in 88% of cases, joining data from the literature [14 ; 15].

Concerning distant metastases, the number of cases presenting synchronous distance metastases in our study is very low and does not enable us to make any conclusion.

In general, The index of tumor proliferation calculated in our study indicates the existence of a relation between the Ki-67 score and the pTNM stage being associated with an unfavorable prognosis in patients with pTNM stage (III or IV).

V. Conclusion

Our results do not show a relation between the Lauren's Classification of gastric adenocarcinomas, the degree of tumor differentiation, lymph node and distance metastases and the Ki-67 score. However, the pTNM stage showed a statistical significance with Ki-67.

In our study, immunohistochemical assessment of the proliferation index (Ki-67) could represent a prognostic factor and seems to be useful in identifying a group of patients with advanced adenocarcinomas.

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Tables

Table 1 : The correlation between Ki67 expression and clinico-pathological features

	Clinico-pathological features	Cas à IP Ki67 low score (n=30)	%	Cas à IP Ki67 high score (n=25)	%
Gender	men	18	60	17	68
	Women	12	40	8	32
Age	< 60 years	17	57	14	56
	≥60 years	13	43	11	44
Size of tumor	Size < 5 cm	21	70	8	32
	Size ≥ 5 cm	9	30	17	68
Histological type	Intestinal type	14	46,70	13	52
	Diffuse type	16	53,30	12	48
Grade	WD	8	27	9	36
	MD	9	30	5	20
	PD	13	43	11	44
Stage	TNM stage (I/II)	11	37	6	24
	TNM stage (III/IV)	19	63	19	76
pT	pT1	4	13	1	4
	pT2	6	20	4	16
	pT3	15	50	11	44
	pT4	5	17	9	36
pN	pN0	10	33	3	12
	pN+	20	67	22	88
pM	pM0	29	97	22	88
	pM1	1	3	3	12

*WD : Well differentiated ; MD : Moderately differentiated ; PD : Poorly differentiated