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**Correlation and prognostic significance of programmed death-ligand 1 and p53 mutational status in eyelid sebaceous gland carcinoma**

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Eyelid sebaceous gland carcinoma (SGC) is an aggressive tumor with increased chances of recurrence and metastasis. The p53 gene is expressed upon cellular stress signals mediating antiproliferative responses. Recent evidences have suggested the role of P53 in blocking the expression of programmed death-ligand 1 (PDL1) protein, an important player responsible for shutting down the immune systems protective response to cancer cells. Circulating cell free DNA (ccfDNA) are fragments of nucleic acids released by apoptosis. Since ccfDNA is known to possess similar mutation signatures as that of the primary tumor it might potentially serve as a prognostic marker in the absence of clinically detectable metastases. In our study we try to correlate the expression of p53 and PDL1 in SGC. Further, ccfDNA was sequenced for *P53* mutations.

ccfDNA from 20 SGC was extracted from the plasma and tumor DNA was isolated by QIAamp tissue DNA midi Kit. The amplified DNA was sequenced and analyzed for P53 gene mutations using Bioedit software. Immunohistochemistry (IHC) was performed to detect tissue level expression of P53 and PDL1.

IHC evaluation revealed nuclear expression of P53 in 55% of cases studied. Direct sequencing analysis revealed mutations in SGC patients (25%). *PDL1* expression found to be significantly overexpressed in *P53* mutated SGC patients and in cases with P53 immunostaining.

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“FRONTIERS IN BIOCHEMISTRY &  
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Study of early stages of osteogenesis in a seel (*Gallus gallus domesticus*) Using a cost-effective and reproducible set-up at undergraduate level

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**ABSTRACT**

Limb development has been a classic model to study pattern formation during embryonic development. The present study deals with anatomical description of skeleton development including appearance of chondrogenesis and the appearance of ossification centers during certain embryological stages of *Gallus gallus domesticus* using Alcian Blue and Alizarin Red S Stain. This study uses chicken embryo biological models for observing development stages using windowed eggs. Six freshly hatched fertilized chicken eggs were procured from the Chattarpur village, New Delhi. The eggs were incubated in 36-37°C and 50-60% humidity in a set up made of plastic cups in a BOD incubator. The development of chick embryo was observed (candling), photographed and videoed without any need to expose the eggs directly to the outside. The developmental stages were correlated with Hamilton-Hamburger (HH) stages. All embryos survived the days of early embryogenesis, and proceeded with further growth & development, latest reaching the 9th post-incubation day. The developmental milestones of the embryos were traced from early to advanced well-developed stages in all the embryos. Then the cartilages and bones of these chick embryos at two different developmental stages were stained by the help of Alcian Blue stain (AB) and Alizarin Red S stain (ARS) respectively. We were able to observe and record the Endochondrial Ossification of the chick embryo in an artificial cost effective experimental culture system starting from an early embryonic stage to a well developed 34th stage with the embryo survival rate of 100%. Thus the model system has the potential to enhance our knowledge about the embryological developmental as well as the fact that it is an excellent model to understand the osteogenesis at both basic and clinical science level. The model system presented in this study proves to be a realistic approach to the subject of embryology but also provide an ideal experimental medium for research at undergraduate level. Larvae. Extracts of *Gymnopetalum chinense* are effective against *Culex* as well as *Albopictus* larvae. Essential oils of *Ipomoea carnea* possess remarkable larvicidal activity against *Culex tritaeniorhynchus*, *Anopheles stephensi* and *Culex quinquefasciatus*.

Formulations can be prepared with different plant extracts and the optimum concentration can be standardized for maximum efficacy. These formulations would not lead to any kind of pollution and would be cost-effective as well, killing multiple species of larvae at once. Thus, by controlling the larval growth, the spread of mosquito-borne diseases can be combated, thereby contributing to public health and overall well being of an individual.

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Preliminary phytochemical screening, estimation of total phenol analysis of human TERT expression in squamous cell carcinoma (SCC) of the skin: its implication in tumor metastasis and differentiation

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**ABSTRACT**

Skin is the largest and the most specialized organ is the site of origin, of a complex array of different tumors. Squamous cell carcinoma (SCC) is a highly invasive malignant tumor showing keratinocytic differentiation and is often associated with chronic exposure to UV light. Telomerase is a RNA dependent DNA polymerase that causes the addition of telomeric repeat DNA sequences to chromosomal ends, however its activity is undetectable with successive cell divisions. Recently, UV signature mutations has been identified in the core promoter region of telomere reverse transcriptase (TERT) gene, which encodes the main catalytic subunit leading to over expression in cutaneous melanoma.

Its role and expression pattern has not been studied in eyelid skin SCC. The aim of the present study was to analyze the presence of TERT in eyelid SCC as its expression pattern and mutational status hasn't been studied in SCC.

A C to T mutation was observed in 3/10 SCC cases. Positive expression of TERT was found in 60 % of the cases analysed and it showed a significant association with keratinocytic differentiation (P=0.04).

TERT has been shown to be overexpressed along with the promoter region and immunohistochemically for the first time in eyelid skin SCC. Our results suggest that over expression of TERT may contribute to the aggressive behaviour associated with SCC and such patients may warrant aggressive treatment.

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Utility of Trichloroisocyanuric Acid (TCCA) Solution as a  
bleaching agent for assessment of conjunctival melanoma;  
an immunohistochemical approach to detect tert  
immunoreactivity

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ABSTRACT

Melanoma is one of the most malignant tumors of the eye and conjunctiva. Excessive amount of melanin pigments in melanomas often hamper immunohistochemical assessment. The standard use of KMnO<sub>4</sub> and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> as bleaching agents has often been shown to alter the specificity and sensitivity of antigenic epitopes of the tissues thereby hindering IHC based investigation. Over expression of telomerase reverse transcriptase (TERT) has often been reported in various carcinomas leading to an increased in the activity of telomerase enzyme which is crucial for survival of cancer cells. TERT promoter mutation are frequent in conjunctival melanoma. However its expression has not been reported in conjunctival melanoma due to presence of heavy melanin pigmentation which often interferes with 3,3'-diaminobenzidine (DAB) based immunohistochemistry. In this study we evaluated the use of Trichloroisocyanuric acid (TCCA) solution as a cost effective and reproducible technique for immunohistochemical evaluation of conjunctival melanoma.

Effective bleaching was achieved using freshly prepared 5% Trichloroisocyanuric acid solution. HMB-45 was localized in cytoplasm and KI-67 positivity was observed in the nucleus. TERT expression was seen 70% cases of ocular melanoma. TERT nuclear positivity was also detected in patients with primary acquired melanosis. The bleaching effect of TCCA solution on melanin pigments did not interfere with immunostaining using TERT antibody. The finding of this study advocates the use of TCCA solution for effective and rapid removal of melanin pigments from paraffin embedded sections to expose the epitopes for immunohistochemical analyses.



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**PD-1 and PD-L1 pathway in sebaceous gland carcinoma of the eyelid: a possible target for curcumin and apigenin?**

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**Introduction:** Eyelid sebaceous gland carcinoma (SGC) is an aggressive tumor with increased chances of recurrence and metastasis. Programmed death-ligand 1 (PDL1) protein is an immunoinhibitory molecule that suppresses the effective T-cell response against tumor antigen leading to the progression of tumors. Inhibition of the interaction of PD-1 and PD-L1 are associated with good clinical response in various carcinomas. The p53 gene is expressed upon cellular stress signals mediating antiproliferative responses. Recent evidences have suggested the role of P53 in blocking the expression of programmed death-ligand 1 (PDL1) protein, an important player responsible for shutting down the immune systems protective response to cancer cells. In addition, recent studies have also shown tumor growth suppressive properties of curcumin and apigenin in melanoma cells by interfering with PD-L1 pathway. However, the value of PD1/PD-L1 axis remains unexplored in eyelid sebaceous gland carcinomas.

**Aim and Objectives:** The purpose of the study was to evaluate the expression of PD-L1 and its correlation with P53 mutational status in eyelid SGC.

**Methods:** ccfDNA from 30 sebaceous gland carcinoma was extracted from the plasma and tumour DNA was isolated by QIAamp tissue DNA midi Kit. The Quality of the DNA was estimated by ALU PCR (Arthrobacter Luteus Polymerase chain reaction). The amplified DNA was sequenced for analyzing P53 gene mutations using the Sanger's Method and analysed using Bioedit software. Immunohistochemistry (IHC) using mAb was performed to detect tissue level expression of P53, PD-1 and PD-L1

**Results:** IHC evaluation revealed nuclear expression of P53 in 55% of cases studied. Direct sequencing analysis revealed mutations in SGC patients (25%). PL-1 expression was restricted to the tumor infiltrating lymphocytes in 16/30 (53%) SGC cases. PD-L1 expression was also found to be significantly over expressed in P53 mutated SGC patients and cases with P53 immunostaining. The rate of co-expression of PD-L1 in tumor cells and PD-1 expression in TILs from the same specimen was 43% (13/30). Tumor PD-L1 positivity, PD-1 expression in TILs and tumor size (>2cm) was associated with reduced disease-free survival. On multivariate analysis only tumor size (>2cm) and a combined positivity of PD-L1 in tumor cells and PD-1 in TILs (with an odds ratio of 5.212 (95% confidence interval 1.449-18.737) continued to be significantly associated with SGC recurrence.

**Conclusions:** PD-L1 is overexpressed in SGC. The combined tumor PD-L1 positivity and TILs showing PD-1 expression within the same SGC patient's samples predict high risk SGC. Routine detection of p53 and PD-L1 may be recommended in the management of eyelid SGC patients.

**Keywords:** Eyelid, Sebaceous Carcinoma, p53, Programmed Death-ligand 1, Immunohistochemistry