

Applied lymphocytes immunophenotyping infiltrated in gastric mucosa of pathologic conditions (Reactive hyperplasia, Dysplasia and Carcinoma)

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Abstract: *Objective:* The aim of this study is to examine the differences among Reactive hyperplasia (RH), Indefinite for Dysplasia (in.dys), Dysplasia (dys) and Gastric Carcinoma through the expression of biomarkers of lymphocytes surrounding the lesions and their possible application in the differentiation diagnosis of these lesions. *Material and Method:* paraffin blocks related to 19 RH samples, 5 In.dys samples, 20 Dys samples and 15 carcinoma samples were selected according to Padova Classification criteria and IHC staining was performed on them for markers by Envision technique; then, the differences between them were analyzed in terms of risk markers. *Findings:* the expression of CD4 marker in lymphocytes surrounding RH, In.dys, Dys and carcinoma is 15, 21, 20 and 31 percent respectively; its expression has no significant difference between In.dys and Dys. But in general, a significant increase has been observed from RH towards carcinoma. The expression of CD8 marker in lymphocytes surrounding RH, In.dys, Dys and carcinoma is 27.4, 28.2, 18.4 and 22.2 percent respectively; Dys and In.dys had no significant difference from each other. But in the progression of lesions from RH towards carcinoma, a significant reduction was observed. The expression of CD20 marker in lymphocytes surrounding RH, In.dys, Dys and carcinoma is 27, 21, 19 and 14 percent respectively; like CD8, a significant reduction was observed from RH towards carcinoma. And the expression of CD56 is 11, 14, 10 and 12 percent respectively with no significant difference. *Conclusion:* from RH towards carcinoma, the expression of CD4 had an increase; the expression of CD8 and CD 20 had a significant reduction and CD56 had no significant difference in the progression of lesions. [Firouzjahi A, Shafaii S, Sharbatdaran M, Homayuni Kelarijani A. **Applied lymphocytes immunophenotyping infiltrated in gastric mucosa of pathologic conditions (Reactive hyperplasia, and carcinoma).** *Life Sci J* 2013;10(8s):381-384] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 61

Key words: Gastric reactive hyperplasia, Dysplasia, Indefinite for Dysplasia, Carcinoma.

1. Introduction

Gastric Carcinoma is the most important and frequent malignant tumor and its incidence varies geographically. Also, it is the most common cancer in Iran. Diagnosis of this cancer in the early stages has a significant impact on its prognosis and treatment (Juan, 2004).

Since the main approach to detect the pre-malignant and malignant lesions in early stages is biopsy by endoscopy, the early histo-pathological diagnosis is essential in order to differentiate the proliferative and malignant lesions. Sometimes, diagnosis and proof the original biological differences of benign and malignant lesions needs parameters other than morphology.

In this study, the expression of CD4, CD8, CD20 and CD 56 markers in lymphocytes surrounding Reactive Hyperplasia (RH), Indefinite for Dysplasia (in.dys), Dysplasia (dys) and Gastric Carcinoma are classified by Padova, based on the international classification criteria of dysplasia and related lesions. Then, these lesions are analyzed in terms of the incidence of these markers.

CD4 marker shows T. helper and represents in 60% of T CD_3^+ mature cells. Th (CD_4^+) acts like an orchestra leader against pathogens and affects almost all the functions of other immune cells including T cells, B lymphocytes, macrophages and NK cells by secreting the cytokines.

Also, CD8 is the marker that exists in T-Cytotoxic and in 30% of T CD_3^+ and it is one of the major factors in fighting against tumor cells.

CD20 marker determines B lymphocytes and B lymphocytes need Th lymphocytes to respond to protein antigens.

CD56 marker is also demonstrator of NK Cell (Natural Killer Cell) and constitutes 10-15 percent of blood lymphocytes. These cells are part of the innate immune system and have the first defense line against infections and tumors (Kumar, 2005).

2. Materials and Method

A) **Sample collecting:** referring to records archived in the pathology departments at Shahid Yahya Nejad and Beheshti hospitals, paraffin blocks of 58 biopsy samples between

2006 to 2009 were extracted; these samples, which were classified based on Padova criterion, are consistent with four studied lesions.

- B) **Staining:** first, slides of selected blocks with 3-4 thickness were prepared and placed on silanized glasses. After deparaffinating the slides by creosol and rehydration in diluted alcohol, samples were washed slowly in water and slides were incubated for 10 min with a solution of 3 percent H₂O₂ (based on PBS) in a dark environment in order to neutralize the peroxidase enzymes within the tissue. After washing in water, tissue slides of the CD4, CD8, CD 20 and CD 56 markers were inserted in citrate buffer (PH:6) in order to antigen retrieval; then, slides were put into Microwave Glass Pressure Reactive (GPR) for 15 min in 120 °C.

Next, slides were stained using antibodies specific to each marker (primary antibody) in the following way:

For CD20, Monoclonal Mouse Antihuman, Clone L26, DAKO was used.

For CD8, Monoclonal Mouse Antihuman, Clone C8/144 B DAKO was used.

For CD56, Monoclonal Mouse Antihuman, Clone 123 C3 DAKO was used.

For CD4, Monoclonal Mouse Antihuman, MSX was used.

Slides were put in the above primary antibodies for 30 min, and put in HRP Conjugated Mouse/Rabbit secondary antibodies for 30 min; then, polymerase for 30 min. At last, they were incubated for 10 min by DAB chromogens. at each step, slides were washed by the fresh TBS solution; after washing and interactive staining (background) by hematoxylin color, slides were dehydrated again (in diluted alcohol) in creosol (for transparency) and ultimately were covered by coverglasses (Dabbs, 2006).

3. Discussion

Cut-off used in this study for the positive or negative above mentioned markers were determined based on previous studies and immunological data. All the lymphocytes were considered as positive when more than 5% lymphocytes had more than moderate staining.

The stainability of different markers is presented quantitatively and in form of the percentage of stained lymphocytes.

The stainability intensity can be classified as follows:

1. Lack of stainability
2. Zero-stainability

3. Weak stainability
4. Moderate stainability
5. Intense stainability

Tonsil tissue was used as positive control because of the plenty of lymphatic follicles; and stomach lymphatic follicles (if available) were used as an internal control. Data analyzing has been done based on Fisher Exact and Chi-square statistical tests and p-value was less than 5% and statistically was considered meaningful.

4. Findings

In normal cases, CD4 marker is expressed in membrane and in para-cortical area, it is expressed in lymphoid follicles. Due to its abundant lymphoid follicles, tonsil tissue was used for all the markers as a positive and negative control. For tonsil tissue, CD4 should be positive in para-cortical follicle; but in germinal center it should be negative. Lymphoid follicles of gastric mucosa (if observed) can be used as an internal control.

CD4 marker in RH, In.dys, Dys and carcinoma is 15, 20.8, 19.9 and 31 percent positive respectively (p=0.65). Percentage of expression in the progression of lesions is increased from RH towards carcinoma.

Normally, CD8 marker in membrane and in para-cortical lymphoid follicles is positive. Also in this case, tonsil tissue was used as external positive and negative control. The expression of CD8 markers in gastric lesions of RH, In.dys, Dys and carcinoma is 27.3, 28.20, 18.4 and 22.2 respectively (p=0.014). The percentage of the expression of this marker is significantly decreased from RH towards carcinoma.

CD20 marker in natural form is cytoplasmic and in the germinal centre of lymphoid follicles is positive and like other markers, tonsil tissues was used as external positive and negative control.

The marker in gastric lesions of RH, In.dys, Dys and carcinoma is 27.47, 21, 19.39 and 14.1 percent respectively (p=0.0131). The expression of this marker is significantly decreased from RH towards carcinoma.

Normally, CD56 marker is expressed in membrane and small-scale in para-cortical area. Tonsil tissue was also used here as external positive and negative control. The expression of this marker in different lesions of RH, In.dys, Dys and carcinoma is 11, 13.8, 10 and 11.2 percent respectively (p=0.839). The percentage of expression for this marker had no significant difference from RH towards carcinoma (table 1).

One of the goals of this study is to investigate the relationship between the co-expressions of the studied markers in the progression of lesions and based on Padova classification. The co-expression

among CD4, CD8, CD20 and CD56 compatible with the progression of the lesion is shown in table 2.

From the content of this table, it can be concluded that there is a significant relationship between the co-expression of CD8 and CD20 (p: 0.000) and CD4 and CD8 (p: 0.000) with the Padova classification of lesions; and also a non-significant relationship was observed between the co-expression of CD4 and CD20, proportionate to the progression of lesions (table 2).

Table 1: Mean and standard deviation of the distribution of the CD56 marker to differentiate the reactions of RH, ID, D45, and Carcinoma.

P-value	Maximum	Minimum	Standard error	Standard deviation	Average	Number	Reaction	Marker type
0.839	27	5	1.992	7.182	11.08	13	RH	CD56
	37	6	5.894	13.180	13.80	5	ID	
	25	5	1.574	5.890	10.07	14	D	
	27	5	3.297	9.326	11.88	8	Car	
	37	5	1.248	7.892	11.23	40	total	

Table 2: Distribution of the correlation coefficient (the expression) between the markers CD4, CD8, CD20 and CD56.

CD4	CD8	CD20	CD56	Marker	
0.230	0.144	0.274	1	Pearson correlation	CD56
0.2559	0.377	0.101		P-value	
0.266	0.614	1	0.274	Pearson correlation	CD20
0.163	0.000		0.101	P-value	
0.608	1	0.614	0.144	Pearson correlation	CD8
0.000		0.000	0.377	P-value	
1	0.608	0.266	0.230	Pearson correlation	CD4
	0.000	0.163	0.259	P-value	

Correlation is significant at the 1% significance level.

5. Conclusion

In this study, the expression of the CD4, CD8, CD20 and CD56 markers in lymphocytes surrounding the lesions was classified and evaluated based on Padova classification.

The expression of CD4 marker is increased from RH lesion towards Dysplasia; But, In.dys and Dys lesions had no significant difference from each other and besides in the progression of lesion, an increase was observed from Dysplasia to carcinoma.

These findings are consistent with the study of Konok et al. in which the incidence of CD_4^+ in increased in proportion to the progression of lesion towards gastric carcinoma. From these findings, it can be concluded that the increased incidence of malignancy will result in an increase of the incidence of CD_4^+ (3).

The incidence of CD8 marker had a significant reduction from RH towards carcinoma. But, there is no significant difference in RH and In.dys. A similar study was carried out by Figuireso Soares et al. in Brazil about the incidence of CD8 marker but in

which the changes in CD_8^+ was investigated according to Duodenal Ulcer (which is actually a type of RH). This study indicated that in children with Duodenal Ulcer DR^+ , the amount of CD_8^+ / HLA increased about 200% (twice) and the amount of $CD_8^+ / CD28^+$ decreased about 34%. But in adults, Duodenal Ulcer was accompanied by

decrease in DR CD_4^+ / HLA reduction which is a symptom of decreased immune function to create Ulcer (Soares et al., 2007).

The final result of this study showed that due to the different immune mechanisms for Ulcer incidence in various ages, the changes of markers also varies. Like CD4, the expression of CD20 marker is also decreased from RH towards Dysplasia and Carcinoma.

Finally, the expression of CD56 marker had also no significant difference in the progression of lesions from RH towards Dysplasia and Carcinoma.

One of the goals of this study was to investigate the relationship between the expressions of the studied markers for the progression of lesions based on Padova classification. The co-expression among CD4, CD8, CD20 and CD56 markers in consistent with the progression of lesions is shown in table2 completely.

It can be concluded from the content of this table that there is a significant relationship between co-expression among CD4, CD8 (p=0.000), CD20 (p=0.000) and CD56 markers and the Padova classification of lesions; and no significant relationship was observed between the co-expression of CD4 and CD20 in consistent with the progression of lesions (table2).

It is important to mention that according to the immunologic books about the innate and acquired immune system, maybe the initial expectation is that an increase should be observed in CD4 (Th) at first from RH towards Dysplasia and Carcinoma in consistent with the progression of lesions and cytokine secretion should also cause proliferation and reproduction of (T-Cytotoxic) CD8 and (CD56) NK Cell and ultimately CD4 decrease towards Carcinoma in consistent with the progression of lesions (Kumar, 2005).

On the other hand, (CD56) NK Cell is known as a first line of innate immunity against tumor cells and

(T-Cytotoxic) CD8 also has a major role in fighting against tumor cells (Kumar, 2005).

But in contrast to the initial expectation, in this study, an increase in CD4 and a significant reduction in CD8 and CD20 were observed in consistent with the progression of lesion from RH towards Dysplasia and Carcinoma and CD56 also showed no significant difference in this progression. So, approve or disapprove of the outlined assumptions needs more widespread and diverse studies with more samples.

6. Final conclusion

Consistent with the progression of lesions from RH (reactive hyperplasia) towards dysplasia and carcinoma, a significant reduction of CD8 and CD20 and significant increase of CD4 was observed.

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