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Profile of Cytokines (IL-1β, TNF- α and IL-10) in Eyelid Tumors

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Abstract

Introduction: Tissue cytokine levels (IL-1β, TNF-α and IL-10) have been utilized for screening, diagnosis, prognostication and treatment of tumors. The current study, therefore, purports to measure the levels of common cancer associated cytokines in histopathologically confirmed tissue samples of eyelid tumors. The compilation of this information is planned with a view to facilitate further studies which will enable the use of specific cytokines for screening, diagnosis, prognostication and treatment of these tumors.

Material and Method: This is a prospective observational cross-sectional case-control study conducted on all immunocompetent cases of eyelid neoplasia that underwent surgical treatment over a one year period. Tissue from other non-inflammatory, non- neoplastic eyelid disorders were recruited as controls. Commercially available "Ready-SET-Go! ELISA Kit" was used to determine the levels of the cytokines (IL-1β, TNF-α, IL-10) in pg/ml. Results were expressed as the mean (SEM). Statistical analysis was done using SPSS software, version 17.0 employing 'Chi square test', 'unpaired t-test' and Pearson's correlation coefficient.

Results: On comparing the mean cytokine levels of the two groups with surgically excised tissue of 25 patients of eyelid neoplasia of either sex as cases and 26 age and sex matched tissue samples as controls, t-test revealed significantly different and higher level of IL- 1β (16.71 ± 2.75 vs. 14.60 ± 3.71, t=2.3, p=0.025), TNF-α (19.11 ± 3.2 vs. 16.73 ± 4.44, t=2.18, p=0.033) and IL-10 (24.86 ± 5.94 vs. 21.60 ± 4.60, t=2.19, p=0.032) in cases as compared to controls.

Discussion: The mean cytokine levels of IL-1β, TNF-α, IL-10 were raised in cases by 12.62%, 12.45% and 13.11% respectively ($p<0.05$) in comparison to controls.

Conclusion: Significantly higher levels of cytokines IL-1β, TNF-α and IL-10 are present in the tissues of eyelid neoplasia. This represents a higher association with tumorigenesis. Development of anti-cytokine molecules, cytokine receptor agonists and antagonists for modifying the disease processes and their application to provide treatment in eyelid tumors is a potential area for research in eyelid tumors.

Keywords: Eyelid tumor; Cytokines; Tumorigenesis

Introduction

Cytokines are important negative and positive regulators of cell replication, differentiation, migration, cell survival, cell death and cell transformation [1-4]. Their role in tumorigenesis has been well established [5]. Limited data is, however, available to substantiate the same for eyelid tumors, specific characterization of which has seldom been done.

The current study, therefore, purports to measure the levels of common cancer associated cytokines, viz. IL-1β, TNF-α and IL-10 in histopathologically confirmed tissue samples of eyelid tumors. The compilation of this information is planned with a view to facilitate further studies which will enable the use of specific cytokines for screening, diagnosis, prognostication and treatment of these tumors.

Material and Method

This is a prospective observational cross-sectional case-control study which was conducted by consecutive recruitment of all immunocompetent cases of eyelid neoplasia that underwent surgical treatment over a one year period from August 2014 to July 2015 in the Department of Ophthalmology, King George's Medical University, Lucknow, India. Tissues from other non-inflammatory, non- neoplastic eyelid disorders [Senile eyelid disorders, Ptosis (non-inflammatory)] were recruited as controls. Both the cases and the controls were Asian – Indians. None of the cases had undergone any prior eyelid surgery.

Small part of the surgically excised tissues were collected in sterile Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotic- antimycotic solution (Gibco BRL, USA), and immediately transported to In Vitro Toxicology Laboratory, Indian Institute of Toxicology Research, Lucknow, India, located at a distance of 2 kms, at a temperature of -4 °C. Tissues were preserved in deep freezer at -80 °C till further processing. Commercially available "Ready-SET-Go! ELISA Kit" (Sigma Aldrich Chemie GmbH,Buchs, St. Gallen) was used to determine the levels of the cytokines (IL-1β, TNF-α, IL-10) in the tissue protein samples. Tissue supernatant (100 µl) were used in triplicate wells for the determination of various cytokines as per manufacturer's instructions. The plates were then analyzed at 450 nm using Multiwell microplate reader (Synergy HT, Bio-Tek, USA). Normal control sets were also run under identical conditions.

Results were expressed as the mean (SEM). Data were summarized as Mean ± SD (standard deviation) from the values obtained from at least three independent experiments, in each of which triplicate samples were used. Categorical groups were compared by chi-square (χ^2) test. Groups were compared by independent Student's 't'test. The Pearson correlation coefficient was calculated to find the direction of the correlation between two continous variables. A two-tailed p-value $p<0.05$ was considered statistically significant. Analyses were performed on SPSS software (Windows version 17.0).

Observation and Results

The present study recruited surgically excised tissues of 25 patients of eyelid neoplasia of either sex as cases and 26 age and sex matched tissue samples as controls. The controls and case samples of our study included a fairly wide range of age distribution spanning five decades. However, there was no significant difference ($p>0.05$) in the mean age of two groups. Gender frequency and demographic factors like smoking, alcohol, tobacco chewing, sun exposure, hygiene and crowding between the two groups, also did not differ statistically ($p>0.05$). In other words, subjects of two groups were demographically matched and comparable, thus, have no influence on the study outcome measures.

The distribution of tissues of controls and types of tumor in cases are summarised in Table 1 and 2 respectively and also depicted in Figure 1 and 2 respectively.

Tissues- controls	Controls (n=26)	Percent (%)
Rectus muscle	18	69.2
Superior oblique muscle	1	3.8
Tarsus	7	26.9

Table 1: Distribution of tissues of controls

Type of tumor	Cases (n=25)	Percent (%)
Basal Cell Carcinoma (BCC)	4	16
Malignant Melanoma (MaligMel)	1	4
Squamous Cell Carcinoma (SCC)	8	32
Sebaceous Cell Carcinoma (SebCC)	12	48

Table 2: Distribution of types of tumors (Cases)

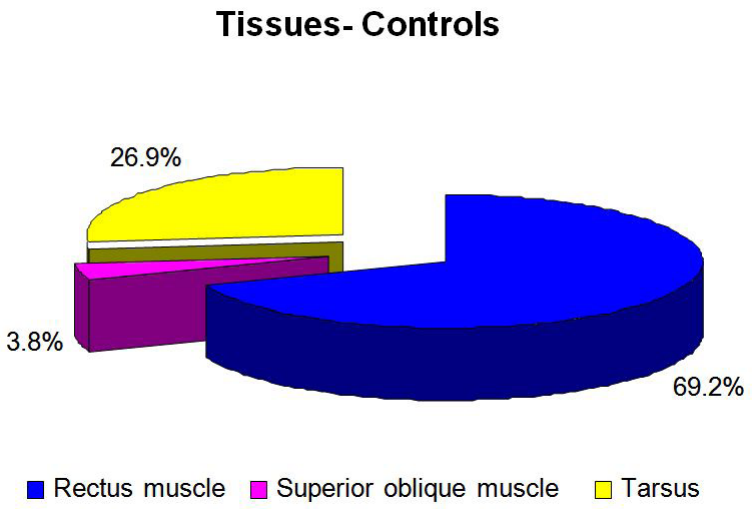

Figure 1: Distribution of tissues of controls

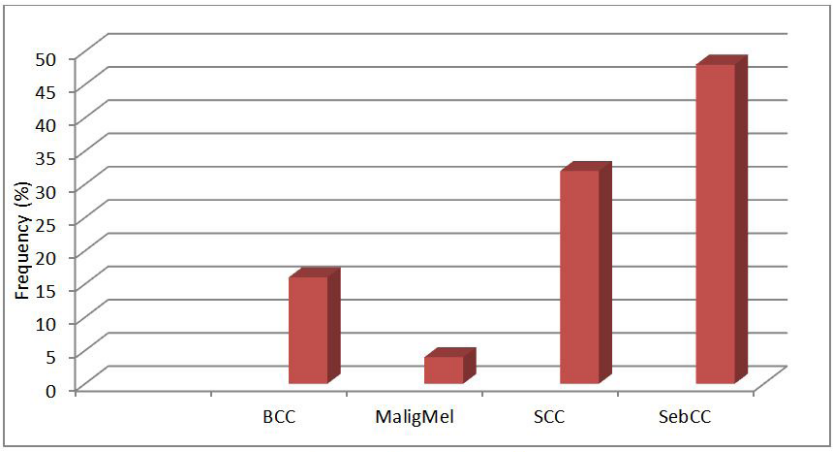

Figure 2: Distribution of types of tumors in cases

The cytokine levels of cases and controls are summarized in Table 3 and also shown in Figure 3. On comparing the mean cytokine levels (in pg/ml) of the two groups, t-test revealed significantly different and higher levels of IL- 1β (16.71 ± 2.75 vs. 14.60 ± 3.71, t=2.3, p=0.025), TNF-α (19.11 ± 3.2 vs. 16.73 ± 4.44, t=2.18, p=0.033) and IL-10 (24.86 ± 5.94 vs. 21.60 ± 4.60, t=2.19, p=0.032) in cases as compared to controls. The mean increase in IL- 1β, TNF-α and IL-10 of cases was found to be 12.62%, 12.45% and 13.11% higher respectively as compared to controls. The degree of correlation amongst the three cytokines themselves was determined for cases using the Pearson correlation coefficient (r) (Table 4). The value of "r" between IL- 1β and TNF-α is 0.276, IL- 1β and IL-10 is -0.182, TNF-α and IL-10 is -0.212. Small sample size being a limitation of our study, individual tumor tissue could not be studied separately.

Cytokine (pg/ml)	Controls (n=26)	Cases (n=25)	% mean change	t- value	p- value
IL-1β	14.60 ± 3.71	16.71 ± 2.75	12.62	2.3	0.025
TNF-α	16.73 ± 4.44	19.11 ± 3.2	12.45	2.18	0.033
IL-10	21.60 ± 4.60	24.86 ± 5.94	13.11	2.19	0.032

Table 3: Cytokine levels (Mean ± SD) of two groups

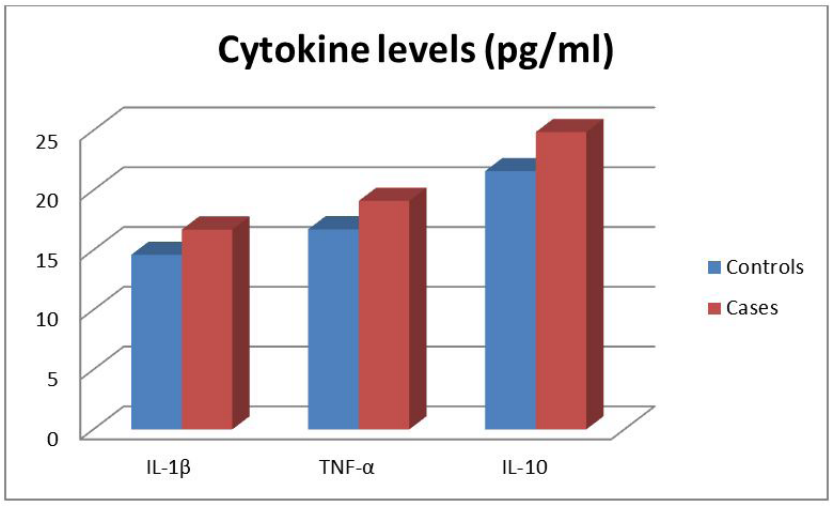

Figure 3: Mean cytokine levels of two groups (pg/ml)

		IL-1β	TNF-α	IL-10
IL-1β	R	1		
	p-value			
TNF-α	R	0.2768	1	
	p-value	0.1804		
IL-10	R	-0.1825	-0.212	1
	p-value	0.3839	0.308	

*Correlation is significant at the p-value < 0.05

Table 4: Pearson correlation coefficient among cytokines in cases

Discussion

To the best of our knowledge, there is no data published till date regarding the correlations of cytokine levels, not only for tumors of eyelid but even for the normal periocular tissues. Therefore, a pilot data with an analysis of the interplay of these cytokines in the local milieu of periocular tissues was developed through this study.

The levels of IL-1β in our study was found to be raised in cases significantly by 12.62% in comparison to controls (t=2.3, p=0.025). IL-1β has been established to be pro-inflammatory pro-tumor cytokine in previous studies in which decrease in tumor cell proliferation correlated with downregulated expression of IL-1β, leading to the possibility that it may directly increase proliferation of tumor cells [6-8].

TNF-α is the oldest and most widely studied cytokine. It has been demonstrated in various studies to be a multi-functional cytokine with different actions in different tissues [9-11]. Our study demonstrated the levels of TNF-α to be higher by 12.45% in cases which has a significant difference with that in control tissues (t=2.18, p=0.033). Thus, suggesting its role in tumorigenesis of eyelid tumors.

IL-10 has a complex biological activity in tumors and has diverse effects regarding its influence on cancer. In our study, its levels in cases were seen to be raised by 13.11% which is significant in comparison to controls (t=2.19, p=0.032). This finding is in accordance with the previous studies in which this cytokine was suggested to serve as a tumor growth factor [12-14].

IL-1β is found to have a weakly positive correlation with the levels of TNF-α in cases (r= 0.276, p=0.18) and a weakly negative correlation with IL-10 (r= -0.182, p=0.383). TNF-α and IL-10 also shows a non-significant weakly negative correlation (r= -0.212, p=0.308). Thereby, meaning that a rise in IL-1β levels would cause a rise of levels of TNF-α in eyelid tumor tissue but the small sample size limits the significance of this correlation.

The application of cytokines as screening and prognostic markers and tumor treatment agents, requires a precise knowledge and a detailed deciphering of the signalling pathways and cellular mechanisms involved in cytokine action in the region of interest. This knowledge will facilitate the development of cytokine analogues, anti-cytokine molecules, cytokine receptor agonists and antagonists for modifying the disease processes. By establishing the role of cytokines in the pathogenesis of eyelid tumors, this study will encourage further undertakings directed towards gaining a better understanding of these mechanisms.

Conclusion

The current study on tissue levels of cytokines IL-1β, TNF-α and IL-10 in eyelid tumors concludes that significantly higher levels of these cytokines are present in the tumor tissue. Thus, having an association with tumorigenesis. Development of anti-cytokine molecules, cytokine receptor agonists and antagonists for modifying the disease processes and their application to provide treatment in eyelid tumors is a potential area for research.

Further research is warranted to identify the precise role of the multifunctional cytokines in this region, to enable their application as disease modifying and prognostication agents.

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