Evaluation of Salivary Interleukin-6 Levels in Oral Leukoplakia and Oral Cancer Patients: - A Comparative Study

Surabhi S. Ausare[1], Daya K. Jangam[2], Pranjali S. Bende[3].

[1]Department of Oral Medicine and Radiology, Dr. D.Y.Patil Dental College and Hospital, Dr.D.Y. Patil Vidyapeeth, Pimpri, Pune-411018.

[2,3,] Department of Oral Medicine and Radiology, SinhgadDental College & Hospital, Vadgaon (Bk), Pune- 411041.

Corresponding author: Dr.Surabhi S. Ausare, Assistant Professor, Department of Oral Medicine and Radiology, Dr. D.Y. Patil Dental College and Hospital, Dr.D.Y. PatilVidyapeeth, Pimpri, Pune-411018.
Email Id: surabhiausare14@gmail.com

ABSTRACT:

Aim:
The aim of the study is to evaluate the salivary IL-6 levels in oral leukoplakia and oral cancer patients and also to determine whether salivary IL-6 can be utilized as a potential salivary biomarker in early detection of potentially malignant lesions and progression of potentially malignant lesions to malignancy.

Objectives:
To assess the salivary interleukin 6 in oral leukoplakia, oral cancer patients and in normal healthy, age and sex matched controls.
To compare salivary interleukin 6 levels in oral leukoplakia patients, in oral cancer patients and in normal healthy controls.

Study design: Sixty-sixpatients (22 with oral cancer, 22 leukoplakia, and 22 healthy controls) were included in this study. Cytokine concentrations were measured by commercial enzyme linked immunoassay.

Results:Salivary interleukin 6 levels were found to be higher in oral cancer and oral leukoplakia group as compared to healthy controls. (P-value<0.001 for both). Salivary interleukin 6 levels were significantly greater in oral cancer as compared to oral leukoplakia. (P-value<0.001)

Conclusions: Patients with oral cancer have elevated levels of inflammatory cytokines in their saliva. Studies yet need to be carried out to standardize a method and a value for salivary IL- 6 to be used as a diagnostic tool in clinical practice. Many studies and some more work is necessary so that it can be envisaged as a simple, fast, portable and cost-effective clinical diagnostic systems in the near future. Hence further studies on salivary interleukin- 6 levels are warranted in the near future.

Key words: *Cytokines, oralleukoplakia, oral cancer, IL-6.*

DOI Number: 10.14704/nq.2022.20.11.NQ66337 **NeuroQuantology 2022; 20(11): 3259-3275**

Introduction:

Oral cancers account for the sixth most common type of cancer, it represents the majority of head and neck cancers with more than half million patients being affected each year worldwide.[1]The majority (~90%) of oral cancers are oral squamous cell carcinoma (OSCC) affecting more than 3,00,000 people annually around the globe. OSCC is a malignant cancer of oral mucosa with increasing incidence and high mortality rate (62%) for 5 years.[2]

Oral Cancer (OC) has the highest prevalence among men. In India the incidence rate is 12.6

eISSN 1303-5150

per 100 000 population, and in other countries of Asia OC remains one of the most common cancers.[3]More than 90% are squamous cell carcinomas, which are mostly attributed to exogenous factors such as tobacco smoking and heavy alcohol consumption. The role of HPV in the oral cavity is still controversial, of interest, new trends are emerging in younger patients with no known risk factors. Leukoplakia is the most common premalignant or "potentially malignant" lesion of the oral mucosa. The incidence and prevalence of leukoplakia in India (0.2-4.9%). The malignant transformation rate of oral leukoplakia varies from 0 to 33%.

In the oral carcinogenesis, several factors have been involved such as age, gender, ethnicity, lifestyle, genetic background, status of health and exposure to carcinogens. Since time immemorial, micro-organisms have been presumed to have an etiological role in the evolution of oral cancer.

Salivary biomarker analysis is one of the newer techniques used in diagnosis of oral cancer, there are numerous salivary biomarkers which are used and studied for years. Cancer related inflammation causes release of certain cytokines in various body fluids like blood, saliva, sweat, tears, urine etc. which can be used for early diagnosis of potentially malignant lesions and oral cancer. Proinflammatory cytokine interleukin 6 (IL-6) exerts various biological functions. Apart from regulating inflammatory response, this cytokine plays significant role in the development of cancer, with a multi-factorial role in cancer related inflammation. Few studies have been conducted with IL-6 as a salivary biomarker for detection of potentially malignanat and malignant lesions. The present study is aimed to evaluate the salivary IL-6 levels in oral leukoplakia and oral cancer and also to determine whether salivary IL-6 can be utilized as a potential salivary biomarker in early detection of potentially malignant lesions and progression of potentially malignant lesions to malignancy.

Subjects and Methods:

The study was undertaken after obtaining permission from The Scientific Advisory Committeeand Institutional Ethical Committee. The study included twenty-two (22) oral cancer patients and twenty-two (22) patients with oral leukoplakia and twenty-two (22) subjects age and sex matched healthy controls with no clinical lesion.

The subjects were grouped as follows: -

Oral Cancer Group: This group included 22 subjects with clinically and histopathologically diagnosed cases of Oral cancer.

Leukoplakia Group: This group included 22 subjects with clinically and histopathologically diagnosed cases of Oral Leukoplakia.

Control Group: This group included 22 subjects age and sex matched healthy controls with no clinical lesion.

Criteria For Patient Selection

Inclusion Criteria:

1. Patients clinically and histopathologically diagnosed with oral leukoplakia and oral cancer
2. Normal healthy controls will be included in the study, who are not suffering from any systemic disease.
3. Patients who have signed the informed consent form.

Exclusion Criteria:

1. Patients with history of previous malignancy.
2. Patients with history of treatment for oral leukoplakia or oral cancer.
3. Patients with chronic inflammatory diseases (such as arthritis, psoriasis, inflammatory bowel disease, Sjogren's syndrome) that might influence the levels of salivary and/or serum cytokines were not considered.

eISSN 1303-5150

4. Patients who have received dental treatment 48 hours prior to the study such as extractions, scaling etc. which might affect the integrity of oral mucosa.
5. Patients with any type of salivary gland disorder.

Methodology:

Based on inclusion and exclusion criteria 24 clinically & histopathologically diagnosed oral cancer patients and 24 oral leukoplakia patients were included in the study. All the participants were explained the need and design of the study and the need for undergoing a thorough clinical examination, biopsy and blood investigations at the start of the study. Only those patients, who gave a signed informed consent on an institutionally approved document, participated in the study.

Patients were made to sit comfortably on a dental chair. Clinical examination was carried out wearing sterile hand gloves and mouth mask under artificial illumination, with patient seated appropriately prior to the procedure being performed.The initial appointment consisted of collecting the demographic data, after confirmation of diagnosis by histopathological report saliva was taken and then this saliva sample was used to measure salivary IL-6.Patients in study group were subjected for biopsy procedure and were explained about the surgical procedure. They were asked to sign the informed consent form before the start of procedure, a chairside Toluidine blue test was done to select the biopsy site.

The subject was asked to rinse the mouth with distilled water thoroughly to remove any food debris and then after 10 minutes, Unstimulated whole saliva measuring 2ml was collected from each of these patients by drooling method in the sterile plastic container.

The saliva was collected in sterile containers and was stored at -20°C. The saliva samples were centrifuged at 3000 rpm for 10 minutes and the supernatants will be taken out. A capture Antibody highly specific for IL-6 was coated to the wells of the microtitre strip plate provided during manufacture. Binding of IL-6 samples and known standards to the capture antibodies and subsequent binding of the biotinylated anti-IL-6 secondary antibody to the analyte was completed during the same incubation period. Any excess unbound analyte and secondary antibody was removed. The HRP conjugate solution was then added to every well including the zero wells, following incubation excess conjugate was removed by careful washing. A chromogen substrate was added to the wells resulting in the progressive development of a blue coloured complex with the conjugate. The colour development was thenstopped by the addition of acid turning the resultant final product yellow. The intensity of the produced coloured complex was directly proportional to the concentration of IL-6 present in the samples and standards. The absorbance of the colour complex was then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of IL-6 in any sample tested. Interleukin 6 levels were measured by using ELISA reader.Data collected was sorted and categorized based on the parameters recorded, the data was then analysed by Frequency analysis, Descriptive analysis and appropriate required test.

Result:

The study included 66 subjects: 22 oral cancer patients, 22 oral leukoplakia patients and 22 patients were in control group.

1. Group I Oral Cancer,
2. Group II Oral Leukoplakia,

eISSN 1303-5150

3. Group III Age –sex matched healthy Controls.

All the three groups, of patients were thoroughly examined clinically and biopsy was taken for every patient and histopathological examination was carried out. After clinical and histopathological examination 2 ml of saliva sample was collected as per procedure described in material methods. The samples were then sent to the laboratory for measuring salivary interleukin -6 levels.

Following the statistical analysis, the various data obtained were tabulated and described in the form of tables and charts.

Age And Average Age Distribution (Table 4 and Graph 1)

The mean age of the cancer group was 56.27 with maximum of 73 and minimum of 40 yrs of age. Similarly, the mean age among leukoplakia group was found to be 41.18, with the maximum age of 71 and minimum age of 25 yrs. The mean age of control group was 36.32 with maximum age of 75 and minimum age of 24 yr.

The distribution of mean ± SD of age in Group 1 (OSCC), Group 2 (OL) and Group 3 (Controls) is 56.27 ± 9.27 years, 41.18 ± 12.47 years and 36.32 ± 13.71 years respectively.

The distribution of mean age is significantly higher in Group 1 (OSCC) compared to the study Group 2 (OL) and Group 3 (Controls) (P-value<0.001 for both).

The distribution of mean age did not differ significantly between Group 2 (OL) and Group 3 (Controls) (P-value>0.05).

Gender distribution (Table 5 and Graph2)

The cancer group consisted of 18 male patients (81.8 %) and 4 female patients (18.2%). The oral leukoplakia group consisted of 21 males (95.5%) and females 1(4.5%) patients. The control group consisted of 17 males (77.3%) patients and 5 females (22.7%).

The gender distribution did not differ significantly between three study groups. (P-value>0.05 for all).

Distribution of type of habit across two study groups. (Table 6 and Graph 3)

Out of 22 patients in Cancer group, all 22 patients (100%) had a habit abuse of which 95.5 % patient had smokeless tobacco habit and 4.5% patient had smoking form of tobacco. In the oral leukoplakia group, all the 22 patients (100%) had a habit of smokeless form of tobacco.

The distribution of type of habit did not differ significantly between two study groups (P-value>0.05).

Distribution of duration of habit across two study groups. (Table 7 and Graph 4)

Of the 22 cases studied In Group 1 (OSCC), 1 (4.5%) had duration of habit less than 10 years, 8 (36.4%) had duration between 11 – 20 years, 7 (31.8%) had duration between 21 – 30 years and 6 (27.3%) had duration more than 30 years.

In Group 2 (OL), out of 22 subjects studied, 8 (36.4%) had duration of habit less than 10 years, 10 (45.5%) had duration between 11 – 20 years, 1 (4.5%) had duration between 21 – 30 years and 3 (13.6%) had duration more than 30 years.

The distribution of duration of habit is significantly higher in Group 1 (OSCC) compared to duration of habit in Group 2 (OL) (P-value<0.05).

Distribution of frequency of habit across two study groups. (Table 8 and Graph 5)

In Group 1 (OSCC), of 22 cases studied 8 (36.4%) had frequency of habit between 5 – 6 time per day, 9 (40.9%) had frequency of habit between 7 – 8 time per day and 5 (22.7%) had frequency of habit for more than 8 times per day.

Of 22 subjects studied in Group 2 (OL), 9 (40.9%) had frequency of habit between 5 – 6

eISSN 1303-5150

time per day, 13 (59.1%) had frequency of habit between 7 – 8 time per day and none had frequency of habit for more than 8 times per day.

The distribution of duration of frequency of habit is significantly higher in Group 1 (OSCC) compared to duration of habit in Group 2 (OL) (P-value<0.05).

Distribution of site of lesion across two study groups. (Table 9 and Graph 6)

In group 1 (OSCC), the most commonly involved site was buccal vestibule and alveolus (22.7%), followed by buccal mucosa and vestibule (18.2%), buccal vestibule (13.6%) and sites like buccal mucosa, buccal mucosa along with vestibule and alveolus involvement and buccal vestibule with retromolar region (9.1%) followed with minimal involvement of buccal mucosa with labial vestibule, labial vestibule and alveolus, alveolus and floor of mouth showing equal distribution. (4.5 %)

Group 2(OL), the site most commonly involved is buccal mucosa and vestibule (45.5%), followed by buccal mucosa (36.4%), and vestibule (4.5%).

The distribution of site of lesion differs significantly between study Group 1 (OSCC) and Group 2 (OL) (P-value<0.01).

Distribution of histopathological findings in Group 1 (OSCC). (Table 10 and Graph 7)

Group 1 (OSCC), consisted of 22subjects out of which 12 (54.5%) had Well differential SCC, 4 (18.2%) had Moderately differential SCC, 6 (27.3%) had Poorly differential SCC.

Distribution of histopathological findings in Group 2 (OL). (Table 11 and Graph 8)

In Group 2 (OL), 22 cases were, studied out of which 17 (77.3%) had Mild epithelial dysplasia, 4 (18.2%) had Moderate epithelial dysplasia and 1 (4.5%) had Severe epithelial dysplasia.

Distribution of median IL-6 levels across three study groups. (Table 12 and Graph 9)

The distribution of median (min – max) IL-6 levels in Group 1 (OSCC) is 41.07 (15.45 – 322.65) pg/ml, Group 2 (OL) 3.86 (0.32 – 60.83) pg/ml, and Group 3 (Controls) is 0.86 (0.53 – 3.35) pg/ml and the mean value is 96.6 ±94.95 pg/ml, 9.11±14.14pg/ml ,1.04±0.63 pg/ml in Group 1 (OSCC), Group 2 (OL) and Group 3 (Controls).

The distribution of mean and median IL-6 is significantly higher in Group 1 (OSCC) compared to the study Group 2 (OL) and Group 3 (Controls) (P-value<0.001 for both).

The distribution of mean and median IL-6 is significantly higher in Group 2 (OL) compared to Group 3 (Controls) (P-value<0.001).

Distribution of median IL-6 across three groups of histopathological stages in Group1 (OSCC). (Table 13 and Graph 10)

The distribution of median IL-6 levels is significantly higher in Group 3 (PDSCC) compared to Group 1 (WDSCC) and Group 2 (MDSCC (P-value<0.001 for both).

The distribution of median IL-6 levels is significantly higher in Group 2 (MDSCC) compared to Group 1 (WDSCC) (P-value<0.001).

Distribution of median IL-6 across three groups of histopathological stages in Group 2 (OL). (Table 14 and Graph 11)

The distribution of median IL-6 levels did not differ significantly between three sub-groups of histopathological stages in Group 2 (OL) (P-value>0.05).

Discussion:

In the present study we have evaluated the levels of salivary Interleukin -6 (IL-6) in oral leukoplakia, oral cancer patients and healthy controls. We included 22 patients of oral leukoplakia, oral cancer and healthy controls each. Based on histopathological examination oral cancer patients were grouped as well

eISSN 1303-5150

differentiated, moderately differentiated and poorly differentiated SCC and leukoplakia patients were grouped as mild, moderate and severe epithelial dysplasia.

The distribution of mean age for oral leukoplakia (Group 2)patients in our study was 41.18 ± 12.47 years with maximum age of 71 and minimum age of 25 yrs. Similar results were given by **Rhodus N.L (2005)[4], Brailo V (2006)[5],Brailo V (2012)[6], Patil S (2013)[7],Juretic M (2013)[8], Khyani I.A.M (2014)[9], Khyani I.A.M (2015)[10], Thayalan Dineshkumar (2017)[11].**

In our study, the distribution of mean ± SD of age in Group 1 (OSCC) is56.27 ± 9.27 years, with a minimum of 40 years and maximum of 73 years. Similar results were found by John **MA (2004)[12],Rhodus N.L (2005)[4], Brailo V (2006)[5], Warnakulasuriya (2009)[13], Hamad (2011)[14], Brailo V (2012)[6],Patil S (2013)[7], Juretic M (2013)[8], Khyani I.A.M (2014)[9], Khyani I.A.M (2015)[10], Kaur J (2015)[15], Selvam P (2015)[16],Rao M (2016)[17], Thayalan Dineshkumar (2017)[11].**Maximum patientsin age group of 45-70 were inflicted with oral cancer. Mean age was found to be more in the study conducted by **SahebJamee (2008)[18] i.e., 71.53 years and Sato J (2010)[19] i.e. 69 years.**The distribution of mean age for Group 3 (Controls) is36.32 ± 13.71 years. The distribution of mean age in oral cancer is significantly higher compared to leukoplakia group and healthy control group and the result was found to be statistically significant. (P- Value <0.001 for both). The distribution of mean age did not differ significantly between Group 2 (OL) and Group 3 (Controls) (P-value>0.05).

The observation regarding the age in the group suggest that most cases of oral cancer and oral leukoplakia were seen above 40 years. This could be attributed to increase duration of oral cancer related habits thus increased exposure to carcinogens.

The oral leukoplakia group consisted of 21 males (95.5%) and females 1(4.5%) patients. Similar results were found in study by **Rhodus N.L (2005)[4], Brailo V (2006)[5],Brailo V (2012)[6], Patil S (2013)[7],Juretic M (2013)[8], Khyani I.A.M (2014)[9], Khyani I.A.M (2015)[10], Kaur J (2015)[15] and Thayalan Dineshkumar (2017)[11].**In our study out of 22 patients, 18 were male patients (81.8 %) and 4 werefemale patients (18.2%) in group 1 (OSCC). Similarly, high incidence of oral cancer in males was reported in studies conducted by **Rhodus N.L (2005)[4], Brailo V (2006)[5], Warnakulasuriya (2009)[13], Hamad (2011)[14], Brailo V (2012)[6],Patil S (2013)[7], Juretic M (2013)[8], Khyani I.A.M (2014)[9], Khyani I.A.M (2015)[10], Selvam P (2015)[16],Rao M (2016)[17], Thayalan Dineshkumar (2017)[11].** Oral cancer ranks number one among men and number three among women in India. The sex distribution did not differ significantly between oral cancer and leukoplakia group (P-value>0.05). Higher incidence of oral cancer and oral leukoplakia was reported in males, this could be attributed to prevalence of habit of tobacco chewing and smoking in the males.

Tobacco both in smoking and smokeless form is considered to be a risk factor for oral cancer and pre- cancer. In the oral leukoplakia group, all the 22 patients (100%) were having habit of smokeless form of tobacco.**Reibel*et al* (2003)[20]**stated that tobacco is a major independent risk factor for the development of oral cancer as well as oral leukoplakia.Of 22 subjects studied in Group 2 (OL), the duration of habit ranging from a period of 10 years to up to 30 years and the frequency ranged from 5 – 8 times a day and none had frequency of habit for more than 8 times per day.

Out of 22 patients in our study, all 22 patients (100%) were habit abuse of which 95.5 % patient had smokeless tobacco habit and 4.5% patient had smoking form of tobacco. **Manjari M (1999)[21], Znaor*et al* (2003)[22]Cnattingius S (2005)[23],RuxandraSfeatcu (2008)[24] ,HariRam (2011)[25], and Maria Arub (2015)[26]**reported that smokeless tobacco is one of the major cause of oral cancer. Out of 22 cases studied

eISSN 1303-5150

in Group 1 (OSCC), the duration of habit ranged from a period of 10 years or less to up to 30 years or more with frequency ranging from 5 times or less to 8 times or more than that in a day.**Murthy *et al*. (1998)**[27]estimated the burden of tobacco related cancers in India by 2001 to be nearly 0.33 million cases annually.

The distribution of habit differs significantly between oral cancer group and healthy control Group (P-Value <0.01). The distribution of type of habit did not differ significantly between two study groups (P-value>0.05). The distribution of duration and frequency of habit is significantly higher in Group 1 (OSCC) compared to duration of habit in Group 2 (OL) (P-value<0.05).

In our study, out of 22 oral leukoplakia patients, the site most commonly involved is buccal mucosa and vestibule (45.5%), followed by buccal mucosa (36.4%), and vestibule (4.5%).Ana in case of 22 oral cancer patients, the most commonly involved site was buccal vestibule and alveolus (22.7%), followed by buccal mucosa and vestibule (18.2%), buccal vestibule (13.6%) and sites like buccal mucosa, buccal mucosa along with vestibule and alveolus involvement and buccal vestibule with retromolar region (9.1%) followed with minimal involvement of buccal mucosa with labial vestibule, labial vestibule and alveolus , alveolus and floor of mouth showing equal distribution.(4.5 %). **Krishnan *et al* (1988)**[28,]**Ogbureke KU*et al* (2012)**[29]**and David G *et al* (2012)**[30]reported that cancer of the buccal mucosa is the commonest malignancy among males and the third most common among female in India, secondary to the use of betel nut chewing, tobacco quid habit.This study proved site specific relationship in areas where tobacco quid is kept in lower buccal vestibule causing cancer of buccal mucosa and also labial mucosa are common with this fact the relevance of this study can be over emphasized considering highest rate of oral cancer specially cancer of buccal mucosa and gingivobuccal sulcus. The distribution of site of lesion differs significantly between study Group 1 (OSCC) and Group 2 (OL) (P-value<0.01).

Cytokines are soluble proteins that play an important role in the initiation and maintenance of inflammatory and immune responses as well as intercellular cross linking. Interleukin-6 (IL-6) is a pleomorphic cytokine involved in a number of physiologic and pathologic processes including response to trauma and development and progression of inflammation and malignancy. As per Virchow ‘s hypothesis, “inflammatory infiltrate might be the origin of cancer in sites of chronic inflammation.” Mantovani et al. stated that the connection between tumorigenesis and inflammation is mediated via intrinsic and extrinsic pathways. Intrinsic pathway is altered by genetic alteration and extrinsic pathway is driven by inflammation. The transformed cells after genetic alterations (DNA damage and inhibition of DNA repair, functional inactivation of tumor suppressor genes, stimulation of angiogenesis and angiogenic factor production.)secrete inflammatory mediators and thus generate an inflammatory micro-environment, this increases risk for development of cancer.

As per our study, the mean distribution of salivary IL-6 levels in oral leukoplakia is found to be 9.11 ± 14.14 pg/ml, which is increased as compared to controls ie 1.04 ± 0.63 pg/ml and was found to be statistically significant. (P-value < 0.001) Similar results were seen in studies conducted by **Brailo V (2012)**[6]**, Patil S (2013)**[7,]**Juretic M (2013)**[8,]**Thayalan Dineshkumar (2017)**[11]**.** In a study conducted by **Brailo V (2006)**[5]the mean value of salivary IL-6 was 14.93 ± 16.48, which was slightly elevated than usual.

Out of the 22 cases in Group 2 (OL), 17 (77.3%) had Mild epithelial dysplasia, 4 (18.2%) had Moderate epithelial dysplasia and 1 (4.5%) had Severe epithelial dysplasia.The distribution of median IL-6 levels for mild,

eISSN 1303-5150

moderate and severe dysplasia 9.03pg/ml, 5.61 pg/ml and 24.32 pg / ml. The distribution of median IL-6 levels did not differ significantly between three sub-groups of histopathological stages in Group 2 (OL) (P-value>0.05).Higher level of IL-6 level was seen in severe epithelial dysplasia similar results were reported by study of **Brailo V (2006)[5], Kaur J (2015)[15]**and **Patil S (2013)[7].** This can be correlated to IL-6, which has a double-edged activity in inflammation, both pro and anti-inflammatory properties. IL-6 in classical signalling activates anti-inflammatory pathway ad helps in regeneration of tissues, whereas trans-signalling activates pro-inflammatory pathway. The regulation of this property of IL-6 is controlled by IL-1b and TNF α. So, the pleiotropic role of IL-6 maintains the host and tumor- homeostasis and further resulting in malignant transformation.

The IL-6 level was found to be higher in Oral Squamous Cell Carcinoma (OSCC), as compared to Oral leukoplakia (OL) and control group, and the results were found to be statistically significant. (P –value < 0.001) The distribution of median (min – max) of IL-6 levels in Group 1 (OSCC) is 41.07 (15.45 – 322.65) pg/ml and the mean value is 96.60± 94.95. Similar results were obtained in study by **Rhodus N.L (2005)[4], Brailo V (2006)[5],Korostoff (2011)[31], Brailo V (2012)[6],Patil S (2013)[7], Juretic M (2013)[8], Khyani I.A.M (2014)[9], Khyani I.A.M (2015)[10], Selvam P (2015)[16],Rao M (2016)[17], Thayalan Dineshkumar (2017)[11].** In a study conducted by **St John MA et al (2004)[12]** no significant difference in salivary IL-6 was found when oral cancer patients and healthy controls were compared.

Out of 22 patients which were studiedin Group 1 (OSCC), 12 (54.5%) had Well differentiatedSquamous Cell Carcinoma (SCC), 4 (18.2%) had Moderately differentiatedSquamous Cell Carcinoma (SCC), 6 (27.3%) had Poorly differentiatedSquamous Cell Carcinoma (SCC).The mean salivary IL-6 values in Well differentiatedSquamous Cell Carcinoma, Moderately differentiatedSquamous Cell Carcinoma and Poorly differentiatedSquamous Cell Carcinoma are 26.09pg/ml, 96.45pg/ml and 237.71 pg/ ml respectively. The IL-6 levels is significantly higher in Poorly differentiatedSquamous Cell Carcinoma compared to Well differentiatedSquamous Cell Carcinoma and Moderately differentiatedSquamous Cell Carcinoma. (P-value<0.001 for both).

The overexpression of IL-6 could be correlated to IL-6 having influence on all hallmarks of cancer like inhibition of apoptosis, promotion of survival, proliferation, angiogenesis, invasiveness and metastasis, and is alsoknown to regulate cancer cell metabolism. So we can say that IL-6 plays an important role in oncogenic pathways.

Many of the studies carried out by various researchers like **Rhodus N.L (2005)[4], Hathaway *et al*.(2005)[32]**, **Brailo V (2006)[5], Katakura et al(2007)[33], Hamad (2011)[14]**, **Brailo V (2012)[6], Patil S (2013)[7], Juretic M (2013)[8], Khyani I.A.M (2014)[9], Khyani I.A.M (2015)[10], Selvam P (2015)[16], Thayalan Dineshkumar (2017)[11]** on serum and salivary IL-6 level comparison have come to conclusion that, though there is increase in serum, salivary levels are more higher. Many authors have concluded and hypothesized that alteration in the production of cytokines in oral cancer occurs chiefly in the oral cavity since the dysplastic oral lesions often accompany with inflammation and epithelial discontinuity, and is not a reflection of serum cytokine concentration.[11] Whereas **Rhodus N.L (2005)[4]**et al*. in his study felt that the increase in salivary IL-6 levels, is due to local* production of this cytokine in cancer tissue. Pries *et al*. **(2006)[34]** in his study also stated that oral cancer cells and tumor infiltrating lymphocytes are capable of producing IL-6.[11]

Thus, we can conclude that, salivary Interleukin -6 (IL-6) levels vary from control,

eISSN 1303-5150

pre-cancer to cancer group. As the carcinogenesis process continues IL-6 level keeps increasing, a Salivary IL-6 level gives us an idea of compromised oral environment. So, it can be considered as early diagnostic marker.The measurement of tumor markers in saliva forms a striking substitute to serum testing due to close contact of the oral cancer lesion with saliva.[35]As very few studies have been done on tumor markers in saliva, this study will definitely be a valuable addition.

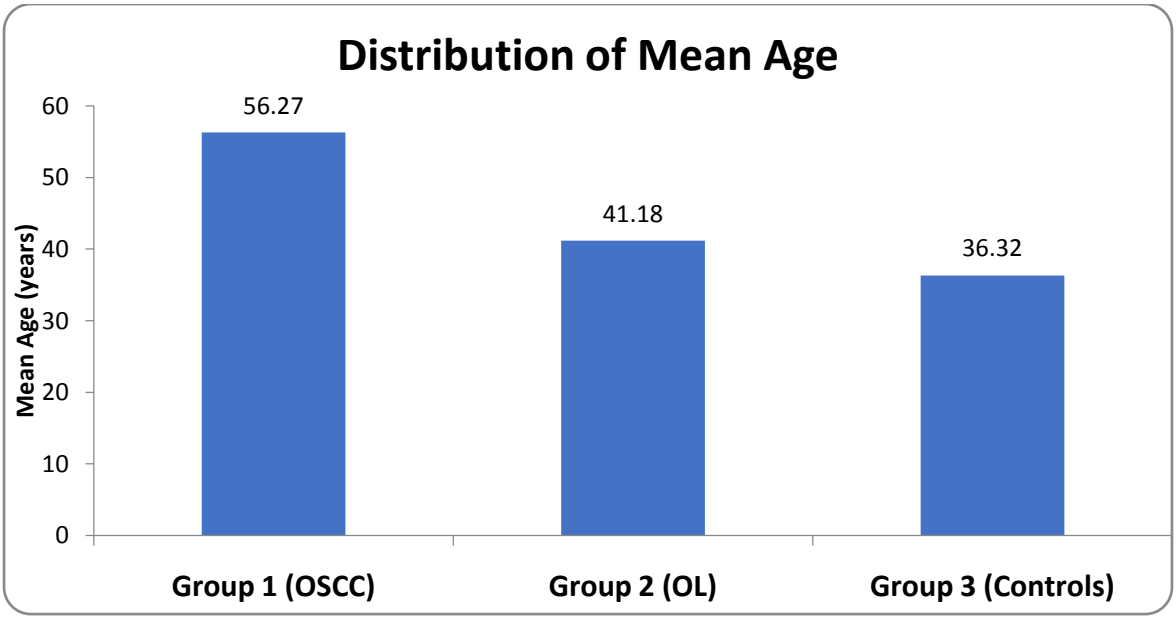
Since our study was done in close dental setting, for better confirmation and standardization of the salivary IL-6 levels, study with larger sample size is needed to be done. Also, very few studies have been done with saliva. In this study we have not considered different forms of IL-6 andalso, we need to compare serum and salivary IL-6 levels. Hence, further studies are required with larger sample size to confirm reliability in screening of oral cancer patients, to e further established as reliable marker.

From above summary, following conclusions were drawn from the present study:

- Salivary interleukin 6 levels were found to be higher in oral cancer and oral leukoplakia group as compared to healthy controls. (P-value<0.001 for both).
- Salivary interleukin 6 levels were significantly greater in oral cancer as compared to oral leukoplakia. (P-value<0.001).
- Saliva collection method is found to be inexpensive, easy and reliable for evaluating of IL-6 value.

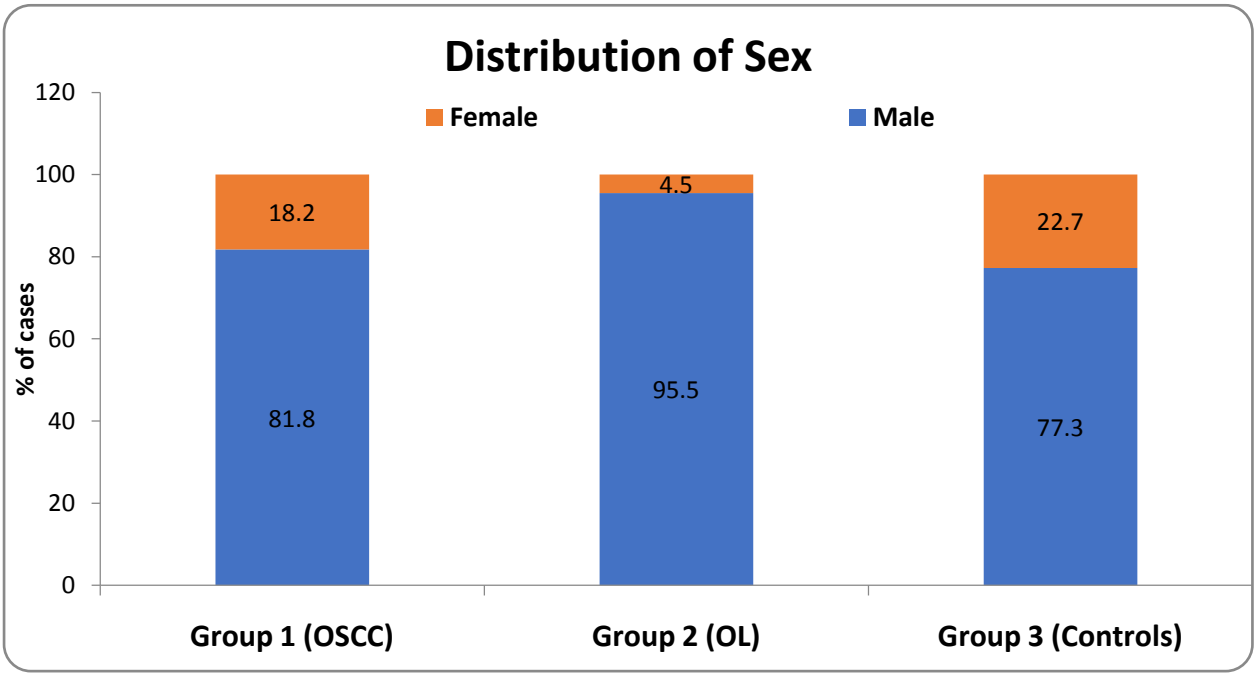
We have also seen significant increase in salivary IL-6 levelsin poorly differentiated SCC and also severe epithelial dysplasia showed higher salivary IL-6 level therefore it can be said that it can be used as progression marker as well as indicator during therapy.Since salivary estimation is non-invasive, and the sample is easily available which can be repeatedly used without causing annoyance to patient, so patient compliance is better and therefore it seems to be a good diagnostic tool. In near future a salivary kit can be formulated to use it as a handy diagnostic tool and a progression marker after therapy. With other salivary markers salivary IL-6 levels can also be included as a marker for screening in oral cancer and pre-cancer.

Graph 1) Distribution of mean age across three study groups.

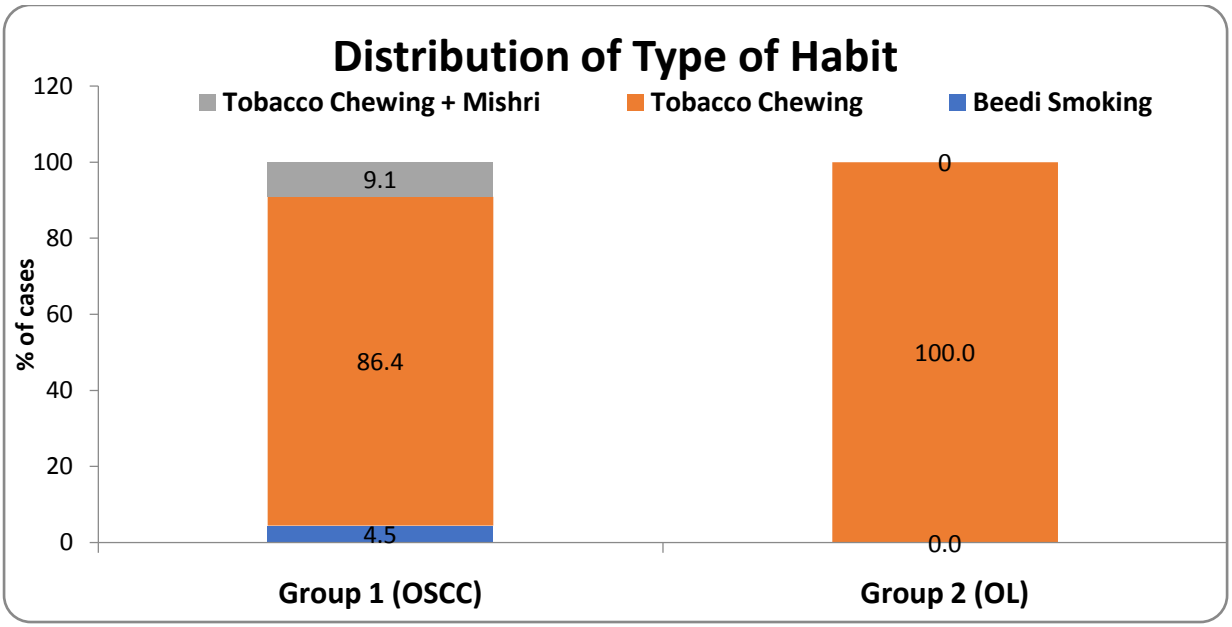


Graph 2) Distribution of sex across three study groups.

eISSN 1303-5150

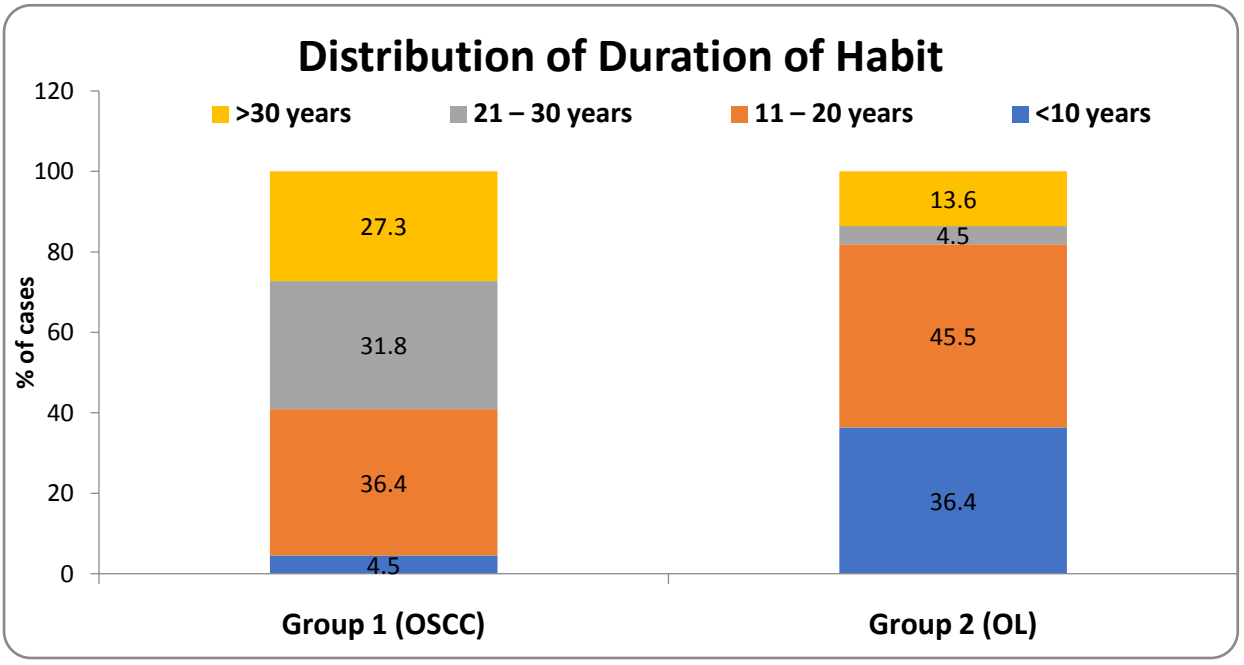


Graph 3) Distribution of of type of habit across two study groups.

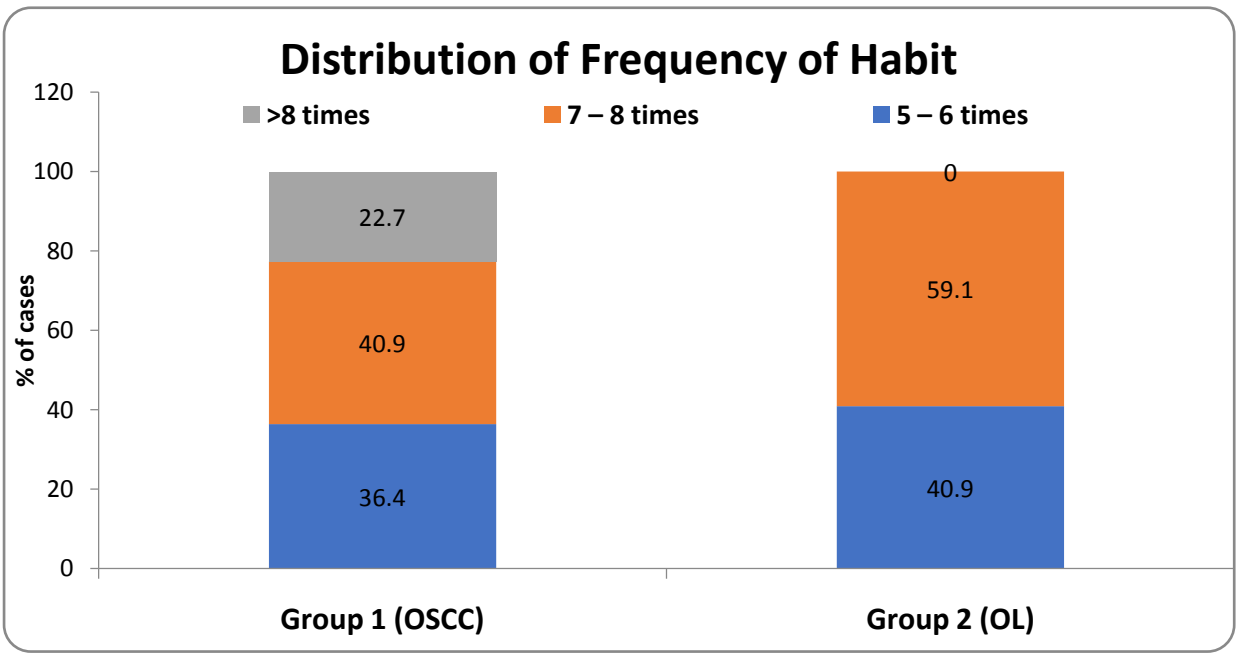


Graph 4) Distribution of duration of habit across two study groups.

eISSN 1303-5150

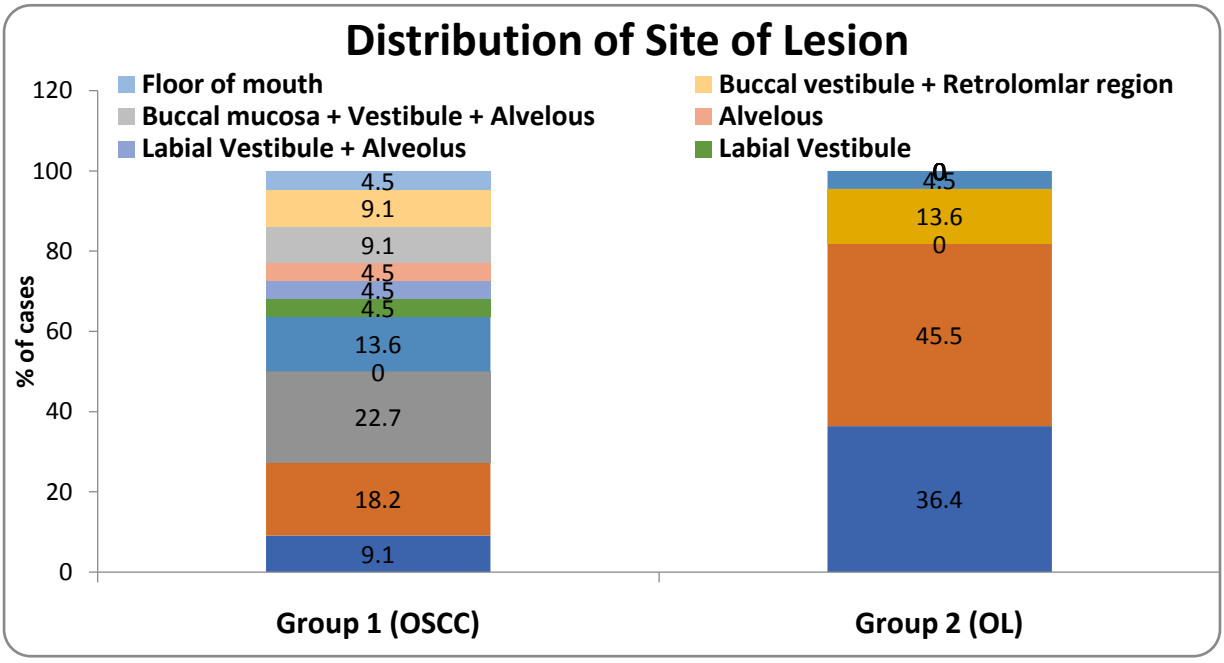


Graph 5) Distribution of frequency of habit across two study groups.

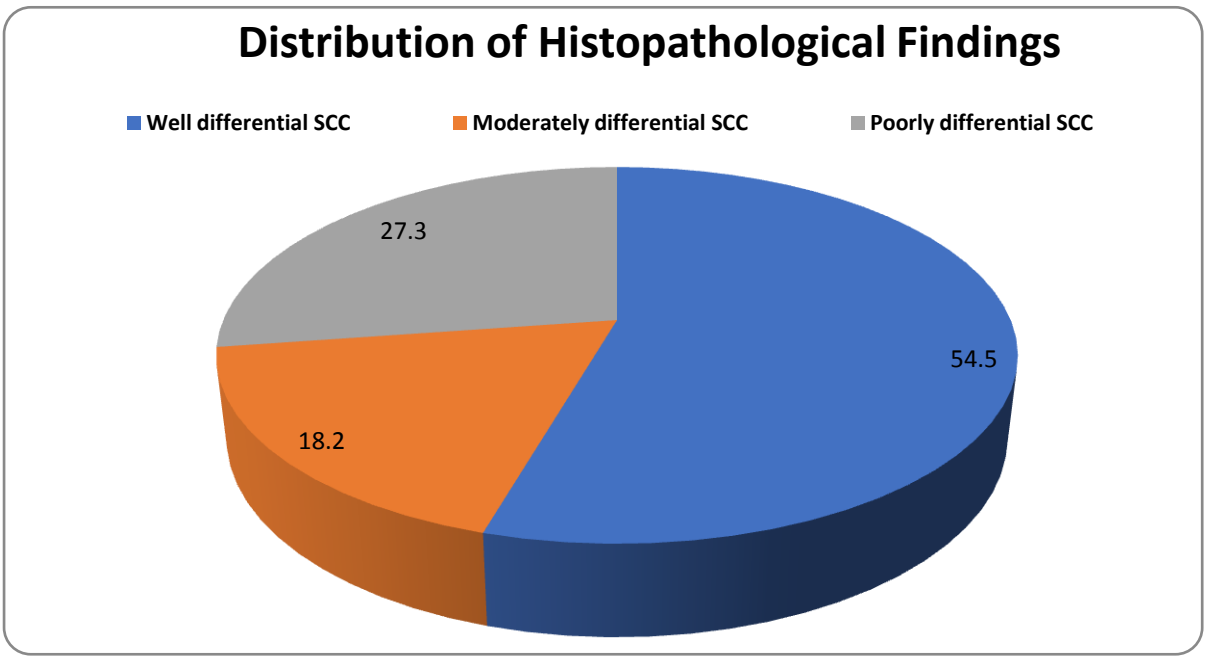


Graph 6) Distribution of site of lesion across two study groups.

eISSN 1303-5150

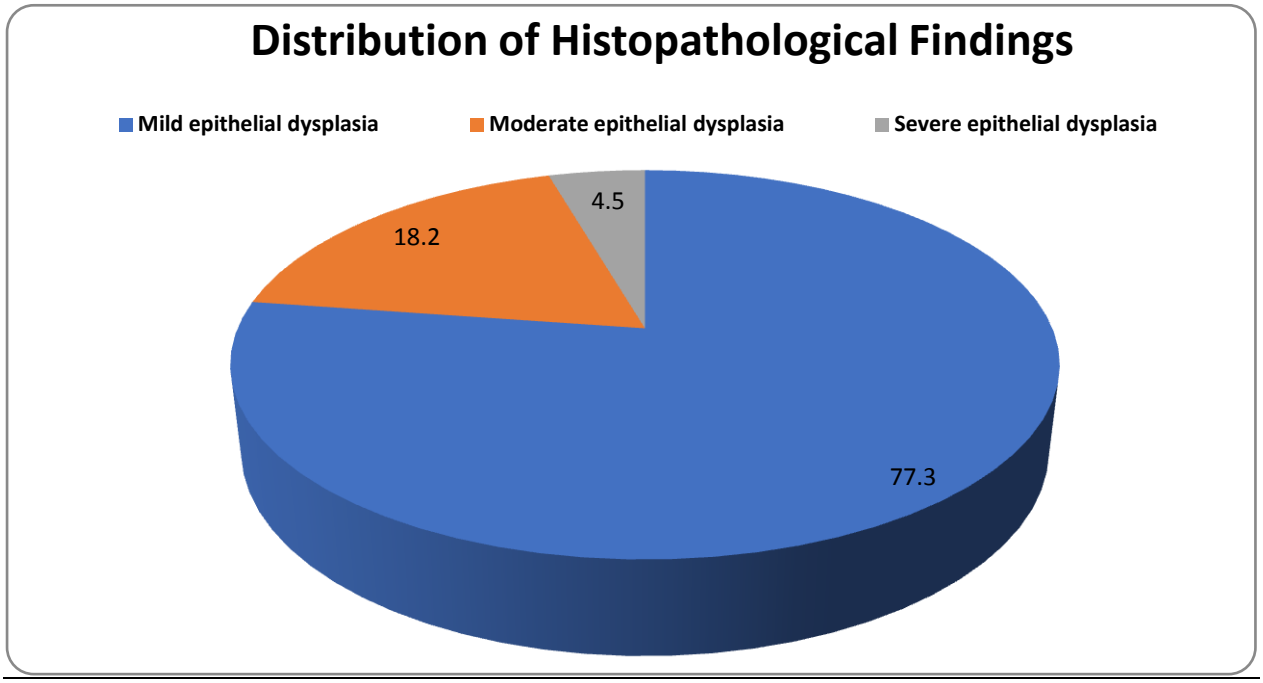


Graph 7) Distribution of histopathological findings in Group 1 (OSCC).

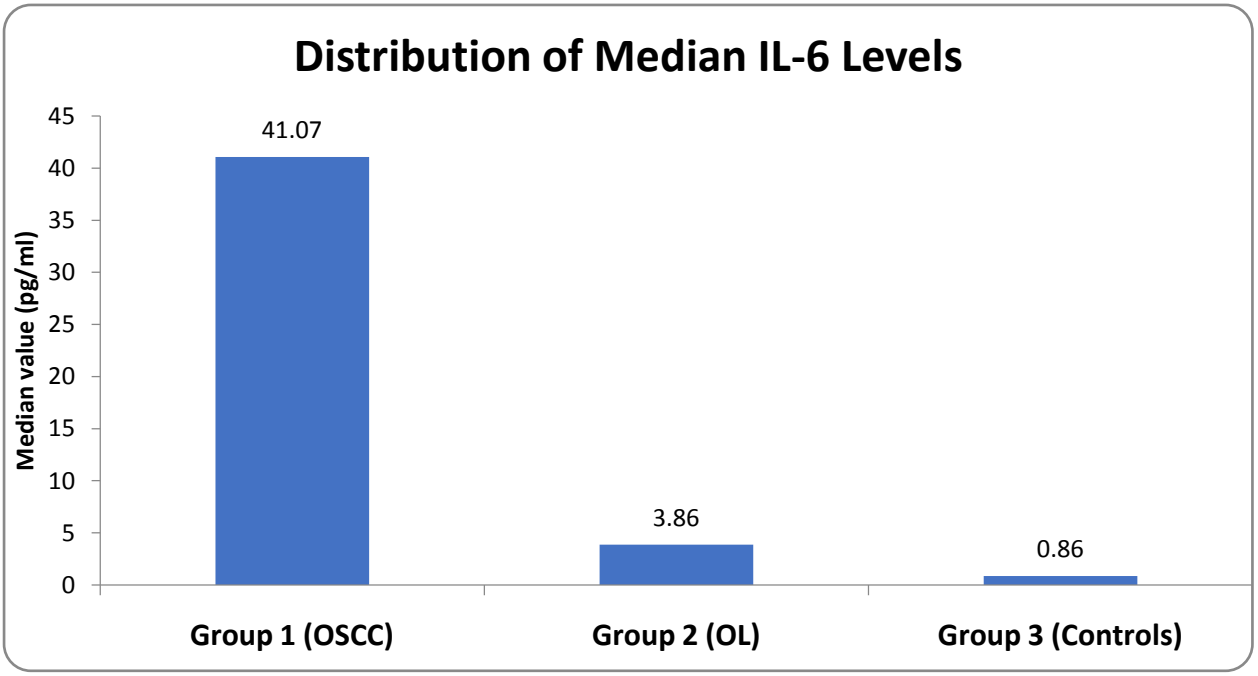


Graph 8) Distribution of histopathological findings in Group 2 (OL).

eISSN 1303-5150

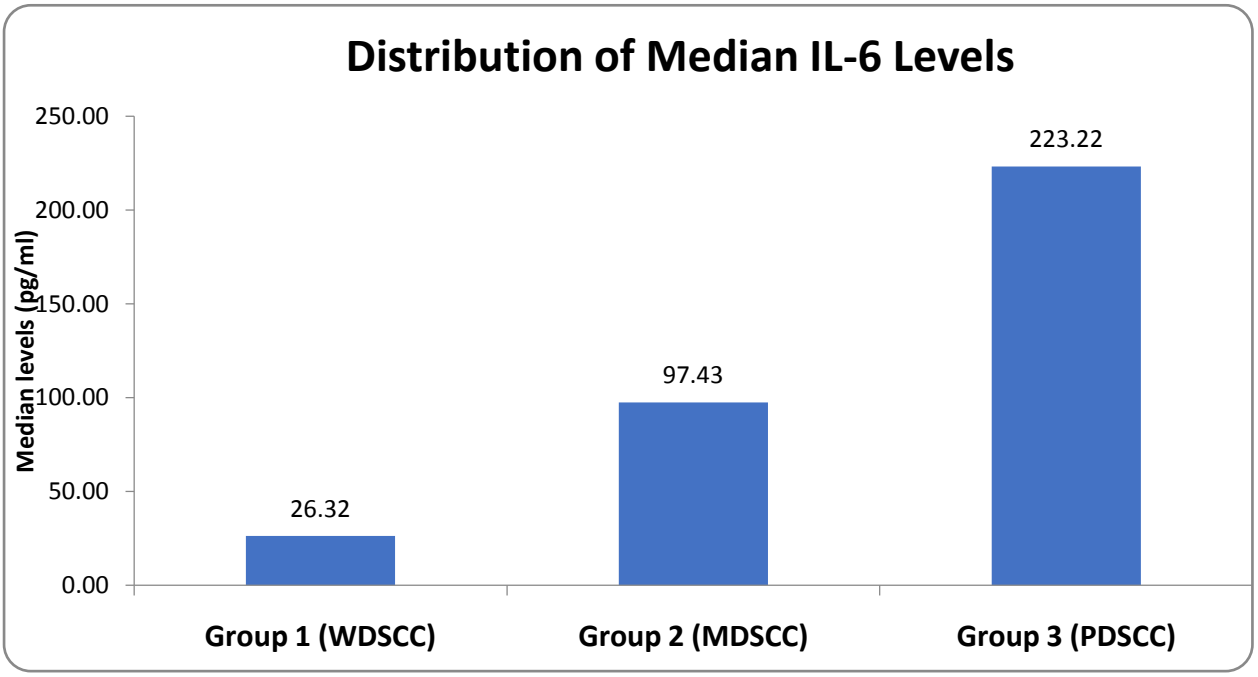


Graph 9)Distribution of median IL-6 levels across three study groups.

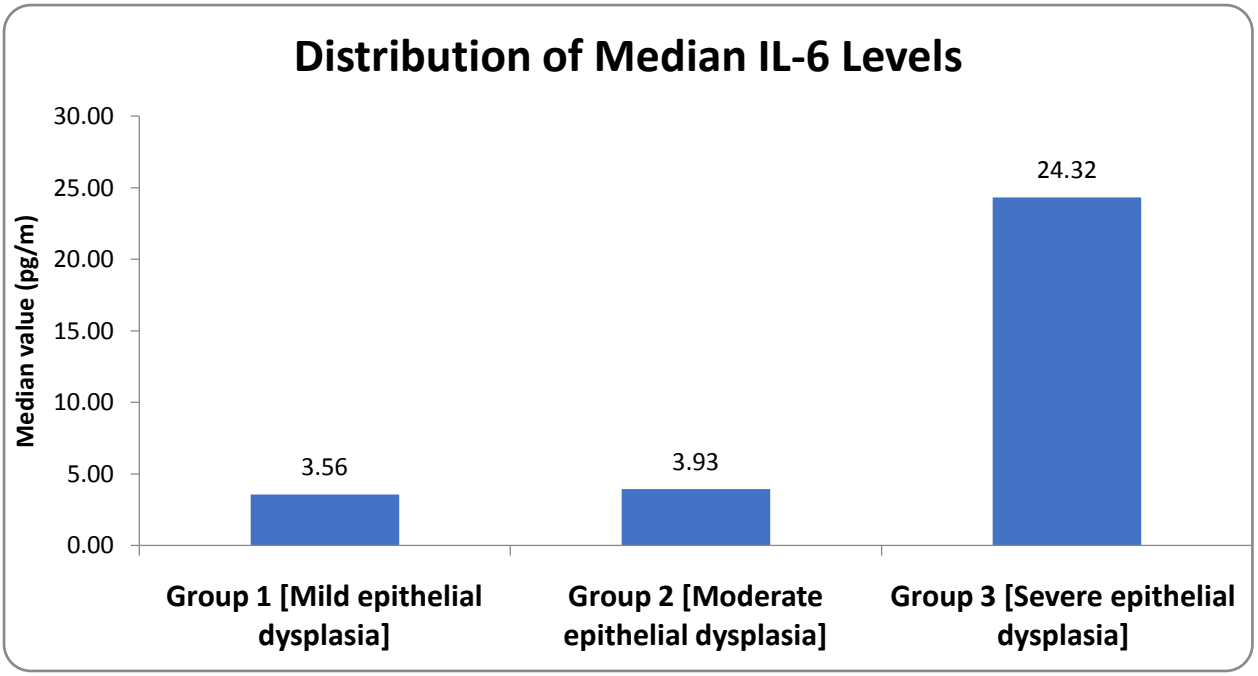


Graph 10) Distribution of median IL-6 across three groups of histopathological stages in Group1 (OSCC).

eISSN 1303-5150



Graph 11)Distribution of median IL-6 across three groups of histopathological stages in Group 2 (OL).



References:

1) Del Corso G, Villa A, Tarsitano A, Gohel A. Current trends in oral cancer: A systematic review. Cancer Cell & Microenvironment. 2016 6;3(3).
2) Zohaib Khurshid, Muhammad S. Zafar, Rabia S. Khan, Shariq Najeeb, Paul D. Slowey, Ihtesham U. Rehman. Role of Salivary Biomarkers in Oral Cancer Detection. Advances in Clinical Chemistry, Volume 86 ISSN 0065-2423 https://doi.org/10.1016/bs.acc.2018.05.002.
3) Ali J, Sabiha B, Jan HU, Haider SA, Khan AA, Ali SS. Genetic etiology of oral cancer. Oral Oncology. 2017 ;70:23-8.

eISSN 1303-5150

4) Rhodus, N. L., Ho, V., Miller, C. S., Myers, S., &Ondrey, F. (2005). NF-κB dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. Cancer Detection and Prevention, 29(1), 42–45. doi:10.1016/j.cdp.2004.10.003
5) Brailo V, Vučićević-Boras V, Cekić-Arambašin A, Alajbeg IŽ, Milenović A, Lukač J. The significance of salivary interleukin 6 and tumor necrosis factor alpha in patients with oral leukoplakia. Oral oncology. 2006 Apr 30;42(4):370-3.
6) Brailo V, Vucicevic-Boras V, Lukac J, Biocina-Lukenda D, Zilic-Alajbeg I, Milenovic A et. al. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. Med Oral Patol Oral Cir Bucal. 2012 Jan 1;17(1):e10-5.
7) Patil S, Kaswan S, Rahman F, Dhoni B, Wadhawan R. Salivary and serum tumor necrosis factor alpha,interleukin 1 alpha, interleukin 1 beta, interleukin 6 and interleukin 8 in patients with oral carcinoma and leukoplakia. JNDA Vol. 13, No. 1, Jan.-June, 2013.
8) Juretic M et.al. Salivary levels of TNF-α and IL-6 in patients with oral premalignant and malignant lesions.Folia Biol (Praha). 2013; 59(2):99-102.
9) Khyani, I.A.M.; Qureshi, M.A.; Farooq, M.U.; Mirza, T. Molecular diagnosis of oral pre-malignant lesions & oral squamous cell carcinoma in saliva-a breakthrough in pakistan. Inter. J. Endorsing Health Sci. Res. 2014, 2, 108–116.
10) Khyani IA, Qureshi MA, Mirza T, Farooq MU. Salivary diagnosis of oral pre-neoplastic and oral squamous cell carcinoma in etiologically distinct population of Pakistan. survival. 2015;15:16.
11) ThayalanDineshkumar et al.*Salivary and Serum Interleukin-6 Levels in Oral Premalignant Disorders and Squamous Cell Carcinoma: Diagnostic Value and Clinicopathologic Correlations.* Asian Pac J Cancer Prev, 17 (11), 4899-4906.
12) John MA, Li Y, Zhou X, Denny P, Ho CM, Montemagno C, et. al. Interleukin 6 and interleukin 8 as potential biomarkers for oral cavity and oropharyngeal squamous cell carcinoma. Archives of Otolaryngology–Head & Neck Surgery. 2004 Aug 1;130(8):929-35.
13) Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral oncology. 2009 May 31;45(4):309-16.
14) Hamad, A.-W.R.; Gaphor, S.M.; Shawagfeh, M.T.; Al-talabani, N.G. Study of serum and salivary levels of proinflammatory cytokines, potential biomarkers in the diagnosis of oral Squamous cell carcinoma. Acad. J.Cancer Res. **2011**, 4, 47–55.
15) Kaur J, Jacobs R.Proinflammatory cytokine levels in oral lichen planus, oral leukoplakia, and oral submucous fibrosis. Journal of the Korean Association of Oral and Maxillofacial Surgeons. 2015 Aug 1;41(4):171-5.
16) PanneerSelvam N, Sadaksharam J. Salivary interleukin-6 in the detection of oral cancer and precancer. Asia-Pacific Journal of Clinical Oncology. 2015 Sep 1;11(3):236-41.
17) Rao M, Ramesh A, Adapa S, Thomas B, Shetty J. Correlation of salivary levels of interleukin-6 and albumin with oral squamous cell carcinoma. J Health Res Rev 2016;3:11-4.

eISSN 1303-5150

18) SahebJamee M, Eslami M, AtarbashiMoghadam F, Sarafnejad A. Salivary concentration of TNFα, IL1α, IL6, and IL8 in oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal. 2008 May1;13(5):E292-5.
19) Sato J, Goto J, Murata T, Kitamori S, Yamazaki Y, Satoh A, Kitagawa Y. Changes in saliva interleukin-6 levels in patients with oral squamous cell carcinoma. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2010 Sep 30;110(3):330-6.
20) Reibel J. Prognosis of oral pre malignant lesions. Significance of clinical, histopathological and molecular biological characteristics. Crit Rev Oral Biol Med 2003;14(1):47-62.
21) Manjari M, Popli R, Paul S, Gupta VP, Kaholon SK. Prevalence of oral cavity, pharynx, larynx and nasal cavity malignancies in Amritsar, Punjab. Indian J Otolaryngol Head Neck Surg 1999;48(3):191-5.
22) Znaor A, Brennan P, Gajalakshmi V, Mathew A, Shanta V, Varghese C*et al*. Independent and combined effects of tobacco smoking, chewing and alcohol drinking on the risk of oral, pharyngeal and esophageal cancers in Indian men. Int J Cancer 2003;105(5):681-6.
23) Karolinska Institute. In: Cnattingius S, Galanti R, Grafstrom R, Hergens M-P, Nyren O, Pershagen G, Wickholm S, editors. Health risks from Swedish snuff; 2005. p. 1–95.
24) Sfeatcu R, Dumitrache A, Burlibasa M, Lonescu L, Lupusoru G. OHDMBSC 2008;VII(2):38-41.
25) Ram H, Sarkar J, Kumar H, KonwarR,Bhatt MLB, Mohammad S. Oral cancer: risk factors and molecular pathogenesis. J Maxillofac Oral Surg 2011;10(2):132–7.
26) Ogbureke KU, Bingham C. Overview of oral cancer. In Oral Cancer 2012. InTech.
27) Murthy NS, Chaudhry K, Rath GK. Burden of cancer and projections for 2016, Indian scenario: gaps in the availability of radiotherapy treatment facilities. Asian Pac J Cancer Prev. 2008 Oct 5;9(4):671-7
28) Nair MK, Sankaranarayanan R, Padmanabhan TK, Padmakumari G. Clinical profile of 2007 oral cancers in Kerala, India. Ann Dent 1988;47(1):23-6.
29) Ogbureke KU, Bingham C. Overview of oral cancer. In Oral Cancer 2012. InTech.
30) Goldstein DP, Bachar G, Barker E, Lea J, O'Sullivan B, Brown DH, Gullane PJ, Gilbert RW, Xu W, Su J, Irish JC. Squamous cell carcinoma of the buccal mucosa: outcomes of treatment in the modern era. The Laryngoscope. 2012 Jul 1;122(7):1552-7.
31) Korostoff, A., Reder, L., Masood, R., & Sinha, U. K. (2011). The role of salivary cytokine biomarkers in tongue cancer invasion and mortality. Oral Oncology, 47(4), 282–287. doi:10.1016/j.oraloncology.2011.02.006.
32) Hathaway, B., Landsittel, D. P., Gooding, W., Whiteside, T. L., Grandis, J. R., Siegfried, J. M., Ferris, R. L. (2005). Multiplexed Analysis of Serum Cytokines as Biomarkers in Squamous Cell Carcinoma of the Head and Neck Patients. The Laryngoscope, 115(3), 522–527. doi:10.1097/01.mlg.0000157850.16649.b8.

33) Katakura A, Kamiyama I, Takano N, Shibahara T, Muramatsu T, Ishihara K, et al. Comparison of salivary cytokine levels in oral cancer patients and healthy subjects. Bull Tokyo Dent Coll. 2007;48:199-203.
34) Pries, R., &Wollenberg, B. (2006). Cytokines in head and neck cancer. Cytokine & Growth Factor Reviews, 17(3), 141–146. doi:10.1016/j.cytogfr.2006.02.001.
35) Nagler, R. M. (2009). Saliva as a tool for oral cancer diagnosis and prognosis. Oral Oncology, 45(12), 1006–1010. doi:10.1016/j.oraloncology.2009.07.005.