IL-1β and TNF-α as a biomarker of recurrence in malignant eyelid tumors

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Abstract

Aim: To establish a correlation between cytokine levels (IL-1β, TNF-α and IL-10**)** and establishing them as a biomarker of recurrence in malignant eyelid tumors.

Materials and Methods: Prospective observational cross-sectional case-control study of 38 consecutive cases of malignant eyelid tumors that underwent surgical treatment over a period of 18 months. 26 age and sex matched controls with other non-inflammatory, non- neoplastic eyelid disorders. The levels of the cytokines [IL-1β (Interleukin- 1β), TNF-α (Tumor necrosis factor-α), IL-10 (Interleukin-10)] (in pg/ml) were determined by using ELISA Kit. Data was analyzed statistically.

Result: On comparing the mean cytokine levels of the two groups, t-test revealed significantly higher levels of IL- 1β (16.39±2.86 vs. 14.62±3.72, t=2.17, p=0.034), TNF-α (19.32±3.47 vs. 16.74±4.45, t=2.61, p=0.011) and IL-10 (24.79±5.87 vs. 21.61 ± 4.61, t=2.33, p=0.023) in cases. ANOVA revealed significantly different levels of IL- 1β (F=7.86, p<0.001), TNF-α (F=7.42, p<0.001) and IL-10 (F=4.36, p=0.006) between tumor and it's three adjacent tissues.

Conclusion: Cytokine levels (IL-1β, TNF-α and IL-10**)** in the adjacent tissues beyond safety margins were normal and comparable to that in controls suggesting the margins to be tumor free. Highly significant lower levels of IL-1β and TNF-α in tissues beyond safety margins can be used as an important predictor for local recurrence of tumor.

Keywords: IL-1β, Malignant eyelid tumor, Recurrence, TNF-α

Introduction

Cytokines, a diverse group of small proteins, are important negative and positive regulators of cell activity. Ample evidence of their role in the diagnosis and treatment of various systemic tumors is present. However, specific characterization of cytokines in malignant eyelid tumors has seldom been done.

IL-1β (Interleukin- 1β) is a crucial mediator of the host inflammatory response in natural immunity with a proinflammatory effect[1]. Expression of IL-1 has been correlated with various systemic tumors[2,3].

TNF-α (Tumor necrosis factor-α) is a multi-functional cytokine having tumor-promoting as well as tumor-inhibiting activity in varying tissue micro-environments and have been demonstrated to promote metastatic behaviour in cancer cells via diverse mechanisms various systemic tumors[4].

IL-10 (Interleukin-10) is an important immunoregulatory cytokine and has been shown to have diverse effects regarding it's influence on cancer. IL-10 has been identified in the serum and tumor with a negative correlation between circulating levels of IL-10 and prognosis[5-8].

The current study aims at estimating the levels of cytokines, viz. IL-1β, TNF-α and IL-10 in histopathologically confirmed tissue samples of malignant eyelid tumors in comparison to other non-inflammatory, non- neoplastic eyelid disorders. Additionally, differences between tumour and its adjacent tissues beyond safety margins will aide in establishing a correlation between cytokine levels and tumor recurrence.

Materials and Methods

Study was conducted according to tenets of declaration of Helsinki after approval from Institutional Ethics Committee. A prospective observational cross-sectional case-control study, was conducted by recruitment of 38 consecutive cases of malignant eyelid tumors that underwent surgical treatment over a period of 18 months from August 2014 to January 2016 in the Department of Ophthalmology, King George's Medical University, Lucknow, India. 26 patients with non-inflammatory, non- neoplastic eyelid disorders [tissues from senile eyelid disorders, ptosis (non-inflammatory)] were recruited as controls. An informed consent was taken from all the patients.

Excised tissues (4 samples were obtained from each case –1 from tumor mass and 3 samples from adjacent tissue including medial, base, and lateral) 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 transported to In Vitro Toxicology Laboratory, Indian Institute of Toxicology Research, Lucknow, India, at a temperature of -4°C immediately and were preserved in deep freezer at -80°C till further processing. The levels of the cytokines (IL-1β, TNF-α, IL-10) in the tissue protein samples (100 µl tissue supernatant) was determined by using commercially available "Ready-SET-Go! ELISA Kit" (Sigma Aldrich Chemie GmbH, Buchs, St. Gallen) in triplicate wells. The analyses of the plates was done at 450 nm using Multiwell microplate reader (Synergy

HT, Bio-Tek, USA). Control samples were also analyzed by identical procedure.

Expression of the results was done as mean (SEM) and data were summarized as standard deviation (Mean ± SD) from the values obtained from at least three independent experiments, in each of which triplicate samples were used. Comparison of the groups was done by independent Student's 't' test, ANOVA and Tukey post hoc test. P-value less than 0.05 was considered statistically significant. SPSS software (Windows version 17.0) was used for statistical analyses.

Result

The present study recruited surgically excised tissues (1 tumor tissue and 3 adjacent tissues) of 38 patients of malignant eyelid tumour of either sex as cases and 26 age and sex matched tissue samples as controls. Subjects of two groups were demographically matched and comparable (Table 1).

In controls, the tissues included were rectus muscle (69.2%), tarsus (26.9%) and muller's muscle (3.8%) and in cases, sebaceous gland carcinoma (47.36%), basal cell carcinoma (26.3%), squamous cell carcinoma (18.4%) and malignant melanoma (7.89%).

i. **Comparison of cytokine levels of cases and controls:** The cytokine levels of cases and controls are summarized in Table 2. Student's t-test for the mean cytokine levels (in pg/ml) of the two groups, revealed significantly higher levels of IL- 1β (16.39±2.86 vs. 14.62±3.72, t=2.17, p=0.034), TNF-α (19.32±3.47 vs. 16.74±4.45, t=2.61, p=0.011) and IL-10 (24.79±5.87 vs. 21.61±4.61, t=2.33, p=0.023) in cases as compared to controls. No definite correlation was seen amongst the three cytokines.

ii. **Comparison of cytokine levels of tumour and it's adjacent tissues:** On comparing the mean cytokine levels of four groups, ANOVA revealed significantly different levels of IL- 1β (F=7.86, p<0.001), TNF-α (F=7.42, p<0.001) and IL-10 (F=4.36, p=0.006) among the groups (Table 3). Further, Tukey post hoc test showed that the mean level of IL- 1β, TNF-α and IL-10 also lowered significantly in medial, base and lateral as compared to tumour tissue [Cases vs. Medial: IL-1β<0.001, TNF-α=0.001 and IL-10=0.005; Cases vs. Base: IL- 1β<0.001, TNF-α<0.001 and IL-10=0.083; Cases vs. Lateral: IL- 1β=0.001, TNF-α=0.001 and IL-10=0.022]. This lowering of cytokine level was highly significant in case of IL-1β and TNF-α (p<0.001) but in case of IL-10, p=0.006.

Table 1: Demographic characteristics of two groups

Demographics characteristics	Controls (n=26) (%)	Cases (n=38) (%)	t/χ^2 value	p value
Age (yrs):				
Mean ± SD	46.23±10.51	48.05±18.21	0.46	0.647
Sex:				
Female	12 (46.2)	15 (39.5)	0.28	0.595
Male	14 (53.8)	23 (60.5)		
Smoking:				
No	22 (84.6)	31 (81.6)	0.10	0.752
Yes	4 (15.4)	7 (18.4)		
Alcohol:				
No	23 (88.5)	31 (81.6)	0.56	0.456
Yes	3 (11.5)	7 (18.4)		
Tobacco:				
No	21 (80.8)	26 (68.4)	1.21	0.272
Yes	5 (19.2)	12 (31.6)		
Sun exposure:				
No	11 (42.3)	11 (28.9)	1.22	0.269
Yes	15 (57.7)	27 (71.1)		
Hygiene:				
Good	21 (80.8)	22 (57.9)	3.66	0.056
Poor	5 (19.2)	16 (42.1)		
Crowding:				
Absent	22 (84.6)	28 (73.7)	1.08	0.299
Present	4 (15.4)	10 (26.3)		

Table 2: Cytokine levels (Mean ± SD) of cases and controls

Cytokine (pg/ml)	Controls (n=26)	Cases (n=38)	% mean change	t- value	p- value
IL-1β	14.62±3.72	16.39±2.86	10.9	2.17	0.034
TNF-α	16.74±4.45	19.32±3.47	13.4	2.61	0.011
IL-10	21.61±4.61	24.79±5.87	12.9	2.33	0.023

Table 3: Comparison of cytokine levels (Mean ± SD) of tumour and it's adjacent tissues

Cytokine (pg/ml)	Cases (n=38)	Adjacent of cases			F Value	P Value
		Medial (n=38)	Base (n=38)	Lateral (n=38)		
IL-1β	16.39±2.86	13.99±2.75	13.85±2.63	14.12±2.35	7.86	<0.001
TNF-α	19.32±3.47	16.87±2.72	16.67±2.78	16.75±2.53	7.42	<0.001
IL-10	24.79±5.87	21.02±4.29	22.10±4.99	22.54±4.49	4.36	0.006

Discussion

The current study analyses the cytokines, viz IL-1β, TNF-α and IL-10 in malignant tumours of eyelid, it's adjacent tissue beyond the safety margins and compares them with the local milieu of periocular tissues. It attempts to study their role as a prognostic marker in eyelid tumours.

IL-1β is a pro-inflammatory pro-tumour cytokine. The levels of IL-1β was significantly raised in tumour tissue. Expression of IL-1β has been correlated with tumour cell proliferation, in previous studies[9,10]. This leads to the possibility that it may directly increase proliferation of tumour cells.

In our study, the levels of TNF-α was demonstrated to be significantly higher in cases than in control tissues. TNF-α is the most widely studied cytokine and has been demonstrated in various studies to be a multi-functional cytokine with different actions in different tissues[11,12]. This, suggests its role in tumorigenesis of eyelid tumours.

IL-10 has a complex biological activity in tumours and has diverse effects regarding its influence on cancer. In our study, its levels in cases were seen to be raised significantly in comparison to controls which is in accordance with the previous studies in which it was suggested to serve as a tumor growth factor[13-15].

Cytokines levels in tissues adjacent to the cut margin beyond safety margins were less than that in tumor tissues in the study. It can be inferred that the surgical margins were tumor free on all the three adjacent sides beyond 5mm of the surgical safety margins of excision. These levels were similar to the control values ($p>0.05$). There was no significant variation in cytokine levels among the three adjacent sides ($p>0.05$), indicating that the three adjacent tissues were without tumor invasion. This indirectly supports the surgical safe margin concept[16-18]. The difference in levels were highly significant in case of TNF- α and IL-1β ($p<0.001$). This decrease in cytokine levels were observed on all the three adjacent sides in case of TNF-α, IL-1β with highly significant lowered levels but not in IL-10. Thus, TNF-α and IL-1β are more important prognostic markers in case of recurrence. Also we can use the side with highest TNF-α and IL-1β levels for vigorous follow-ups to look for any evidence of recurrence.

No recurrence was noted in the study period. The fact that the peritumor cytokine level beyond safety margin were normal can be used as an indicator of tumour Free State, therefore, be utilized as a prognostic marker. Similar use of cytokines (IL-6 and TNF-α) as prognostic markers for prostate cancer was suggested by Michalaki *et al.* (2004) and were correlated directly with the extent of malignant disease[19].

Conclusion

The current study on tissue levels of cytokines (IL-1β, TNF-α and IL-10) in malignant eyelid tumors and it's adjacent tissues beyond safety margins concluded that significantly higher levels were present in the tumor tissues. Cytokines were found to be normal in the adjacent tissues beyond safety margins and comparable with controls, thus establishing the margins to be tumor free. This finding enables us to use IL-1β and TNF-α as an important predictor for local recurrence of tumor. This observation at the biomolecular level establishes the surgical excision margin of 5mm as safe.

The prognostic significance of cytokine levels in adjacent tissues beyond safety margins, will encourage further undertakings directed towards the development of tumor treatment agents.

References

1. Kurzrock R, Kantarjian H, Wetzler M, Estrov Z, Estey E, Troutman-Worden K, Gutterman JU, Talpaz M. Ubiquitous expression of cytokines in diverse leukemias of lymphoid and myeloid lineage. Exp Hematol 1993 Jan;21(1):80-5.
2. Sims JE, March CJ, Cosman D, Widmer MB, MacDonald HR, McMahan CJ, Grubin CE, Wignall JM, Jackson JL, Call SM. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. Science. 1988 Jul 29;241(4865):585-9.
3. Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M. IKKβ links inflammation and tumorigenesis in a mouse model of

colitis-associated cancer. Cell. 2004 Aug 6;118(3):285-96.
4. Mantovani G, Macciò A, Mura L, Massa E, Mudu MC, Mulas C, Lusso MR, Madeddu C, Dessì A. Serum levels of leptin and proinflammatory cytokines in patients with advanced-stage cancer at different sites. J Mol Med. 2000 Dec 1;78(10):554-61.
5. Dummer W, Becker JC, Schwaaf A, Leverkus M, Moll T, et al.Elevated serum levels of interleukin-10 in patients with metastatic malignant melanoma. Melanoma Res. 1995 Feb 5(1):67-8.
6. Gotlieb WH, Abrams JS, Watson JM, Velu TJ, Berek JS, Martínez-Maza O. Presence of interleukin 10 (IL-10) in the ascites of patients with ovarian and other intra-abdominal cancers. Cytokine. 1992 Sep 30;4(5):385-90.
7. Khatri VP, Caligiuri MA. A review of the association between interleukin-10 and human B-cell malignancies. Cancer Immunol Immunother.1998 Jul 16;46(5):239-44.
8. Klein B, Zhao-Yang L, Jiang GZ, Costes V, Jourdan M, Rossi JF. Interleukin-10 and Gp130 cytokines in human multiple myeloma. Leuk Lymphoma.1999 Jan 1;34(1-2):63-70.
9. Taurone S, Bianchi E, Attanasio G, Gioia CD, Ierinó R, Carubbi C, Galli D, Pastore FS, Giangaspero F, Filipo R, Zanza C. Immunohistochemical profile of cytokines and growth factors expressed in vestibular schwannoma and in normal vestibular nerve tissue. Molecular medicine reports. 2015 Jul 1;12(1):737-45.
10. Cozzolino F, Torcia M, Aldinucci D, Rubartelli A, Miliani A, Shaw AR, Lansdorp PM, Di Guglielmo R. Production of interleukin-1 by bone marrow myeloma cells. Blood. 1989 Jul 1;74(1):380-7.
11. Pitiakoudis M, Bounovasi A, Simopoulosi K. Serum Levels of Tumor Necrosis Factor-oi and Nutritional Status in Pancreatic Cancer Patients. Anticancer Res. 2001;21:1355-8.
12. Yoshida N, Ikemoto S, Narita K, Sugimura K, Wada S, Yasumoto R, Kishimoto T, Nakatani T. Interleukin-6, tumour necrosis factor α and interleukin-1β in patients with renal cell carcinoma. Br J Cancer. 2002 May 6;86(9):1396-400.
13. Yue FY, Dummer R, Geertsen R, Hofbauer G, Laine E, Manolio S, Burg G. Interleukin-10 is a growth factor for human melanoma cells and down-regulates HLA class-I, HLA class-II and ICAM-1 molecules. Int. J. Cancer. 1997 May 16;71(4):630-7.
14. Dummer W, Becker JC, Schwaaf A, Leverkus M, Moll T, et al.Elevated serum levels of interleukin-10 in patients with metastatic malignant melanoma. Melanoma Res. 1995 Feb 5(1):67-8.
15. Jammal MP, Araújo da Silva A, Martins Filho A, de Castro Côbo E, Adad SJ, Murta EF, Nomelini RS. Immunohistochemical staining of tumor necrosis factor-α and interleukin-10 in benign and malignant ovarian neoplasms. Oncol lett. 2015 Feb 1;9(2):979-83.
16. Cigna E, Tarallo M, Maruccia M, Sorvillo V, Pollastrini A, Scuderi N. Basal cell carcinoma: 10 years of experience. J Skin Cancer. 2010 Dec 1;2011.
17. Nemet AY, Deckel Y, Martin PA, Kourt G, Chilov M, Sharma V, Benger R. Management of periocular basal and squamous cell carcinoma: a series of 485 cases. Am J ophthalmol. 2006 Aug 31;142(2):293-7.
18. Griffiths RW, Suvarna SK, Stone J. Do basal cell carcinomas recur after complete conventional surgical excision? Br J Plast Surg. 2005 Sep 30;58(6):795-805.
19. Michalaki V, Syrigos K, Charles P, Waxman J. Serum levels of IL-6 and TNF-α correlate with clinicopathological features and patient survival in patients with prostate cancer. Br J Cancer. 2004 Jun 14;90(12):2312-6.