# An Immunological Evaluation of Squamous Cell Carcinoma Oral Cavity, Pharynx and Larynx by Skin Window Test

## Medical Science

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## ABSTRACT

*Neoplastic cells are antigenically different from normal cells. The antigens of tumour cells induce cellular and humoral responses. Squmaous cell carcinoma of head and neck are associated with the impairments of cellular immunity. Cellular response is mainly responsible in effecting cytotoxic immunological reaction against the neoplasm. The assessment of cellular response in vivo is being done with skin sensitisation test such as the Skin window test, Mantoux test and DNCB (dinitrochloroben-zene) test. The responses in the patients are assessed and co-related with histological grading, clinical staging and mode of treatment of the tumour. This assessment helps in understanding the immunological condition and prognosis of the disease in patients suffering from cancer of the oral cavity, pharynx and larynx.*

## Introduction:

The immune system protects the body against illness and infection caused by bacteria, virus, fungi or parasites. Immune response is the collection of reaction and responses that the body makes to damage cells or infective agents.

In India, approximately 40% of all cancers recorded annually are cancers of the oral cavity, pharynx and larynx based on hospital registry. This high incidence of cancers of the oral cavity, pharynx and larynx has been attributed to the common habit of chewing betel leaf with areca nut with or without tobacco, ill fitting denture and smoking. Laryngeal carcinoma accounts for a small fraction of all human malignancies (less than 2%), but the incidence varies among geographically. Laryngeal squamous cell carcinoma occurs most often in the sixth and seventh decades. Men are more frequently affected than women. The etiology is not well known, but exposure of the mucosa to a wide variety of ingested and inhaled exogenous carcinogenic agents, such as tobacco smoke, alcohol, and HPV infections greatly increases the risk of developing these tumors. Avoiding cigarettes and alcohol could prevent about 90% of laryngeal SCC. Despite a predominance of oral cancers and increasing morbidity due to laryngeal cancer there have been only few investigations of the immune status of these patients.

*John Rebuck and Crowley* (1955) first time uses skin window technique in immunology where the top layer of skin is scraped off making it possible to identify the immune response that would occur with a diminished physical barrier [6,9,12and13].

Cancer immunosurveillance is a theory formulated in by *Burnet and Thomas,* (1957) [15]. Who proposed that lymphocytes and macrophage act as sentinels in recognizing and eliminating continuously arising, newly appearing neoplastic cells as a result of the new antigens they tend to express. This newly formed cell recognized as non-self by the immune system. Immune response develops against these antigenic cells and destroyed by immune system.

Quantisation of circulating immune complexes levels was established in patients with oral cancer and carcinoma pharynx and larynx. The levels were compared with that in normal controls with no signs of any disease. Patients with oral cancer and carcinoma larynx and pharynx had elevated or decreased circulating immune complexes when compared to the control groups.

## Materials and methods: [figure-1]

In the present review only patient with mucosal derived squmaous cell carcinoma of oral cavity, pharynx (oropharynx and laryngopharynx) and larynx are taken. Nasopharyngeal squmaous cell carcinomas are excluded due to different biology of cancer.

## Immunological test:

A) **Xylocaine sensitivity-**It was tested with 0.2 cc of 2% xylocaine subcutaneously.

B) **Prepration of antigen-** biopsy piece from each case was crushed in mortar and pistel and soluble antigen was prepared by thermal treatment at 44C for 20 min so antigenicity preserved but tumerogenicity of sample lost. Total protein content of each preparation was estimated by biuret method (King *and Wooton*, 1975). For skin window test tumor protein should be adjusted to 2.0 gm%.

X) **Skin window technique-**

- Flexor aspect of both forearm cleaned using aseptic technique with sprit, betadine and normal saline, drapping done.
- Local anaesthesia in the form of xylocaine 2% given at the test site.
- The "skin window" technique permits investigation of the immediate and delayed inflammatory response *in vivo* in humans. This typically involves creation of a lesion in the stratum corneum of the skin. 6 Abrasions each of 2 cm diameters was created on the flexor aspect of fore-arm using sandpaper (no 120) [Figure-1] until capillaries appear on exposure of dermis.
- Care was taken to avoid bleeding.
- Abraded area were covered with naked cover slips (2.2cm×2.2cm) and fixed with leucoplast.
- Cover slips were removed after 6, 24 and 48 hours. 3 cover slips were layered with one drop of tumour antigen (0.1 ml) before covering the abraded skin over one arm known as test arm and 3 naked cover slip without tumour antigen applied over other arm known as control arm. In patient without lesion, act as control only 3 naked cover slip over one arm applied.
- One cover slips from each arm was removed after 6 hrs, 24 hrs and 48 hrs and stained by leishman method.
- Differential leukocyte count was done at 5 different sites on each coverslip and mean value was determined.
- Difference >15% in differential leukocyte count on naked

and autologous tumor containing coverslips ware considered to be significantly changed.



**Figure 1- material used in skin window technique**

**Observation:**

A total number of 43 cases of squamous cell carcinoma of oral cavity, pharynx and larynx underwent biopsy; chemotherapy and surgery within a period of one year six months i.e. from Jan 2014 to September 2015 were taken under study. All the cases undergo follow-up for a period of one to six months and their particulars are given below:

**Table-1: Showing the age distribution of cases**

| Age group(years) | No. of patients | Percentage |
|---|---|---|
| 20-35 | 5 | 12 |
| 36-50 | 17 | 39 |
| 51-65 | 15 | 35 |
| 66-80 | 6 | 14 |
| Total | 43 | 100 |

Maximum no of patients (17) in this series was in the age group of 36-50 years (39%) and in age group of 51-65 years (35%). So most of the cases (74%) belong to age group of 36-65 years. [Table-1]

**Table-2: Showing the sex distribution of cases**

| Sex | No. of patients | Percentage |
|---|---|---|
| Male | 36 | 84 |
| Female | 7 | 16 |
| Total | 43 | 100 |

Maximum no of patients were male i.e. (84%) and male to female ratio in present case series was approximately 5:1 as shown in [table II].

**Table-3: Showing site distribution of cases**

| Site | No. of patients | Percentage |
|---|---|---|
| Buccal mucosa | 12 | 28 |
| Tongue | 16 | 37 |
| Oropharynx | 2 | 5 |
| Hypopharynx(pyriform fossa) | 6 | 14 |
| Larynx | 7 | 16 |
| Total | 43 | 100 |

Criteria for deciding the site were as follows

1. **Buccal mucosa**: Refers to the inside lining of the cheeks, in present study we included the lesion occupying epithelial lining of hard and soft palate, retromolar area, floor of mouth in this section.
2. **Tongue**: Lesions involving dorsal and ventral surface, base of tongue.
3. **Oropharynx**: Refers to lesions involving tonsillar fossa and part of pharynx behind the oropharyngeal isthmus.
4. **Hypopharynx**: Refers to part of pharynx extending between lower level of oropharyngeal isthmus to crico-oesophageal

sphinctor including pyriform sinus and post cricoids area.

5. **Larynx**: Involving lesions of supraglottic, glottis and sub-glottic larynx.

In present case series most of the patient presented with squamous cell carcinoma of oral cavity (buccal mucosa 28%+tongue 37%). 19% patients presented with squamous cell carcinoma of pharynx (oropharynx 5%+hypopharynx14%). 16% patients were presented with squamous cell carcinoma of larynx. [Table-3]

**Table-4: Showing mode of treatment.**

| Mode of treatment | No of patients | Percentage |
|---|---|---|
| Biopsy | 43 | 100 |
| Chemotherapy | 14 | 32.56 |
| Surgery | 7 | 16.28 |
| Chemotherapy + surgery | 3 | 13 |

All the patients in present case series were biopsied. 14 (32%) underwent chemotherapy, seven (16%) undergone surgery and three (13%) patients receive both chemotherapy and surgery. [Table-4]

**Table-5: Showing relation of skin window test to clinical staging**

| Staging | Skin window test result | |
|---|---|---|
| | + | - |
| 0 | 0 | 0 |
| I | 12 | 4 |
| II | 4 | 2 |
| III | 1 | 1 |
| IVA | 12 | 7 |
| IVB | 0 | 0 |
| IVC | 0 | 0 |
| TOTAL | 29 | 14 |

Positive skin window test result (greater than 15% differences in cell count between case and control) indicate that patient have significant immune reaction against tumour antigen. In present case series out of 43 cases 28 (65%) shows positive correlation. Table-5 also shows that with advancing stage of tumour immune status of patient decreases. Patients in stage I show maximum (73%) positive relation on skin window test.

**Table-6: Showing relation of skin window to age of patients**

| Age group | Skin window test result | |
|---|---|---|
| | + | - |
| 20-35 | 5 | 0 |
| 36-50 | 12 | 5 |
| 51-65 | 9 | 6 |
| 66-80 | 2 | 4 |

Above table [6] showing that patient with increasing age group has decreasing positivity of skin window test due to increase morbidity and decreased immune status. In our study all the five patient of age group 20-35 years shows maximum (100%) immune reaction to tumor antigen and cases with age group 66-80 years shows minimum (33%) immune status.

**Table-7: Showing skin window test result after chemotherapy.**

| Test result | No of patient | Percentage |
|---|---|---|
| + | 0 | 0 |
| - | 14 | 100 |

Current study shows decrease immune reaction against tumor antigen after chemotherapy due to adverse effect of chemotherapeutic agents. All the cases shows depressed immune status after chemotherapy [Table-7]

**Table-8: Showing skin window test result after surgery.**

| Test result | No of patient | Percentage |
|---|---|---|
| + | 5 | 72 |
| - | 2 | 14 |

In present case series seven patient underwent surgery. After surgery by skin window test 72% patient shows improvement in immune status. [Table-8]

**Discussion:**

The present study was undertaken to evaluate the immunological status of squamous cell carcinoma patients of oral cavity, pharynx and larynx and also to access the response of various modes of treatment modalities.

Immune response is known to develop against malignant tumours. Malignant tumours express newer antigen on their cell surface membrane which elicit both T-cell and B-cell mediated immune response. There is definite invitro evidence that Tcell senstization develops in patients bearing malignancies. T cell eliminate tumour by releasing short range of mediators lymphokines like migratory inhibitory factor, chemotactic factor etc. In early part of immune reaction neutrophil migrates followed by monocyte-macrophage cell.

The assessment of cellular response in vivo is being done with skin sensitisation test such as the Skin window test, Monteux test and D.N.C.B. test. The responses in the patients are assessed and co-related with histological grading and clinical staging of the tumour. This assessment helps in understanding the prognosis of the disease, response of chemotherapy, response of surgery in patients suffering from cancer of the oral cavity and larynx and pharynx.

Table 1 showing 43 cases, ranging from 24 to 75 years of age of either sexes, with squamous cell carcinoma oral cavity, pharynx and larynx underwent skin window test. Maximum number of cases 32 (74%) belongs to age group of 35 to 65 years. The mean age of all the cases together is 52.1 years with median age of 50 years. Similar finding were noted in the study of *Fahmi et.al.* [7] In which median age of oral cancer was 55 years of age. *Umesh kapil et a.* (2005) shown her study that Nearly 89.8 percent of the patients were in the age group of 41-80 years. In another study *Bhattacharjee A* (2006) [4] shows that 71.9% of these tumors were reported in this age group is closely related to our study.

Table two shows the ratio between male (84%) and female (16%) was 5:1. The male to female ratio reported by large scale epidemiological studies by Mehanna H et, al.(2010) and national cancer registries varies from 2:1 to 15:1depending on the site of disease [10]. Recently Alvarenga et,al (2008) studied that the incidence of head neck carcinoma in women has been increased significantly, probably due to changes in environmental exposure [2]. For example, Abiose B et al(1991) studied in Argentina that man/woman ratio for oral cancer has been decreased from 7.1:1 (1950-1970 period) [1] to 1.24: 1 (1992-2000 period) [11]. In the USA, 5% of the male and 2% of the female population is affected by oral cancer with a sex ratio of male to female of 2:1. Suba Z et,al (2007) found that hormone replacement therapy in postmenopausal women has increased in some countries and it is suggested as a factor for increasing head and neck cancer incidence in women [14].

Table 3 shows 65% cases belong carcinoma oral cavity, out of which 37 % were carcinoma tongue which is consistent with the study of *Branddizzi D et al* (2008) that tongue was the most frequent involved site of oral cavity (35%) that was consistent with other reports [5].

In our study table 4 shows that with advancing age immune status of the patient deteriorate. All the patients in our study below 35 years of age show positive immune reaction against against tumour antigen which becomes 33% in above age group of 66 years. Biomedical studies by *Vera E et. al. and Armanios M et. al.* have revealed age-related declines in the average length of immune cell telomeres (protective nucleoprotein complexes found at the ends of eukaryotic chromosomes) in humans and laboratory models [14,15], *Fossel M et,al (2012).* indicate that immune cell telomere lengths can act as biomarkers of age-related disease [8]. Telomeres consist in part of repetitive sequences of DNA $(TTAGGG)^n$ that often decrease in length over time (largely due to oxidative damage and the end replication problem during cell division; and may trigger cellular senescence once they become critically short. While the enzyme telomerase can recover telomere length.

Chemotherapy weakens the immune system as shown by table 7 in our study. The entire patient presented with deterioration in immune status after chemotherapy. Chemotherapeutic drugs tend to lower the number of white blood cells because it destroys any cells in your body that grow quickly. These include cancer cells, but also the rapidly growing healthy cells in your hair, digestive system, and bone marrow, where blood cells are produced. According to *Gardner,* (1999); *van der Most, Currie, Robinson, & Lake*, (2006) the immune system is a major regulatory mechanism in the defence of the body. Although cancer diagnosis and adjuvant chemotherapy are known to alter immune responses significantly surprisingly little has been investigated about how altered immune responses recover and how the type of cancer therapy and cancer stage interact with immune recovery over time. *J. P. Purohit et.al.* (1986) studied immunopathological correlation with lymph node metastasis in squamous cell carcinoma of larynx and pharynx. [11]

**Table 8 showed that after surgery immunological status of the patient were improved.**

**Conclusion:**
**The following conclusions were drawn from the present study:-**

- The incidence of squmous cell carcinoma oral cavity, pharynx and larynx was most common in age group of 36-65 years.
- Betel chewing (with or without tobacco), smoking, alcohol, poor hygiene and sharp teeth were the important risk factor of the disease progression.
- In head and neck region oral cavity is the most common site of SCC, and larynx is least common site.
- Immune status of the patient significantly decreases with advancing age.
- Immune reaction against tumour antigen declines with higher staging of tumour.
- Immunity is declined after chemotherapy.
- Surgery improves immune status of the patients.
- Skin window test positivity (polymorph and macrophage migration) significantly associated with the good prognosis of the patient.

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