RESEARCH ARTICLE

Editorial Process: Submission:03/13/2023 Acceptance:06/16/2023

Topoisomerase IIalpha (TopoIIa) A New Promising Marker for Early Detection of Laryngeal Squamous Cell Carcinoma: An Immunohistochemical Study

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Abstract

Objective: the aim of this study is evaluation of Topo IIa expression in laryngeal squamous cell carcinomas and correlation of this expression with various clinicopathological parameters. **Methods:** ninety cases of laryngeal squamous cell carcinoma archived paraffin blocks were collected in the form of total laryngectomies. Each paraffin block was re-cut by rotatory microtome at 4 µm thickness and stained by hematoxylin and eosin for routine histopathological examination and on charged slides for immunohistochemistry using an automated staining system with antibodies against Topo IIa. Mainly nuclear and slightly cytoplasmic staining was considered positive. Percentage of positive Topo IIa cells was graded then grouped into low expression and overexpression. **Results:** Topo IIa overexpression was seen in 91.1% of cases, while low expression was noticed in the remaining 8.9% of cases. There were statistically significant correlations between Topo IIa expression and tumor histological grade, lymph node metastasis and T stage as well as statistically significant positive correlation between Topo IIa expression as we progress from normal to dysplastic/in situ up to malignant transformation. **Conclusion:** High expression of Topo IIa may indicate more aggressive tumor and may play a role in tumorogenesis in laryngeal squamous cell carcinoma.

Keywords: Laryngeal squamous cell carcinoma - Topo IIa - immunohistochemistry

Asian Pac J Cancer Prev, **24 (6)**, 2135-2139

Introduction

Head and neck cancers account for about 500,000 new cases of cancers every year worldwide, and they particularly arise in oral cavity, larynx, and pharynx of which 90% of cases is squamous cell carcinoma (Meireles et al., 2021). In The United States, based on American cancer society, 2020, laryngeal carcinoma is the second most common cancer of respiratory tract after lung cancer with estimated death rate of 3.750 deaths (Siegel et al., 2020). In Egypt, the study done by the Cancer Pathology Registry of National Cancer Institute of Cairo University, laryngeal carcinoma came third among the respiratory cancers, representing 0.2% of total primary tumors with high male predominance (male : female ratio 14 : 1), (Mokhtar et al., 2016). Laryngeal carcinoma is basically related to chronic heavy smoking which explains the male predominance especially in Egypt. However, a change in disease epidemiology occurred with a rapid rise in laryngeal carcinoma incidence among females mainly adolescents rather than adults dropping the male-to-female ratio to 4.5:1. This might be attributed to changing socials habbits in the Egyptian population, increasing the incidence of cigarette and water-pipe smoking among adolescent females (Eltelety and Nassar 2022). Accordingly, laryngeal carcinoma represents a major problem in Egypt that needs to be contained. Since, the prognosis of laryngeal cancer patients is closely related to the tumor size, histological grade, patient age and the presence of lymph node or distant metastasis (Marioni et al., 2006), therefore finding an easy affordable screening test for early detection of laryngeal carcinoma becomes a crucial matter. One of the very promising immunohistochemical markers that could be used as screening test is the Topoisomerase II alpha (Topo IIa) which belongs to a class of nuclear enzymes necessary for cell survival, by regulating the DNA topology and managing replication, transcription, proliferation, and chromosome segregation during the cell cycle. Topo IIa is important for actively dividing cells, and its protein levels are regulated throughout the cell cycle, with peak concentrations at the G2/M phase (Matias-Barrios and Dong 2023). It acts on both strands of DNA, removing knots or tangles (Greco et al., 2022). Since increased cell proliferation is a prerequisite for cancer development and progression, thus overexpression of Topo IIa seems

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to be a very important factor in evolution of malignancy making it a candidate for screening tests. Moreover, Topo IIa inhibition results in permanent links between the DNA strands, eventually blocking cell transcription and replication with resultant apoptosis of damaged cells which means that Topo IIa could be a molecular target for antineoplastic drugs as well (Kyrodimos et al., 2021).

For this reason, this work was planned to evaluate the immunohistochemical expression of Topo IIa as a crucial enzyme that regulates DNA topology in laryngeal squamous cell carcinoma cases in order to correlate its expression with available clinico-pathological data and compare it with non-neoplastic adjacent epithelium and adjacent dysplastic epithelium whenever possible. This aims at using it as a screening test for early detection of laryngeal carcinoma as well as being a target for antineoplastic therapy.

Materials and Methods

This is a preliminary study in which 90 laryngeal squamous cell carcinoma archived paraffin blocks were collected in the form of total laryngectomies, 74 cases of which were associated with selective neck dissection and 16 cases were not associated with neck dissection. Data collected from the patients' sheets included: age, sex, tumor site and size, histological type, tumor grade, depth of invasion and presence of lymph node metastasis. The study was approved by the Medical Ethics Committee.

Staining procedures

Each paraffin block was re-cut by rotatory microtome at 4 μm thickness then mounted on a glass slide and stained by hematoxylin and eosin (H&E) for routine histopathological examination and on charged slides for immunohistochemistry (IHC). IHC was performed using an automated staining system (Dako autostainer link 48) with antibodies against Topo IIa (rabbit polyclonal anti-Topo IIa antibody (1:100 to 1:200), abx133572, Abbexa Ltd, Cambridge, UK).

Immunohistochemical evaluation

Tumor tissue sections were examined under the microscope at high power magnification. Positive expression was accepted in predominantly nuclear and slightly cytoplasmic staining, as declared in the manufacturers' data sheet. Three high power fields were randomly chosen and the mean percentage of positive cells was defined as the percentage of positive cells in each tissue section and graded as follows: negative (-): <10% positive cells; Weak (+): 10-25% positive cells; Moderate (++): 26-75% positive cells; and strong (+++): 76-100% positive cells. The grades between (+) and (+++) were considered to be positive. For statistical reasons, the grades between (-) and (+) were grouped as low Topo IIa expression and grades between (++) and (+++) were grouped as Topo IIa over expression (Papanikolaou et al., 2020).

Statistical analysis

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) vs. 27. Numerical data were summarized using means and standard deviations or medians and ranges. Categorical data were summarized as percentages. Comparisons between 2 groups with respect to normally distributed numeric variables were done. For categorical variables, differences were analyzed with (chi square) test. All p-values are two-sided. P-values < 0.05 were considered significant.

Results

This is a retrospective study, done on randomly selected 90 cases of laryngeal squamous cell carcinoma obtained from total laryngectomy specimens. Selective neck dissection was documented in 74 cases only while the rest of cases (16 cases), lymph nodes could not be assessed. Males represented 84 cases while 6 cases were females, with a male to female ratio of 14:1. Age ranged from 20 to 83 years, with mean age of 61 year ± 10.52. Fourty nine cases (54.4%) were ≥61 years and 41 cases (45.6%) were <61 years. The commonest location was the glottic region. Maximal tumor diameter ranged from 0.5 cm to 6 cm; with mean diameter 3.59 cm ± 1.11. Forty nine cases (54.4%) showed maximal tumor diameter < 3.59 cm and 41cases (45.6%) were ≥ 3.59 cm. As regards the histological grade of tumors, the majority of cases (63.3%) were moderately differentiated (grade 2), followed by poorly differentiated (26.7%) (grade 3) and only 10 % were well differentiated (grade 1). Most of our cases (66.7%) showed cartilage infiltration and 73.3% show positive infiltration of resected margins. Lymph node metastasis was seen in 37 cases out of the 74 cases with selected neck dissection. Most of cases were of T2 and N1 stages.

Topo IIa expression and its correlation with histopathological parameters

All the studied tumor cases showed positive Topo IIa expression. Strong expression was seen in 61.1% of cases, 30% of cases showed moderate expression, 8.9 % of cases showed weak expression. Consequently, Topo

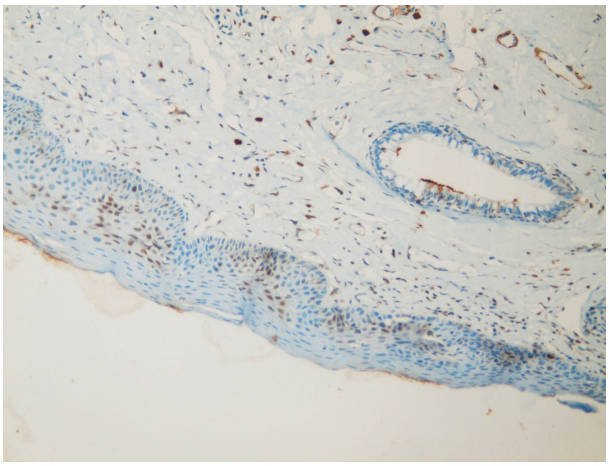

Figure 1. A Case of Normal Laryngeal Epithelium Exhibiting Nuclear and Cytoplasmic Topo IIa Immunostaining (Mainly Nuclear) in Less than 25% of Epithelial Cells Representing Weak Expression (x200 Original MagnificatioAn).

Table 1. Topo IIa Expression in Cases Showing Normal, Dysplastic Epithelium/Insitu Component and Malignant Tissue Altogether

		Epithelium						
		Normal	%	Dysplastic	%	Malignant	%	p value
TopoIIa	Negative	6	20.7	0	0.0	0	0.0	< 0.001
	Weak	10	34.5	7	24.1	2	6.9	
	Modrate	8	27.6	13	44.8	9	31.0	
	Strong	5	17.2	9	31.0	18	62.1	
total		29	100.0	29	100.0	29	100.0	

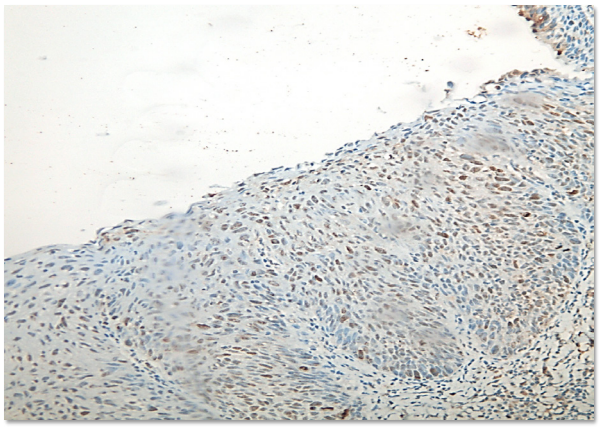

Figure 2. A Case of Laryngeal Epithelium Showing High Grade Dysplasia Exhibiting Nuclear and Cytoplasmic Topo IIa Immunostaining (Mainly Nuclear) in Slightly more than 50% of Epithelial Cells Representing Moderate Expression (x200 Original Magnification).

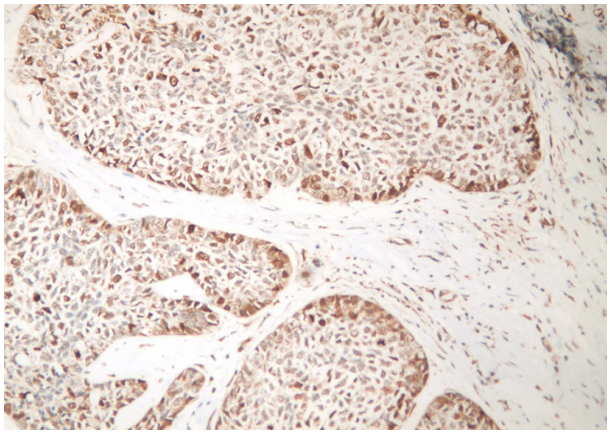

Figure 3. A Case of Laryngeal Squamous Cell Carcinoma Grade II Exhibiting Nuclear and Cytoplasmic Topo IIa Immunostaining (Mainly Nuclear) in more than 75% of Epithelial Cells Representing Strong Expression (x400 Original Magnification).

IIa overexpression was seen in 91.1% of cases while low expression was noticed in the remaining 8.9% of cases.

Topo IIa overexpression was noticed to positively correlate with higher histological tumor grade with statistically significant correlation (p value <0.001); as 3.3% of grade 1 cases, 61.1% of grade 2 cases and 85% of grade 3 cases showed Topo IIa overexpression. As for tumor stage, Topo IIa overexpression was noticed to be more with higher tumor stage (T) with statistically significant correlation (p value <0.001); as 2.2% of T1 cases, 37.8% of T2 cases, 34.4% of T3 cases and 16.7% of T4 cases showed Topo IIa overexpression. Concerning lymph node metastasis, Topo IIa overexpression was noticed to be higher with cases showing positive lymph node metastasis with statistically significant correlation (p value = 0.008). Furthermore all cases with positive nodal metastasis showed Topo IIa overexpression. Although Topo IIa overexpression was noticed to be higher with higher N stage but the results were not statistically significant.

Topo IIa expression in normal, dysplastic/insitu and malignant tumor

Normal epithelium was encountered in 34 cases, 20.6% showing negative Topo IIa expression in the normal epithelium in contrast to 79.4% showing positive expression mostly weak (44.4%) as shown in Figure 1, while 100% of malignant tumor tissue showed positive expression mostly strong (58.8%). There was a statistically significant relationship (p value < 0.001) between Topo IIa expression in normal epithelium and underlying malignant tissue.

Dysplastic epithelium/insitu component, was seen in 54 cases all of them showed positive expression of Topo IIa mostly moderate (46.3%) as shown in Figure 2, besides 100% of concomitant malignant tumor tissue mostly strong expression (63%) as shown in Figures 3 and 4 in the malignant tissue showing statistically significant correlation (P value=0.008).

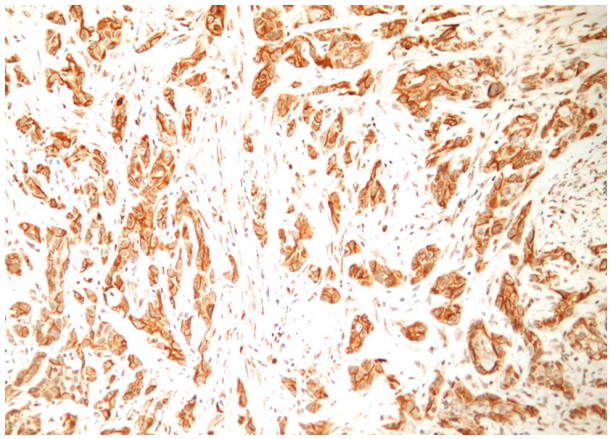

Figure 4. A Case of Laryngeal Squamous Cell Carcinoma Grade III Exhibiting Nuclear and Cytoplasmic Topo IIa Immunostaining (Mainly Nuclear) in more than 75% of Epithelial Cells Representing Strong Expression (x200 Original Magnification).

Normal, dysplastic epithelium/insitu component and invasive malignant tissue were present altogether in 29 cases only in the current study, 76.9% of normal epithelium showed positive expression mostly weak, while 100% of them showed positive expression of Topo IIa in the dysplastic epithelium/insitu component mostly moderate and 100% in the malignant tumor tissue with stronger expression in the malignant tissue with statistically significant correlation (P value < 0.001) as shown in Table 1.

No other statistically significant correlation could be achieved between Topo IIa expression and any other clinicopathological parameter.

Discussion

Laryngeal carcinoma is the most common head and neck cancer worldwide and is the second most prevalent malignancy of the respiratory system (Zhang et al., 2021). It is considered a major problem in Egypt due to the increasing incidence among adolescent females. In the current study we tried to evaluate the expression Topo IIa in laryngeal carcinoma cases in order to use it as a screening test for early cancer detection. Our study was done on 90 cases of laryngeal carcinoma removed by laryngectomy. All our studied cases showed positive Topo IIa expression, with predominance of overexpression (91.1%) while low expression was noticed in the remaining 8.9% of cases. Likewise Topo IIa overexpression was dominant in the study done by Papanikolaou et al., (2020), but with a lower percentage (64%). In the current study, a statistically significant correlation was observed between Topo IIa expression and tumor histological grade (more in higher grades) (p value < 0.001). These results go with those reported by Papanikolaou et al., (2020), and Feng et al., (2014). In our study, there was statistically significant correlation between Topo IIa expression and T stage (p value < 0.001) as well as lymph node metastasis (p value = 0.008). Likewise Shvero et al., (2008) also found a significant correlation between Topo IIa expression and T stage (P value =0.0001), and close to the results of Papanikolaou et al., (2020) who found borderline correlation with the T stage (p=0.053)). These similarities between our study and theirs emphasise the role of Topo IIa in laryngeal carcinoma progression and prognosis which could be used in evaluating advanced or early stage of cancer and may be used as early predictor of its prognosis. In our study we couldn't observe any statistically significant correlations between Topo IIa expression and age, sex, tumor location, maximal tumor diameter, state of cartilage infiltration, infiltration of resected margins or N stage. These findings were consistent with studies done by Papanikolaou et al., (2020) and Shvero et al., (2008).

In the current study, normal epithelium was encountered in 34 cases, 20.6% showed negative expression of Topo IIa in the normal epithelium and 79.4% showed positive expression mostly weak compared to 100% of malignant tumor tissue showing positive Topo IIa expression mostly strong. There was a statistically significant relationship between Topo IIa expression in normal epithelium and underlying malignant tissue (P value < 0.001). This was supported by the result of Horibe et al., (2000) who performed an immunohistochemical analysis on 63 cases of early glottic laryngeal carcinoma and 10 cases of normal laryngeal mucosa, and showed that the expression of Topo IIa was significantly increased in early glottic laryngeal carcinoma tissue when compared to normal laryngeal mucosa.

The current study also revealed 54 cases that showed dysplastic epithelium/insitu component, all of them showed positive expression of Topo IIa in the dysplastic epithelium or the insitu component mostly moderate compared to 100% concomitant malignant tumor tissue showing positive expression mostly strong with statistically significant correlation (P value=0.008). Normal, dysplastic epithelium/insitu component and invasive malignant tissue were present altogether in 29 cases only in the current study, 76.9% of them showed positive expression of Topo IIa in the normal epithelium mostly weak, 100% of them showed positive expression of Topo IIa in the dysplastic epithelium/insitu component mostly moderate and 100% of them showed positive expression of Topo IIa in the malignant tumor tissue mostly strong. Accordingly a statistically significant correlation was found between concomitant Topo IIa expression in normal, dysplastic epithelium/insitu component and invasive malignant tissue (P value < 0.001). This significant correlation was within our expectations knowing the role of Topo IIa in tumor progression, being an important regulator of the cell cycle. To our knowledge no other comparable studies were spotted comparing Topo IIa expression in normal, dysplastic epithelium/insitu component and invasive malignant tissue. In a study done by Shamaa et al., (2008) and Tsukinoki et al., (2001) on expression of Topo IIa in oral squamous cell carcinomas cases a significant correlation was found between Topo IIa expression and T stage, lymph node metastasis, tumor grade and significant different expression between malignant cells and adjacent normal epithelium was observed. These findings suggest that Topo IIa plays a role in carcinogenesis and development of oral carcinoma and probably laryngeal carcinoma as well, being both exposed to similar carcinogenic environment.

To sum up, the relation between Topo IIa expression on one hand and tumor histological grade, lymph node metastasis and T stage on the other hand was proved to be statistically significant emphasizing its prognostic role. The relation between Topo IIa expression and the transition between normal, dysplastic/in situ and invasive tumor was statically significant, highlighting the role of Topo IIa in tumorogenesis of laryngeal carcinoma.

On that account, Topo IIa expression could be a used as a screening test for predicting laryngeal squamous cell carcinomas particularly patients who are at higher risk of metastatic carcinoma as well as being a possible target for antitumor therapy. Nevertheless, more studies using immunohistochemistry and molecular analysis with larger sample size including benign, dysplastic and malignant laryngeal lesions are encouraged for further justification.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

The authors declare that there is no conflict of interest and received no financial support for this research. The authors solely developed the theory, verified the analytical methods and wrote the manuscript. The material obtained in this study was collected in the form of archived paraffin blocks and clinical data were taken from pathology request sheets designated by numbers, therefore no consent from patients was required. All steps of this research were approved by the ethical committee.

Ethics approval

All steps are approved by the ethical committee.

Conflict of interest

The authors declare no conflict of interest.

References

Eltelety AM, Nassar AA (2022). Analysis of the pathological characteristics of locally advanced laryngeal carcinoma in Egyptian females: 7-year review. *Egypt J Otolaryngol*, **38**, 37.

Feng AL, Le A, Johnson DN, Varvares, MA (2018). Multiple simultaneous head and neck cancers in Lynch syndrome: Case report and literature review. *Laryngoscope*, **128**, 2759-1.

Greco G, Pellicioni V, Cruz-Chamorro I, et al (2022). Marine-Derived Compounds Targeting Topoisomerase II in Cancer Cells: A Review. *Mar Drugs*, **20**, 674.

Horibe Y, Murakami M, Komori K, et al (2000). Expression of topoisomerase II alpha, Ki-67 and p53 in early stage laryngeal carcinomas not featuring vocal cord fixation. *Apmis*, **108**, 689-6.

Kyrodimos E, Chrysovergis A, Mastronikolis N, et al (2021). Impact of Topoisomerases Complex Deregulation on Head and Neck Carcinoma Genomic Instability. *Anticancer Res*, **41**, 2773-9.

Marioni G, Marchese-Ragona R, Cartei G, et al (2006). Current opinion in diagnosis and treatment of laryngeal carcinoma. *Cancer Treat*, **32**, 504-5.

Matias-Barrios VM, Dong X (2023). The Implication of Topoisomerase II Inhibitors in Synthetic Lethality for Cancer Therapy. *Pharmaceuticals*, **16**, 94.

Meireles Da Costa N, Mendes FA, Pontes B, et al (2021). Potential therapeutic significance of laminin in head and neck squamous carcinomas. *Cancers*, **13**, 1890.

Mokhtar N, Salama A, Badawy O, et al (2016). Cancer Pathology Registry: A 12-Year Registry, 2000-2011. Vol. 13. Bethesda: National Cancer Institute; Cairo, pp 192-208.

Papanikolaou V, Chrysovergis A, Mastronikolis N, et al (2020). Topoisomerase IIa protein expression patterns in laryngeal squamous cell carcinoma. *Anticancer Res*, **40**, 807-1.

Shamaa A, Zyada M, Wagner M, et al (2008). The significance of Epstein Barr virus (EBV) and DNA topoisomerase II alpha (DNA-Topo II alpha) immunoreactivity in normal oral mucosa, oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC). *Diagn Pathol*, **3**, 1-3.

Shvero J, Koren R, Shvili I, et al (2008). Expression of Human DNA topoisomerase ii-α in squamous cell carcinoma of the larynx and its correlation with clinicopathologic variables. *Am J Clin Pathol*, **130**, 934-9.

Siegel RL, Miller KD, Jemal A (2020). Cancer statistics, 2020. *CA Cancer J Clin*, **70**, 7-30.

Tsukinoki K, Kamoi Y, Watanabe Y (2001). Immunohistochemical study of DNA topoisomerase type II alpha and Ki-67 in oral squamous cell carcinoma. *Oral Med Pathol*, **6**, 79-4.

Zhang Q, Wang H, Zhao Q, et al (2021). Evaluation of risk factors for laryngeal squamous cell carcinoma: a single-center retrospective study. *Front Oncol*, **11**, 224