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Association of CTLA-4 A/G polymorphism with occurrence of ocular squamous cell carcinoma

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Abstract

Introduction: The association of polymorphisms of genes controlling the immune regulation with development of cancers and autoimmune diseases, has been a heavily researched area with the hope of probable discovery of a biomarker or antitumour vaccine. The most commonly investigated polymorphism is CTLA-4 +49A/G. However, no research has been carried out to find out association between eye cancers and the same polymorphism till date, to the best of our knowledge.

Purpose: To find out association between CTLA-4 +49A/G polymorphism and ocular surface squamous neoplasia (OSSN).

Methods: 12 histologically proven OSSN patients were taken as subjects. 20 cataract patients were taken for comparison. This data was further compared with the published data on 103 individuals from an Indian study. DNA extraction, SNP selection and genotyping was done. Odds ratio, confidence interval and statistical significance were found out.

Results: 95% confidence interval was 0.044-1.521 between OSSN cases and cataract group (p=0.144). 95% confidence interval of the OSSN cases and healthy control group was 0.14-2.375 (p=0.544).

Conclusions: Statistically significant difference was not found in the frequency of polymorphism between OSSN cases and groups taken for comparison, in this pilot study. Larger number of OSSN cases need to be evaluated for the polymorphism.

Keywords: CTLA4; OSSN; +49 A/G Polymorphism; PCR-RLFP; Immune regulation

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Introduction

Ocular surface squamous neoplasia (OSSN) is the third most common conjunctival malignancy Worldwide. It is most common in tropical regions of the world. It has an incidence of 37.3per 106 amongst all eye cancers and 8.4 per 106 amongst squamous cell carcinomas of eye [1]. Several factors have been implicated in development of OSSN. It may arise from dysfunctional limbal stem cells having been altered by various mutagenic agents such as ultraviolet (UV) radiation, infection with human papillomavirus (HPV) etc. [2]. Light pigmentation of hair and skin, vitamin A deficiency, heavy smoking, ocular injury are other risk factors. However, majority of susceptible peopledo not develope OSSN. Host genetic factors must also play a role in persistence of mutagenic effects of UV radiation, HPV and human immunodeficiency virus (HIV) infection etc., in subsequent development of OSSN. The genetic factors haven't received nearly the same amount ofresearch attention as the mutagenic agents.

The human body generates an immune response to tumours, but it is generally ineffective at causing tumour destruction. One possible reason is that T-cell activation will not occur until two separate signals are received by the cell. The first signal is specific antigen recognition, which requires T-cell receptors to recognise and bind to major histocompatiblity complex (MHC) molecules of antigen presenting cells (APC). The second signal is Ag independent, generated by interaction between CD28 on the T-cell surface and its ligands CD80 and CD86 on APCs [4-7].

CTLA-4, a CD28 homologue is a glycoprotein expressed on activated T-cells and its function is to down regulate T-cell response [8-10]. CTLA-4 blockade leads to enhancement of immune response [11], rejection of tumours [12] or even cure of tumours in mice when used incombination with antitumour vaccines [13]. One of the most frequently studied polymorphisms is A/G transition in exon 1 at position +49 [14]. Results of studies suggest that a G-allele instead of an A allele at position +49 can attenuate the CTLA-4 driven down-regulation of T-cell response [15-17].

There have been three meta-analysis which have tried to assimilate all studies on association of CTLA-4 polymorphisms with various cancers. None of the studies include any eye cancer. Hence, the present study was undertaken to evaluate if there is any association between CTLA-4 polymorphism with OSSN.

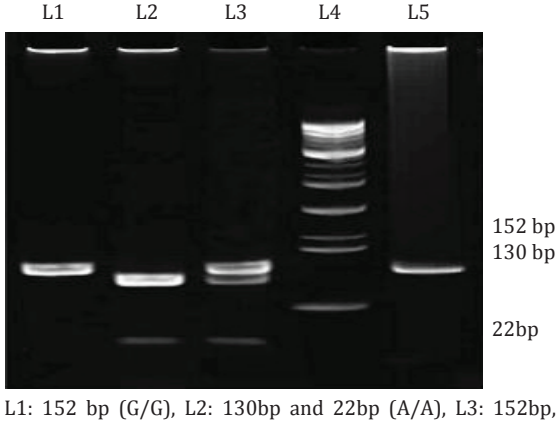
Materials and methods

Study subjects: This was a hospital based study taking histologically proven OSSN patients and patients with cataract, for comparison. The OSSN cases and cases with cataract were age-matched. Data for comparison from a published report of one of the authors on a group of healthy individuals, who didn't suffer from any co-morbidities or cancers was included for further comparison [18]. All patients (OSSN and cases with cataract) were provided written informed consent. Ethical clearance was obtained from both the institutes.

DNA extraction, SNP selection and genotyping

Blood samples (2ml) were collected by using EDTA vacutainers. The QIAamp DNA Blood Mini Kit (Qiagen, Berlin, Germany) was used to isolate genomic DNA from peripheral blood lymphocytes and DNA samples were frozen at -80oC. Genotypes of CTLA4 +49G>A site were analysed by using polymerase chain reaction–restriction fragment length polymorphisms (PCR-RLFP) method (Figure 1) [18]. The association of CTLA4 +49A/G genotypes with the risk 45 of OSSN was evaluated by odds ratio (OR) and corresponding 95% confidence intervals (CIs). An internet based Hardy-Weinberg equilibrium (HWE) calculator was used to measure the deviation from the HWE among the controls.

Representative picture of RFLP of CTLA-4 +49 A/G pohymorphim



L1: 152 bp (G/G), L2: 130bp and 22bp (A/A), L3: 152bp, 130bp and 22bp (A/G), L4: Ladder(100bp), L5: Unout (152bp)

Figure 1: RFLP of the DNA in blood samples.

Statistical analysis was implemented using SPSS version 16.0 and Epi-info version 6.0. Statistical significance was defined as P<0.05 with two tailed for all statistical analyses.

Results

There were 12 histologically proven OSSN patients and 20 patients with cataract. Of the 12 histologically proven cases, 9 were intraepithelial OSSN (Figure 2) and 3 were invasive OSSN. Mean age of the OSSN patients and cataract patients was 54.1 years. Among the OSSN patients 8 were males and 4 were females. Among the cataract patients 14 were males and 6 were females. Published data available from 103 healthy individuals, who didn't suffer from any eye problems, co-morbidities or cancers were taken for comparison. Among them 58 were males and 42 were females. All were of western Indian ethnicity.

A/G polymorphism was seen in 4 out of 12 cases of OSSN, in 13 out of 20 cases of cataract, while this was reported in 47 of 103 healthy individuals. The 95% confidence interval was 0.044-1.521, which suggests no difference in the frequency of the polymorphism between case and cataract group (Table 1). The 95% confidence interval of the cases and healthy group was 0.14-2.375 which again suggests no difference in the frequency of the polymorphism (Table 2). Because of small number of samples available for

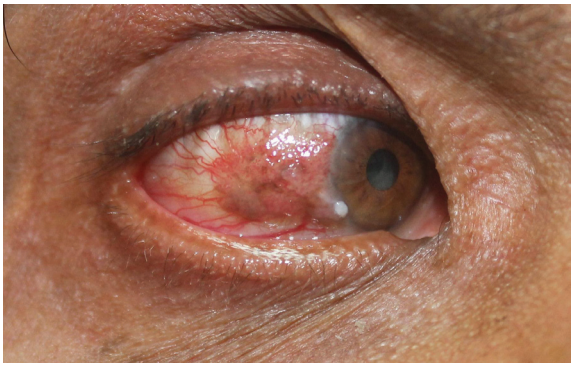


Figure 2: Ocular Surface Squamous Cell Neoplasia (OSSN). Note the tortuous dilated feeder vessels leading upto the mass. This is a mixed (gelatinous and papilliform) intraepithelial type of OSSN.

Table 1: Comparision of CTLA4 +49A/G polymorphism in OSSN cases and cataract patients.

CTLA-4 (+ 49A/G)	*OSSN cases N=12*	*Percentage*	*Cataract cases N=20*	*Percentage*	*OR*	*P-value*	*95% CI*
A/A	4	33.33	5	25	1.5	0.696	0.241-9.429
A/G	4	33.33	13	65	0.269	0.144	0.044-1.521
G/G	4	33.33	2	10	4.5	0.165	0.526-45.985
Allele							
A	12	50	23	57.5			
G	12	50	17	42.5			
		0.2482(HW)		0.1401(HW)			

Table 2: Comparision of CTLA4 +49A/G polymorphism in OSSN cases and healthy individuals.

CTLA-4 (+ 49A/G)	*Cases N=12*	*Percentage*	*Healthy individuals [Ref.18] N=103*	*Percentage*	*OR*	*P-value*	*95% CI*
A/A	4	33.33	26	25.24	1.481	0.508	0.34-6.078
A/G	4	33.33	47	45.63	0.596	0.544	0.14-2.375
G/G	4	33.33	30	29.12	1.217	0.747	0.282-4.943
Allele							
A	12	50	99	48.06			
G	12	50	107	51.94			
		0.2482 (HW)		0.3828 (HW)			

each tumour grade, we could not get any statistically significant correlation with +49A/G polymorphism.

Discussion

In 2010, Zheng J et al. [19] found out in their metanalysis that presence of CTLA4 +49A/G was associated with increased risk of developing solid tumours (lungs, breast, colorectal, skin, nasopharyngeal, cervical, esophageal and oral squamous cell carcinoma) but not non-solid tumors. They also found that the risk was higher in Asians and Caucasians. Gao X et al. [20] published in their metanalysis that +49A/G polymorphism was associated with decreased risk of cancer amongst Asians but not among Europeans. However no eye cancer were included in either of the metanalysis. Publication bias could be a reason why studies with negative result have been missed out of metanalyses.

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Conflicts of interest

Authors declare no conflicts of interest.

Conclusion

In this pilot study, we investigated a specific SNP (+49 A/G) of the CTLA 4 gene in patients suffering from OSSN. We did not find any statistically significant difference between the presence of +49A/G polymorphism in OSSN patients, cataract patients and normal healthy individuals. Small sample size is limitation of the study. Further studies to determine the association of +49A/G polymorphism in OSSN in large number of cases are required to confirm the contribution of this polymorphism.

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