Monoclonal anti-IgA antibody expression in the posterior commissure mucosae of laryngeal cancer patients

Original Article

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ABSTRACT

Objective: It has been reported that the interarytenoid fold of the larynx (IAFL) is rarely affected by malignancy 1. It is therefore crucial that research be undertaken to delineate its structural differences of the posterior commissure of the larynx from other laryngeal sub-sites.

Study Design: Comparing the expression of anti-IgA antibody in laryngeal tumor cells and the mucosa of the PC (free of tumor). The study aims to detect a specific immune response mounted by the host that might explain the previous observation.

Setting: Twenty-seven specimens from patients suffering from T3 and T4 squamous cell carcinoma were included in the present study.

Patients and Methods: Sample biopsies were procured from the tumor, the PC, and the tumor-free mucosae of the larynx, and were subjected to immunohistochemical study using a commercially prepared rabbit-hosted monoclonal anti-IgA antibody. Several criteria were applied to evaluate the expression of IgA in all of the samples.

Results: The posterior commissure was free of tumor involvement in all twenty-seven of the excised larynges. IgA expression was significantly higher in the PC epithelium than in the neighboring laryngeal epithelium devoid of tumor. One interesting result is the highly significant expression of IgA in the immunocytes located in the PC as compared to those in the tumor, where it is almost negligible.

Conclusion: Immunohistochemical study further revealed a significant characteristic of the PC regarding its higher expression of IgA. Research should be directed toward revealing the factors that provide immunity and resistance in the PC area.

Key Words: Anti-IgA antibody, larynx, posterior commissure.

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INTRODUCTION

Articles reporting involvement of the posterior commissure are few. Series including two cases[2], sixty cases[3], 40 T1 carcinoma[4] and two cases[5] were published between 1959 and 2014. In all the reviewed articles, the vocal process of the arytenoid was included as part of the PC.

Controversy exists regarding the incorporation of the vocal process of the arytenoids as a part of the posterior commissure, hence the study focused solely on the plica interarytenoidea[1]. A retrospective case-control study included 437 laryngectomies of 394 patients with cancer larynx. In all cases the IAFL was free of tumor involvement[1].

Immunoglobulins are considered among tumor markers in human cancers. IgA is the most predominant immunoglobulin in the human mucosa and plays a major role in its host-pathogen immunity. Authors speculated it may be used as tumor marker[6]. Others suggested IgA monoclonal antibodies that are directed against tumor antigens may be effective as cancer treatment[7&8].

Several studies were conducted to study the reaction of immunoglobulins in malignancy[9,10&11] Serum IgA level was reported to be significantly higher in non treated patients of oral SCC patients when compared to that of treated patients and the normal healthy individuals[12]. Another study on oral cancer the levels of serum immunoglobulins were

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found to increase with the progression of the disease.[13], IgA was detected in tumor cells in a wide range of cancers e.g. breast cancer[14]. Its presence is correlated with poor prognosis as in bladder cancer[15].

A study was conducted in Cairo medical school to localize IgA and C3 in cancer larynx, using indirect immune-histochemical technique. Immunoglobulins were significantly localized in non-metastatic as compared to metastatic laryngeal cancer[16].

AIM OF THE WORK:

To confirm non affection of the mucosa of the posterior commissure of the larynx by malignant cells, and to delineate its structural difference from other laryngeal sub-sites. Comparing the expression of anti-IgA antibody on laryngeal tumor cells and mucosa of the PC (free of tumor) aims to detect a specific immune response mounted by the host that might explain the previous observation.

PATIENTS AND METHODS:

The study was conducted in Cairo University Hospital.

Twenty seven patients suffering from T3 and T4 squamous cell carcinoma were randomly selected. Twenty five patients were T3 and two patients were T4. All patients were not legible for cancer preservation protocols and were subjected to total laryngectomy.

From each of excised larynges samples of laryngeal biopsies were procured from the tumor, the posterior commissure and larynx free tumor mucosae.

Commercially prepared rabbit hosted monoclonal anti-IgA antibody, was purchased from Chongking Biopsies Co., Ltd. (IHC).

All samples were processed in 4 mm thickness sections of formalin fixed paraffin embedded blocks (FFPEB), dewaxed in xylene then immersed in graded series of alcohol and finally rinsed with water. Following the incubation at 4°C, using the automated Ventana immunostainer, sections were incubated for 30 min at room temperature.

Then heat- mediated antigen retrieval was performed with citrate buffer pH 6, using monoclonal anti-IgA antibody (#YMA1195), 100ul with dilution 1:25- 100. Sections were incubated with diaminobenzidine peroxidase (DAB) substrate as chromogen to give a brown stain, and Hematoxylin as a counterstain.

Criteria to evaluate expression of anti-IgA antibody

Several criteria were applied to evaluate the expression of the IgA both in and tumor tissues and the posterior commissure and tumor free mucosae. These included the staining intensity, the percentage of cells stained and the percentage score of cell staining. IGA marker expression within the cytoplasm of epithelial cells and immunocytes was further evaluated applying the immunoreactive score (IRS) to appraise both the intensity of immunohistochemical staining and proportion of stained cells. The immunoreactive score (IRS) gives a range of 0–12 as a product of multiplication between positive cells proportion score (0–4) and staining intensity score (0–3)[17].

Positive cells were quantified as a percentage of the total number of cells, and assigned to one of five categories: 0, no positive cells; 1, <10; 2, 10–50; 3, 51–80; 4, >80%.

The staining intensity was sub classified as 0 (No stain), 1 (weak), 2 (moderate) and 3 (strong). The percentage of positivity of the tumor cells and staining intensity were multiplied to generate the immunoreactive score for each tumor specimen (Table 3). For a diagnostic purpose, a cut-off of 10% stained tumor cells was chosen to define a positive tumor[17].

Statistical Analysis:

The data were analysed using the Statistical Package of Social Science (SPSS) (version 26). Data were presented as median and range. The comparison between groups was done using Friedman test followed by post hock test for pairwise comparison between groups all tests were two tailed a *p- value < 0.05* was considered significant.

Table 1: Sub-classification of percentage of positive cells, intensity of staining, and the IRS score designated for IGA marker expression[13]

A (percentage of positive cells)	B (intensity of staining)	IRS score (multiplication of A and B)
0 = no positive cells	0 = no color reaction	0-1 = negative
1 = <10% of positive Cells	1 = mild reaction	2-3 = mild
2 = 10-50% positive cells	2 = moderate reaction	4-8 = moderate
3 = 51-80% positive cells	3 = intense reaction	9-12 =strongly positive
4 = >80% positive cells	Final IRS score (A × B): 0-12	

RESULTS:

Table 2 Showing percentage of positive cells, intensity of staining and the IRS score designated for IGA marker expression in the tumor mucosa, epithelium and immunocytes of tumor free and posterior commissure mucosae

Table 2: Percentage of positive cells, intensity of staining, and the IRS score designated for IgA marker expression in the tumor mucosa, epithelium, and immunocytes of tumor-free and posterior commissure mucosae

No.	Grade	Tumor				
		intensity	%	% score	IRS	interpretation
1	2	1	5	1	1	negative
2	1	0	0	0	0	negative
3	3	0	0	0	0	negative
4	2	1	5	1	1	negative
5	2	1	5	1	1	negative
6	2	1	10	2	2	mild
7	2	0	0	0	0	negative
8	2	1	5	1	1	negative
9	2	1	5	1	1	negative
10	1	0	0	0	0	negative
11	1	0	0	0	0	negative
12	1	0	0	0	0	negative
13	2	0	0	0	0	negative
14	3	0	0	0	0	negative
15	2	1	10	2	2	mild
16	2	1	10	2	2	mild
17	2	1	10	2	2	mild
18	1	0	0	0	0	negative
19	2	1	10	2	2	mild
20	2	1	5	1	1	negative
21	3	1	5	1	1	negative
22	2	0	0	0	0	negative
23	3	0	0	0	0	negative
24	3	1	10	2	2	mild
25	2	0	0	0	0	negative
26	2	1	20	2	2	mild
27	2	1	10	2	2	mild

No.	Grade	Tumor-free epithelium					Tumor-free immunocytes				
		intensity	%	% score	IRS	interpretation	intensity	%	% score	IRS	interpretation
1	2	1	1	1	1	negative	3	20	2	6	moderate
2	1	1	20	2	2	mild	3	10	2	6	moderate
3	3	3	5	1	3	mild	2	50	3	6	moderate
4	2	0	0	0	0	negative	3	10	2	6	moderate
5	2	1	1	1	1	negative	3	20	2	6	moderate
6	2	1	20	2	2	mild	3	10	2	6	moderate
7	2	1	15	2	2	mild	3	30	2	6	moderate
8	2	0	0	0	0	negative	3	10	2	6	moderate
9	2	1	1	1	1	negative	3	20	2	6	moderate

10	1	1	20	2	2	mild	3	10	2	6	moderate
11	1	0	0	0	0	negative	3	30	2	6	moderate
12	1	0	0	0	0	negative	2	10	2	4	moderate
13	2	1	20	2	2	mild	2	20	2	4	moderate
14	3	0	0	0	0	negative	1	10	2	2	mild
15	2	0	0	0	0	negative	3	30	2	6	moderate
16	2	1	10	2	2	mild	3	30	2	6	moderate
17	2	1	10	2	2	mild	3	20	2	6	moderate
18	1	1	20	2	2	mild	3	5	1	3	mild
19	2	0	0	0	0	negative	3	50	3	9	strong
20	2	1	20	2	2	mild	3	20	2	6	moderate
21	3	1	5	1	1	negative	3	30	2	6	moderate
22	2	0	0	0	0	negative	2	20	2	4	mild
23	3	0	0	0	0	negative	1	10	2	2	mild
24	3	0	0	0	0	negative	3	20	2	6	moderate
25	2	0	0	0	0	negative	3	50	3	9	strong
26	2	0	0	0	0	negative	3	30	2	6	moderate
27	2	0	0	0	0	negative	3	50	3	9	strong

No.	Grade	PC epithelium					PC immunocytes				
		intensity	%	% score	IRS	interpretation	intensity	%	% score	IRS	interpretation
1	2	2	80	4	8	moderate	3	90	4	12	strong
2	1	2	20	2	4	moderate	3	50	3	9	strong
3	3	2	10	2	4	moderate	3	90	4	12	strong
4	2	3	10	2	6	moderate	3	80	3	9	strong
5	2	2	80	4	8	moderate	3	90	4	12	strong
6	2	2	20	2	4	moderate	3	90	4	12	strong
7	2	2	5	1	2	mild	3	90	4	12	strong
8	2	3	10	2	6	moderate	3	80	3	9	strong
9	2	2	80	4	8	moderate	3	90	4	12	strong
10	1	2	20	2	4	moderate	3	50	3	9	strong
11	1	0	0	0	0	negative	3	30	2	6	moderate
12	1	1	30	2	2	mild	3	90	4	12	strong
13	2	0	0	0	0	negative	3	80	3	9	strong
14	3	3	10	2	6	moderate	3	70	3	9	strong
15	2	2	10	2	4	moderate	3	90	4	12	strong
16	2	2	20	2	4	moderate	3	90	4	12	strong
17	2	1	1	1	1	negative	3	100	4	12	strong
18	1	1	5	1	1	negative	3	30	2	6	moderate
19	2	0	0	0	0	negative	3	90	4	12	strong
20	2	3	5	1	3	mild	3	90	4	12	strong
21	3	1	10	2	2	mild	3	90	4	12	strong
22	2	0	0	0	0	negative	3	50	3	9	strong
23	3	0	0	0	0	negative	3	70	3	9	strong
24	3	1	10	2	2	mild	3	70	3	9	strong
25	2	1	10	2	2	mild	3	70	3	9	strong
26	2	0	0	0	0	negative	3	70	3	9	strong
27	2	1	10	2	2	mild	3	50	3	9	strong

Table 3: Difference in IRS score and cell percentage between posterior commissure and other parts

Characteristics	IRS	*p-value*
Tumour vs. Tumour-free epithelium	1 (0-2) vs. 1 (0-3)	1.000
Tumour vs. Posterior commissure epithelium	1 (0-2) vs. 2 (0-8)	0.001
Tumour free epithelium vs. Posterior commissure epithelium	1 (0-3) vs. 2 (0-8)	<0.001
Tumour vs. Posterior commissure immunocytes	1 (0-2) vs. 9 (6-12)	<0.001
Tumour free immunocytes vs. Posterior commissure immunocytes	6 (2-9) vs. 9 (6-12)	<0.001

Data presented as median (range)

Table 4: Difference in cell Percentage between posterior commissure and other parts

Characteristics	Percentage	*p-value*
Tumour vs. Tumour-free epithelium	5 (0-20) vs. 1 (0-20)	0.443
Tumour vs. Posterior commissure epithelium	5 (0-20) vs. 10 (0-80)	0.008
Tumour free epithelium vs. Posterior commissure epithelium	1(0-20) vs. 10 (0-80)	0.042
Tumour vs. Posterior commissure immunocytes	5(0-20) vs. 80 (30-100)	<0.001
Tumour free immunocytes vs. Posterior commissure immunocytes	20 (5-50) vs. 80 (30-100)	<0.001

Data presented as median (range)

The IRS scores: No significant difference between tumour and tumour free epithelium *p-value =1.000*. The Posterior commissure epithelium IRS was significantly higher than tumour IRS score.

(2 versus 1, *p-value= 0.001*). The Posterior commissure epithelium was significantly higher than tumour free epithelium (2 versus 1, *p-value < 0.001*).

In addition, a highly significant higher posterior commissure immunocytes compared to tumour (9 versus 1, *p-value <0.001*), and a highly significant higher posterior commissure immunocytes than Tumour free immunocytes (9 versus 6, p-value <0.001) (Table 1).

The percentage of positive cells stained: No significant difference between tumour and tumour free epithelium *p-value =0. 443*. Posterior commissure epithelium was significantly higher than tumour (10 versus 5, *p-value =0.008*). The Posterior commissure epithelium was significantly higher than Tumour free epithelium, (10 versus 1, *p-value= 0.042*). Nonetheless, posterior commissure immunocytes was significantly higher than tumour (80 versus 5, *p- value <0.001*). Posterior commissure immunocytes was also significantly higher than Tumour free immunocytes (80 versus 20, *p-value <0.001*).

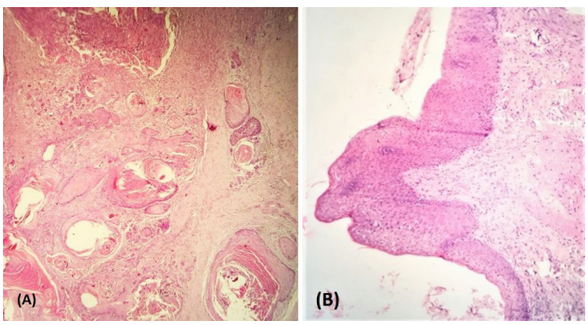

Fig. 1: Photomicrographs showing (a) Moderately differentiated keratinizing squamous cell carcinoma. (B) Posterior commissure mucosa free of tumor cells. [H&E staining, original magnification x100]

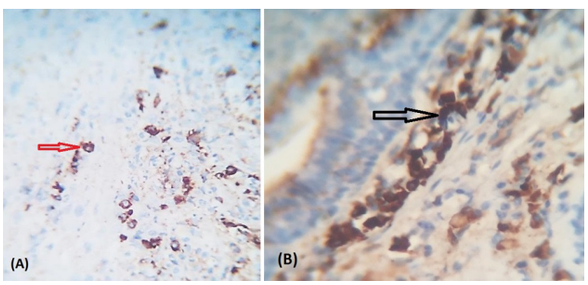

Fig. 2: Photomicrographs showing (a) High power view showing mild expression (red arrow) of the positive staining in the stroma of the tumor (b) High power view showing strong expression (black arrow) of the positive staining in the posterior commissure. [Anti IgA immunostaining, magnification x200 and x400].

In all the twenty seven excised larynges the posterior commissure was free of tumor involvement grossly and by histopathological examination.

DISCUSSION

A study of four hundred and thirty-seven postoperative laryngeal specimens reported rarity or almost complete absence of involvement of the posterior commissure with malignancy mainly squamous cell carcinoma[1] irrespective of the size or aggressiveness of tumor extension within the laryngeal box. The PC was considered in these reports as the mucosal fold extending across the midline between the arytenoid cartilages at the base of the interarytenoid notch, forming the postero median portion of the laryngeal inlet[18 and 19].

The nihility of affection of the PC in all 27 specimens examined endorses the previous study reporting uncommon affection of the PC by squamous cell carcinoma[1].

Immuno histochemical study further revealed a significant characteristic of the PC regarding its relevant and higher expression of IgA. Using monoclonal anti-IgA antibody IgA expression was significantly higher in the PC epithelium than in the neighboring laryngeal epithelium devoid of tumor. Interesting is the highly significant expression of IgA in the immunocytes located in the PC as compared to those in the tumor where it is almost negligible. There is also a highly significant difference between Tumor free immunocyte and posterior commissure immunocyte.

The distinct expression of IgA in the PC mainly in the immunocytes is a promising finding. This exclusive observation regarding the PC should be further studied to identify its realm. The anatomy of the larynx including its mucosal and submucosal compartments, and its ligamentous structure has been already thoroughly studied. Researches should be directed to reveal the factors inhibiting the posterior extension of the tumor or those providing immunity and resistance to the area.

CONFLICT OF INTEREST

There are no conflicts of interest.

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