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STUDY OF INFLAMMATORY CYTOKINE IL-10 IN CLINICO- PATHOLOGICAL PARAMETERS TOBACCO INDUCED ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Objective. Inflammation is an important symbol of all cancers and inflammatory response is determined by a slight balance between pro inflammatory- and anti-inflammatory cytokines, which may be affected by tobacco exposure, so the present study was designed to explore the effect of various parameters of tobacco exposure on interleukin-10 (IL-10) inflammatory cytokine levels and their survival rate in oral cancer patients. ***Methods*.** 30 oral cancer patients and pre-cancerous controls with were recruited; baseline levels of serum IL-10 were measured and analysed in various tobacco exposed groups by appropriate statistical implements. ***Results***. The expression of serum anti-inflammatory (IL-10) cytokines was associated with tobacco exposed group as smokers, tobacco chewers, and pan-masala as well as alcohol users have shown significantly levels (P< 0.001) with significantly lower median survivals, clinical grade III and IV of tobacco addicted patients have also shown significantly increased levels of IL-10. Conclusions. IL-10 seem to be affected by various modes of tobacco exposure and inflammation also affects median survival of cancer patients.

Key words: Cancer, Cytokines, Clinical Grade, Inflammation, Interleukin-10

INTRODUCTION

Oral cancer is a major worldwide health problem. Around 300,000-400,000 patients are annually evaluated to have this chronic disease all over the world. Our main interest reductions in head and neck squamous cell carcinoma (HNSCC) in general and oral squamous cell carcinoma (OSCC) in definite because of its distressing incidence and mortality **[1].** Oral squamous cell carcinoma is a multifactorial disease. It is influenced by genetic alterations of various genes; as well as various environmental factors, such as tobacco substituents and consumption of cigarette smoking, tobacco chewing, alcohol infection by oncogenic viruses, and low intake of fruits and vegetables **[2]**. During the last decade, a clear evidence has been obtained that inflammation plays a critical role in tumorigenesis **[3]**. There are various Cytokines are important components of inflammation. They are cell-signalling protein molecules which affect the intercellular networks **[4]**. They are detected to be produced in the tumor microenvironment, signifying their suggestive role in cancer pathogenesis **[5]**. Interleukin-10 is a potent pleiotropic cytokine with immunosuppressive and anti-inflammatory functions, as well as anti-cancer activities **[6]**. Various cell populations are able to produce it. However, the most important cause of IL-10 production is macrophages Increased IL-10 expression in tumor tissues, serum and saliva was noted in different cancer types (Moreover, the high concentration was proposed as an indicator of poor prognosis . Oral cancer progression is a complex process that involves host-tumor interactions, which occur via multiple molecular and cellular factors within the tumor microenvironment. The genetic irregularities of human cancer to a certain degree depend on geographical location, cultural and environmental backgrounds and also cell lines in which it is expressed. IL10 activates through the Janus kinase–signal transducer and activator of transcription (JAK–STAT) signaling pathway by blocking nuclear factor-kappa-B (NF-κB) nuclear translocation. Interruption of the interactions of IL-10 with its receptors IL10RA and IL10RB and α-2-macroglobulin (A2M) may lead to improved inflammation.

Which could encourage tumor growth, blockage of the A2M-APP communication may lead to cancerous cellular proliferation through free APP, blockage of A2M-KLK13 (Hk13) interaction can increase free Hk13, which can promote cancer cell growth, metastasis and invasion through damage in the extracellular matrix **[7]**. The balance of immune responses between Th1/Th2 regulates consequences of diseases. Th1 cells produce interferon (IFN) γ, IL2, IL12 and tumor necrosis factors α cytokines, which are complex in the cell-mediated pro-inflammatory response **[8]**. Th2 cells secrete IL4, IL5, IL6, IL10 and IL13 cytokines, which facilitate anti-inflammatory humoral response and immune suppression via the inhibition of Th1 cytokine production. TGFβ1, a member of the TGFβ family that is predominantly secreted by regulatory T-cells (Tregs), is another multi-functional cytokine. It promotes tumor progression by inducing mesenchymal transition, tumor outflow by antagonizing IL2 functions and inducing immune suppression, tumor invasion and metastasis. Previous study concluded, that there was a highly significant contributions from IL6 and TNF-α in occurrence of OSCC than other interleukins like IL4, 8 and 10 IL10 also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine identified in 1989. It mainly controls immune response and if not available, inflammation reaction becomes possible. The main source of IL10 construction is mainly from macrophage and is encoded by a gene located on chromosome 1 at 1q31-32 which has five exons and four introns **[9]**. IL10 has numerous functions, such as reserve of cytokine production, T-cells proliferation, angiogenesis and regulation of inflammatory responses. The active role of IL10 in tumorigenesis and tumor inhibition is of debate. Elevated levels of IL10 production have been identified in oral cancers and solid tumors **[10, 11, 12]**.

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation among humans. It is the alteration of a nucleotide, namely adenine (A), thymine (T), cytosine (C) and guanine (G), between a pair of chromosomes. The etiology of a specific cancer might be associated with genetic variants. IL10 polymorphisms like A1082G/AA/AG/GG genotypes may not only contribute to differential appearance levels of IL10 among individuals but may also lead the risk oral cancer susceptibility **[13]**.

Apart from oral cancers, studies showed IL10 polymorphisms to various cancer types like breast carcinoma, hepatocellular carcinoma **[12].** The aim of the present work was to evaluate the expression of IL-10 in different grades of OSCC, and to correlate this expression to the disease stage. The assessment of IL-10 serum and salivary concentrations using ELISA analysis was also aimed. Finally, correlating the tissue, serum and salivary concentrations of IL-10 was done.

Objective:

The present study was designed to study and correlate: To evaluate and correlate the expression of IL-10 in different histopathological grades of OSCC, as well as to assess its serum level.

MATERIALS AND METHODS

Patient and Control Selection:

All thirty patients newly diagnosed, previously untreated patients with tumour or early stage of tumour attending department of dentistry of Eras Medical College and Hospital Lucknow or oncology clinics of king George medical college Lucknow Lucknow, India, between 2015 to 2018 blood samples were collated for this study. During the same period, patients has divided into groups on the basis of demographical and clinicographical parameters like gender, age, tumor stage, node involvement and clinical and pathological grades. 30 patients of oral-carcinoma subjects were taken. OSCC patients collected from Eras Medical College and Hospital Lucknow All patients signed informed consents for the agreement to participate in the study. the ethical clearance was received from the Institutional Ethics committee. Following an informed consent, information was obtained from the subjects as age, gender, habitual attributes (recall basis), and family history for any cancer. All the study participants were instructed to refrain from eating, drinking, smoking, or carrying out oral hygiene procedures for at least one hour before sample collection. 3to 4 ml of blood samples were taken from the patients and controls before surgery. Blood samples were allowed to clot for at least 30 minutes, and then centrifuged for 10 -15 minutes at 1500 rpm. The serum layer was removed and stored at -20°C. Serum levels of IL-10 were measured using a human IL-10 ELISA Kit (ElabSciences,) according to the manufacturer`s instructions.

Environmental Factors:

Exposure Factors. The exposure factors were recorded in cases and controls, which included tobacco use (smoking and chewing tobacco) and alcohol intake. Tobacco habit was categorized into smokers and chewers. All patients signed informed consents for the agreement to participate in the study. Patients and controls who presented with signs of active infection, autoimmune diseases, or having a history of radiotherapy, chemotherapy or other cancers were excluded from the study. Tobacco Exposure by Various Modes May alter Anti-Inflammatory (IL-10) Levels and Affects the Survival of oral Carcinoma Patients:

Exclusion criteria:

1.The patients and controls suffering from diabetes, arthritis, cardiovascular disease, hepatitis, AIDS, and other inflammatory diseases including prostatitis were excluded.

2. Patients and controls who presented with signs of active infection, autoimmune diseases, or having a history of radiotherapy, chemotherapy or other cancers were excluded from the study

Statistical Analysis:

Data were potted as mean ± SD and in percentages. Initially the analysis of variance ANOVA (one-way and two-way) was applied among various groups of tobacco users, smokers ,age, gender , if found statistically significant among groups, then pair wise comparison was performed between groups by using independent unpaired t-test. All the analysis was carried out by using SPSS 15.0. The P value < 0.05 was considered as statistically significant Tobacco/pan-masala Exposure by Various Modes May Alter Anti-Inflammatory (IL-10) Levels and Affects the Survival of oral Carcinoma Patients:

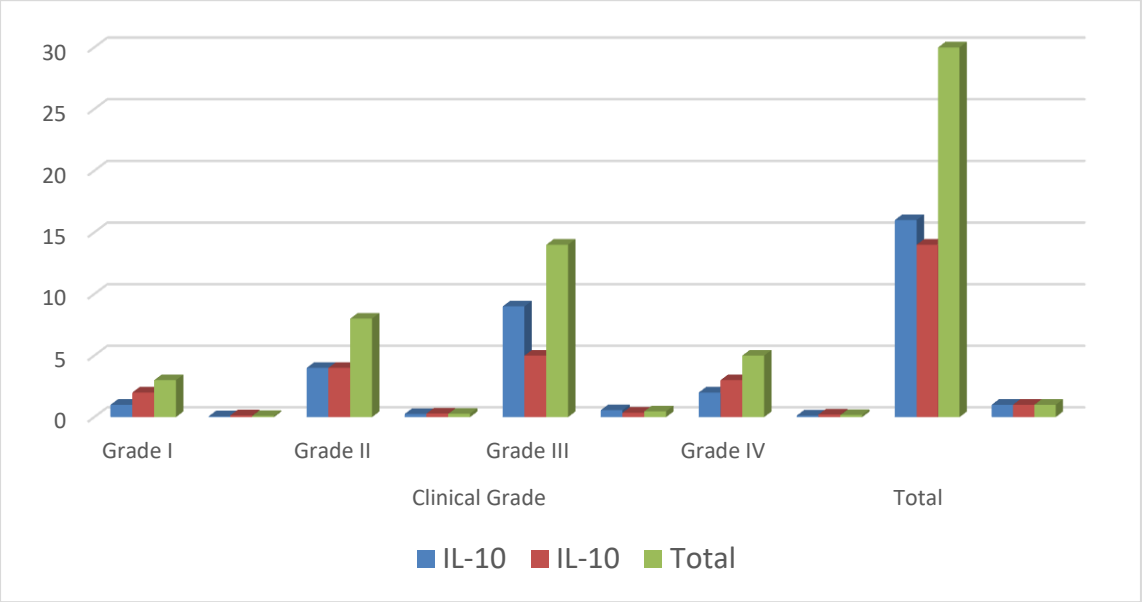
Grading system:

Staging looks at the size of tumor, depth of (tumour) and whether it has spread anywhere else in the body. There are different grading systems can use for mouth cancer. The TNM staging system is one of these showing data as follows-

Table-1, The results showed that as clinical grade increases mean Il-10 concentration in serum increases. Mean IL-10 concentration was Grade I (10.0%), grade II (26.7%), grade III (46.7%), and grade IV (16.7%), respectively. On comparing, ANOVA revealed significantly different mean il-10 concentrations among the groups (χ^2 =1.550, P<0.671).

		IL-10		Total
Clinical Grade	Grade I	1	2	3
		6.2%	14.3%	10.0%
	Grade II	4	4	8
		25.0%	28.6%	26.7%
	Grade III	9	5	14
		56.2%	35.7%	46.7%
	Grade IV	2	3	5
		12.5%	21.4%	16.7%
Total		16	14	30
		100.0%	100.0%	100.0%

Applied χ^2 test for significance. χ^2 value=1.550; p-value=0.671; consider not significant.



Mean IL-10 concentration in cases of various clinical grades of oral squamous cell carcinoma cases,

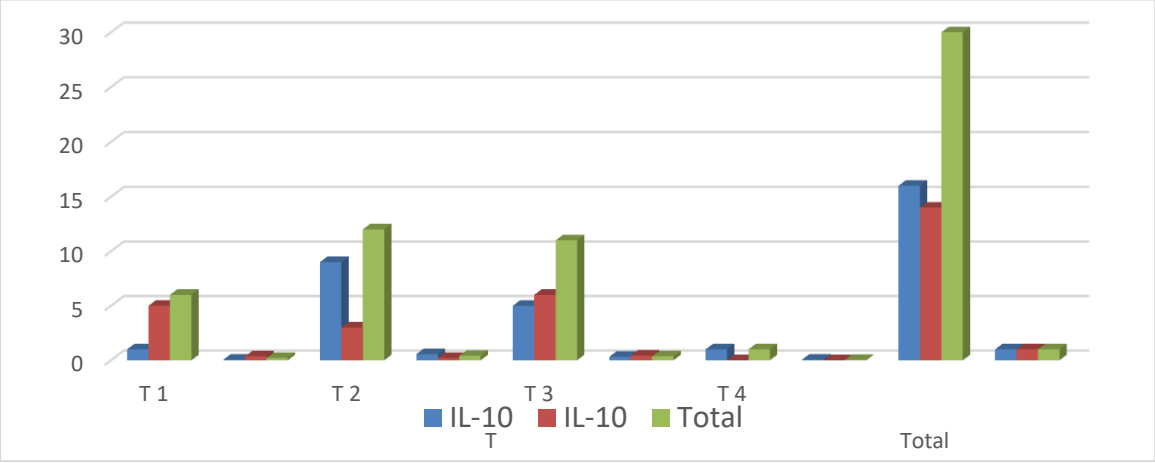
TNM stands for Tumour, Node, Metastasis. It describes:

- the size and depth of the primary tumour (T)
- whether the cancer has spread to the lymph nodes (N)
- whether the cancer has spread to another part of the body (M)

Table-2, The Results showed that as size of tumor increases and mean Il-10 concentration in serum increases. Mean IL-10 concentration was T1 (20.0%), T2 (40.0%), T3 (36.7%), and T4 (3.3%) respectively. On comparing, ANOVA revealed significantly different mean il-10 concentrations among the groups (χ^2 value=6.654; p-value=s0.084).

		IL-10		
		Positive	NEGATIVE	Total
T	T 1	1	5	6
		6.2%	35.7%	20.0%
	T 2	9	3	12
		56.2%	21.4%	40.0%
	T 3	5	6	11
		31.2%	42.9%	36.7%
	T 4	1	0	1
		6.2%	.0%	3.3%
Total		16	14	30
		100.0%	100.0%	100.0%

Applied χ^2 test for significance. χ^2 value=6.654; p-value=0.084; consider not significant.

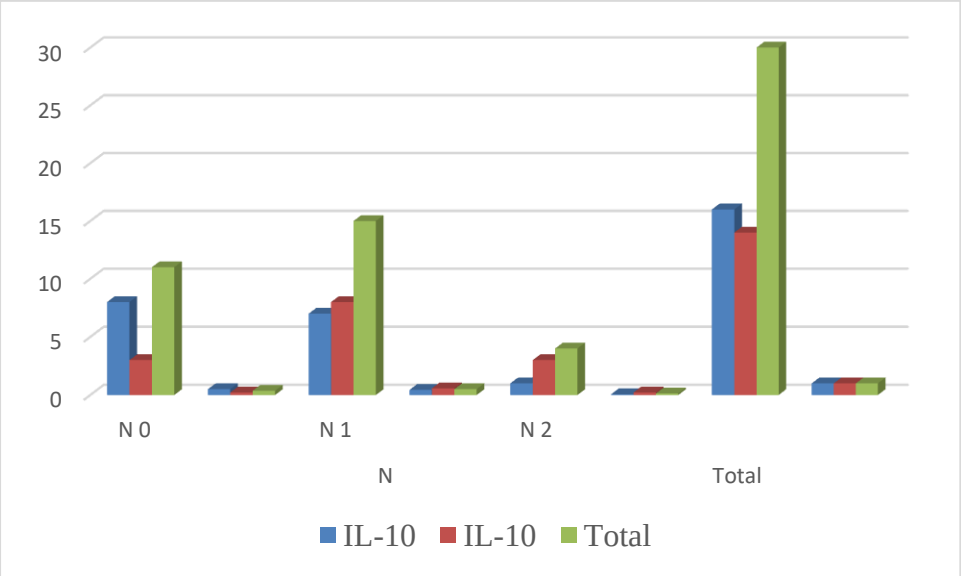


Mean IL-10 concentration in cases of tumor size of oral squamous cell carcinoma cases.

Table 3. The results showed that as Node Involvement and mean of Il-10 concentration in serum. mean il-10 concentrations among the groups (χ^2 value=3.220; p-value=0.200;).

		IL-10		
		Positive	NEGATIVE	Total
N	N 0	8	3	11
		50.0%	21.4%	36.7%
	N 1	7	8	15
		43.8%	57.1%	50.0%
	N 2	1	3	4
		6.2%	21.4%	13.3%
Total		16	14	30
		100.0%	100.0%	100.0%

Applied χ^2 test for significance. χ^2 value=3.220; p-value=0.200; consider not significant.

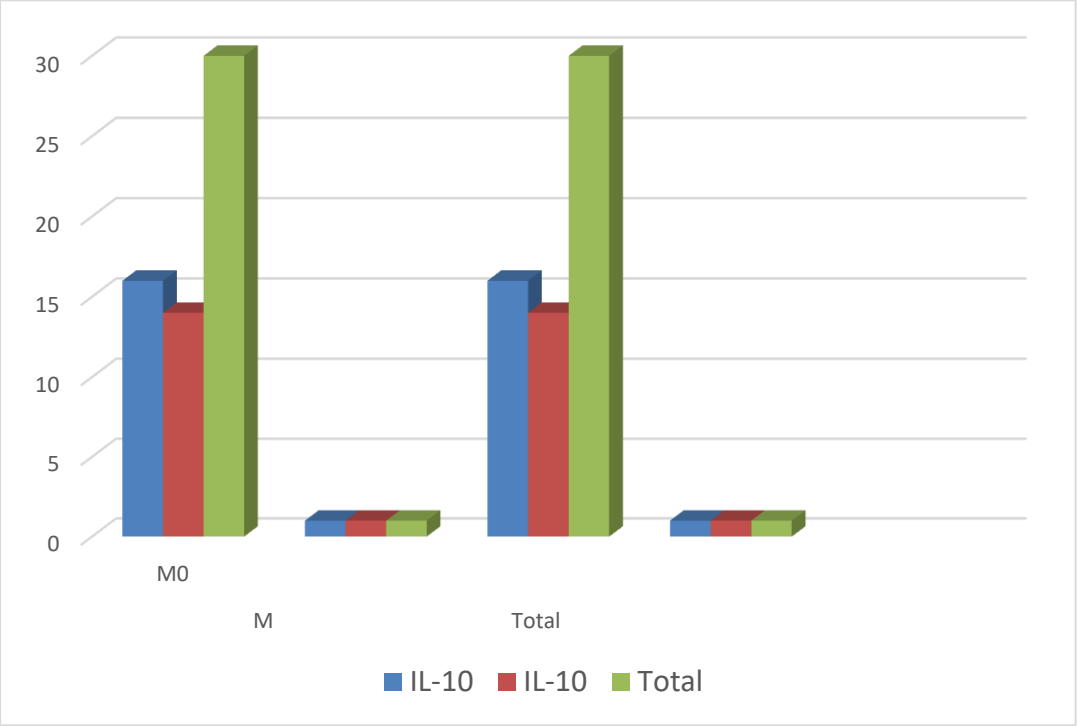


Graphical data showing Mean IL-10 concentration of Node involvement in oral squamous cell carcinoma cases.

Table-4. The Results showed that as Tumor Metastasis the value of mean Il-10 concentration in serum.

		IL-10		
		Positive	NEGATIVE	Total
M	M0	16	14	30
		100.0%	100.0%	100.0%
Total		16	14	30
		100.0%	100.0%	100.0%

Applied χ^2 test for significance. χ^2 value=6.654; p-value=0.084; consider not significant.

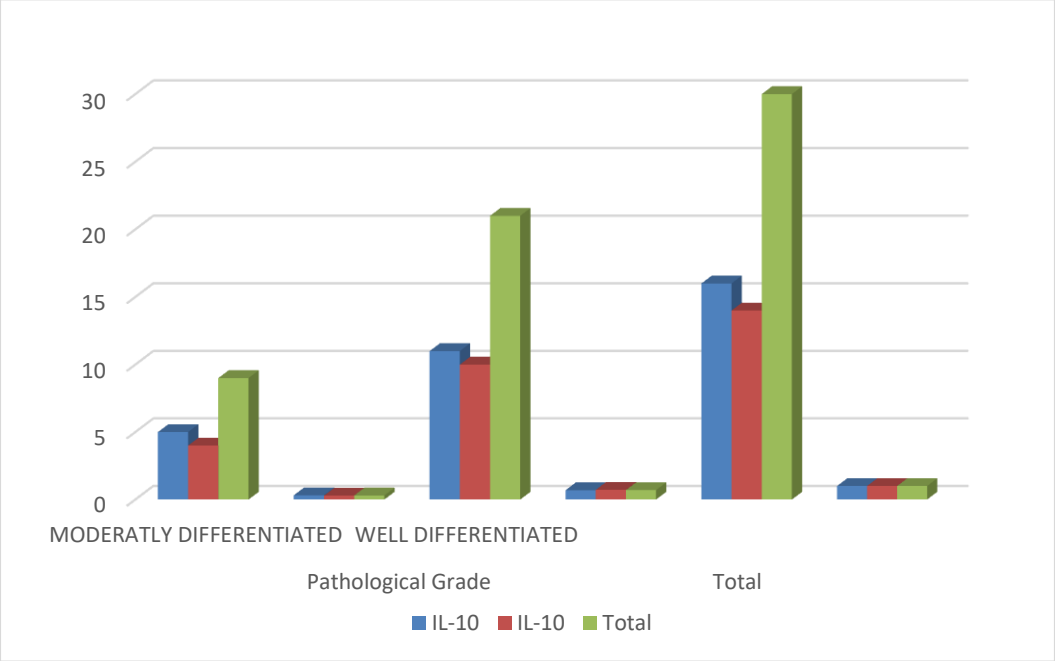


Graphical data showing Mean value of IL-10 concentration Tumor in Metastasis of oral squamous cell carcinoma cases.

Table-5. The Results showed that as Pathological Grade increases mean Il-10 concentration in serum increases. Mean IL-10 concentration of Moderately Differentiated (30.0%), well differentiated (70.0%), respectively. On comparing, fisher exact test significantly p value=1.000.

		IL-10		
		Positive	NEGATIVE	Total
Pathological Grade	MODERATLY DIFFERENTIATED	5 31.2%	4 28.6%	9 30.0%
	WELL DIFFERENTIATED	11 68.8%	10 71.4%	21 70.0%
Total		16 100.0%	14 100.0%	30 100.0%

Applied fisher exact test for significance. p-value=1.000; consider not significant.

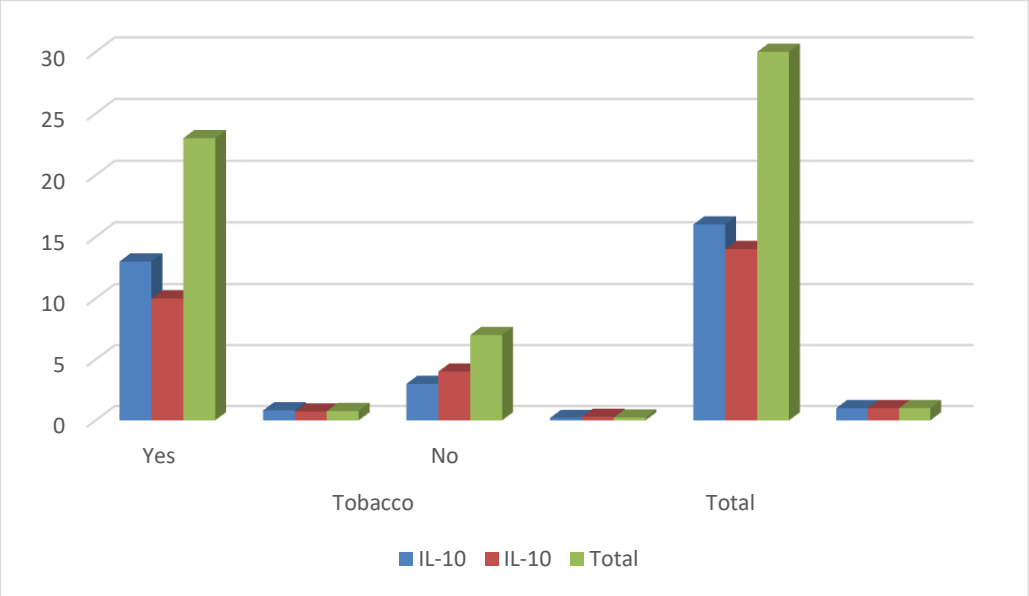


Graphical data showing Mean value of IL-10 concentration in Pathological Grades of oral squamous cell carcinoma cases.

Table-6. The Results showed that as Tobacco mean Il-10 concentration in serum increases. Mean IL-10 concentration), Applied fisher exact test for significance. p-value=0.675.

		IL-10		
		Positive	Negative	Total
Tobacco	Yes	13 81.2%	10 71.4%	23 76.7%
	No	3 18.8%	4 28.6%	7 23.3%
Total		16 100.0%	14 100.0%	30 100.0%

Applied fisher exact test for significance. p-value=0.675; consider not significant.

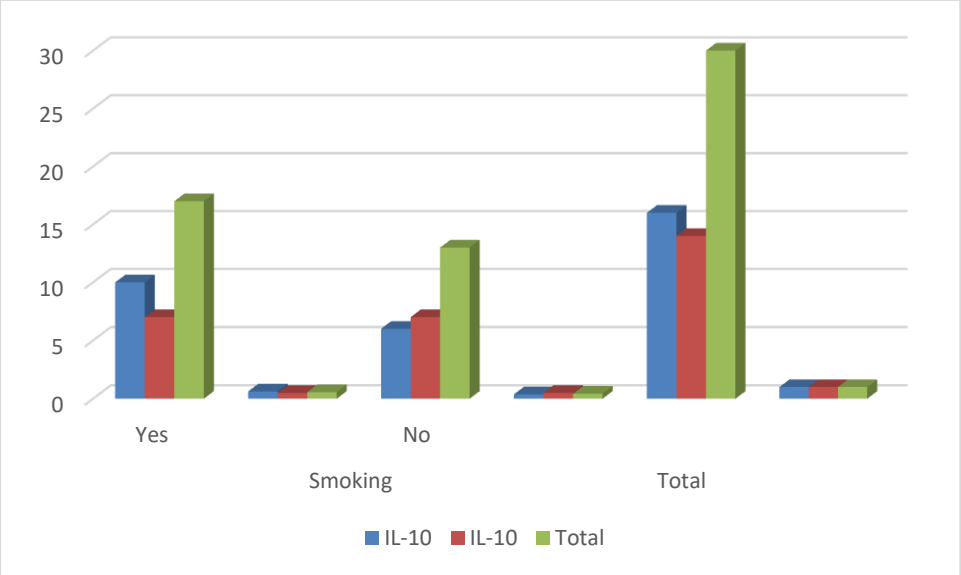


Graphical data showing Mean value of IL-10 concentration in tobacco of oral squamous cell carcinoma cases.

Table-7. The results showed that as concentration of smoking and mean value of Il-10 concentration in serum increases. Mean IL-10 concentration was (χ^2 value=0.475; p-value=0.491).

		IL-10		
		Positive	NEGATIVE	Total
Smoking	Yes	10 62.5%	7 50.0%	17 56.7%
	No	6 37.5%	7 50.0%	13 43.3%
Total		16 100.0%	14 100.0%	30 100.0%

Applied χ^2 test for significance. χ^2 value=0.475; p-value=0.491; consider not significant.

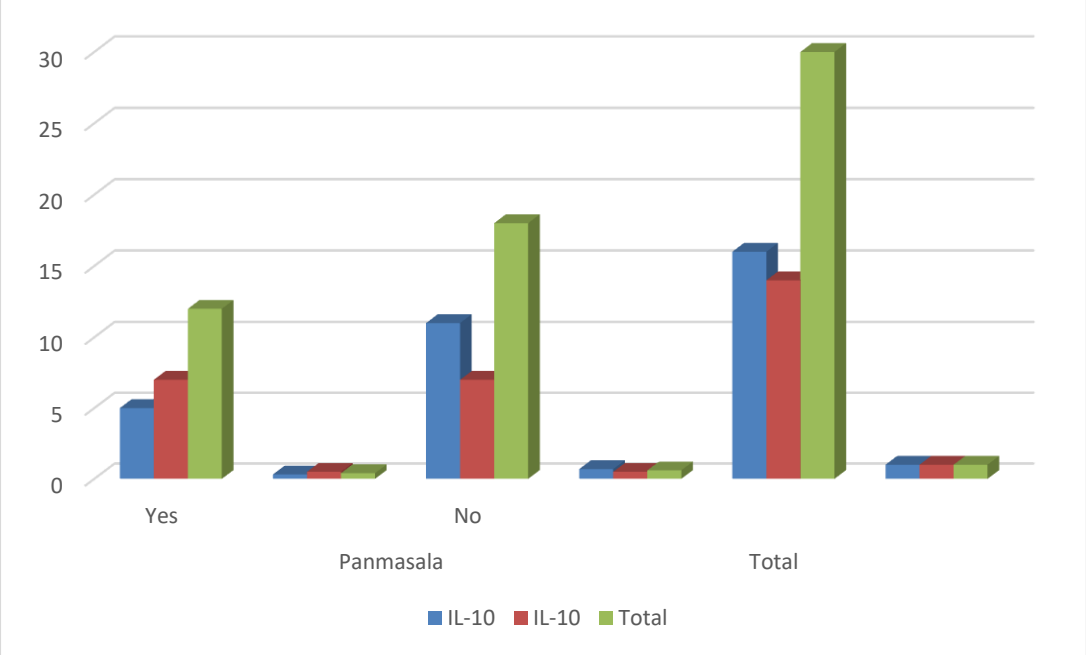


Graphical data showing Mean value of IL-10 concentration in smoking of oral squamous cell carcinoma cases.

Table-8. The results showed that as concentration of pan-masala and mean value of Il-10 concentration in serum. Mean IL-10 concentration was (χ^2 value=1.094; p-value=0.296).

		IL-10		
		Positive	NEGATIVE	Total
Panmasala	Yes	5	7	12
		31.2%	50.0%	40.0%
	No	11	7	18
		68.8%	50.0%	60.0%
Total		16	14	30
		100.0%	100.0%	100.0%

Applied χ^2 test for significance. χ^2 value=1.094; p-value=0.296; consider not significant.

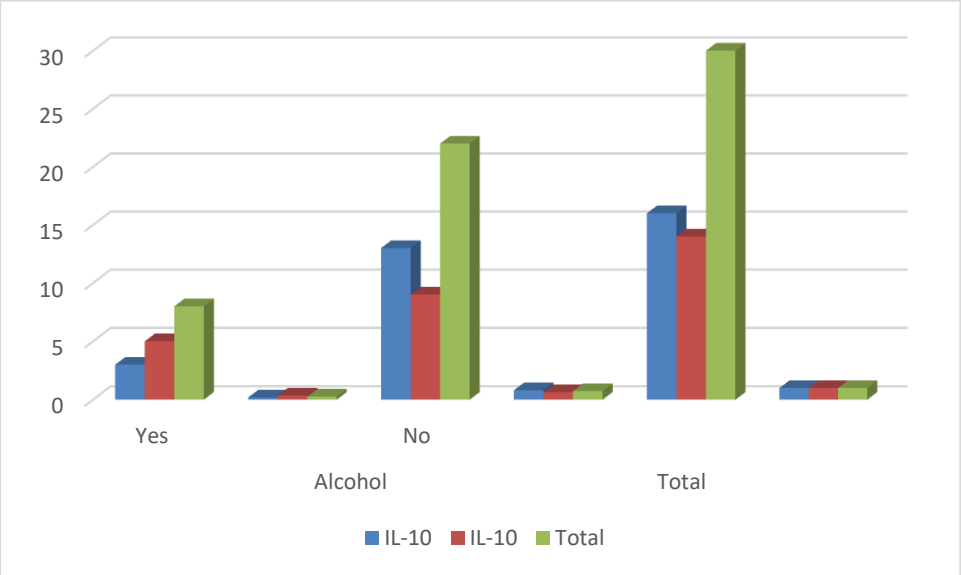


Graphical data showing Mean value of IL-10 concentration in pan-masala of oral squamous cell carcinoma cases.

Table-9, The results showed that as concentration of Alcohol and mean value of Il-10 concentration in serum increases. Mean IL-10 concentration was Applied fisher exact test for significance (p-value=0.417).

		IL-10		
		Positive	NEGATIVE	Total
Alcohol	Yes	3	5	8
		18.8%	35.7%	26.7%
	No	13	9	22
		81.2%	64.3%	73.3%
Total		16	14	30
		100.0%	100.0%	100.0%

Applied fisher exact test for significance. p-value=0.417; consider not significant.



Graphical data showing that Mean value of IL-10 concentration in alcohol of oral squamous cell carcinoma cases.

RESULTS

During the research period, were identified 30 patients aged under 25-70 years with oral SCC in the institutions surveyed. Patients of OSCC visit our hospital in advanced stage and many of them have also palpable regional lymph node. Histological grading quite often fails to indicate the actual types of lesion and its relationship with metastasis. of these 30 patients, with mean value (47.9000) met the inclusion criteria. Patients with different clinical grades were also included in this study with different mean value, 3 patients were in clinical grade 1 (10.0%), 8 were in Grade II mean was (26.7%), 14 were Grade III with mean (46.7%), and 5 were in Grade IV with their mean (16.7%). Regarding the social habits,17 (56.7%) reported smoking and 8 (26.7%) alcohol ,23 was tobacco consumption. The mean age of the control group patients was 63.14 years (± 8.62), ranging from 50 to 84 years. Most were male 11 (78.57%) and 3 (21.43%) were female. According to the habits, 8 (57.14%) reported tobacco and 6 (42.87%) alcohol consumption 23 were tobacco consumers (76.7%) and pan-masala were 12 and mean value was (40.0%). Mean Value of Pathological grades showed that as Pathological Grade increases mean Il-10 concentration in serum increases. Mean IL-10 concentration of Moderately Differentiated (30.0%), well differentiated (70.0%), respectively.

DISCUSSION

Interleukin-10 is an immunoregulatory cytokine with biological functions of anti-inflammation and immunosuppression. There is an indication that this cytokine may play a role in cancer pathogenesis **[11]**. A lot of studies observation that high levels of IL-10 produced by the tumor cells in different types of malignancy **[18]** reported IL-10 production by gastric cancer cells Ali et al. Tissue, Serum and Salivary Expressions of IL-10 in OSCC Alexandria Dental Journal. (2018)). The present study revealed that IL-10 was expressed in all OSCC samples, while it was not found in the normal mucosal tissues. In this study, a cytoplasmic expression of IL-10 was revealed in the malignant epithelial cells. Interestingly,. This was in accordance with the study conducted by Chandler et al **[19]**. They observed the localization of IL-10 in the cancer cells only. Similar results were found by Smith et al **[20]**.

Consequently, it is assumed that IL-10 could be primarily tumor-derived rather than being a product of the immune cells. On the other hand, many authors detected IL-10 expression in the inflammatory cells which are distributed over the tumor stroma **[21,22,23,24].** The present study revealed that IL-10 expression was significantly correlated with the tumor grade. The poorly differentiated grade is associated with the highest expression, followed by the moderately and well differentiated types respectively. Contradictory results were found by Chandler et al **[25].** They mentioned that IL-10 expression was inversely related to the grade. On the other hand, Chen et al **[26]** found that no significant association was found between IL-10 expression and the tumor grade. The discrepancy between the present data and the previous reports may be due to the limited sample size with few cases of the poorly differentiated grade. In the current work, the expression of IL-10 had no association with the clinical staging of OSCC. This is in agreement with Fujieda et al **[27]** and Wang et al **[28].** On the other hand, Hamzavi et al **[29]** stated that a significant correlation was found between IL-10 and the disease stage; such that the incidence of IL-10 decreases in higher stages. This discrepancy could be due to the mixed sample from different head and neck locations in the previously mentioned study. Conversely, high IL-10 expression was associated with advanced clinical stages in the study conducted by Arantes et al **[30].** This diversity of the results may be explained as, 68% of the patients studied by those authors had an advanced stage of OSCC, in comparison to only 25% of the cases encountered in the present work. According to the present research, IL-10 was detected in the serum of OSCC patients. However, the detectable IL10 concentrations showed no significant difference between patients and controls. This goes with the results of Hamzavi et al **[7],** Alhamarneh et al **[31]** and Czerninski et al **[32]** Moreover, in the study conducted by Linkov et al **[33]** there was no difference between IL-10 serum level of patients with HNSCC and that of the control group who were smokers. In contradiction to the current research results, Interleukin-10 was not detected in the serum of patients with SCC and adenoid cystic carcinoma of oral carcinoma patients Conversely, De Vita et al **[34]** detected elevated levels of serum IL-10 in gasterointestinal carcinoma patients compared with healthy controls.

that serum IL-10 overproduction may be a shared observation in certain types of human malignancies. It is observed that high IL-10 serum levels were detected in the advanced stages of malignancy **[35]**. Unfortunately, the number of patients who were in the advanced disease stages (stage III and IV) was low in the present study. This could be a possible explanation for the decreased IL-10 serum levels. Another reason for the contradictory results is related to the difference in the methods of measuring IL-10 in serum **[36]**. In addition, having radiotherapy or chemotherapy before giving blood samples could be also a cause for this discrepancy. In the current work, the serum levels of IL-10 in OSCC patients were slightly higher than in the healthy individuals. However, the difference between the two groups was not statistically significant. In a study conducted by Nelson et al **[37]** on cervical cancer, salivary IL-10 level was evaluated. It showed no significant difference between patients and controls. These results may assume that the presence of IL-10 in the serum is most likely to exert a physiological role and it might not constitute a significant serum biomarker for OSCC **[38]**.These studies concluded that IL-10 has a great potential for becoming a salivary biomarker for OSCC. Furthermore, Polz-Dacewicz et al **[39]** stated that the level of IL-10 in serum and saliva was higher in oropharyngeal SCC patients than in controls. The authors added that the concentration of IL-10 in the patients group was higher in saliva than in serum. The conflicting data regarding the salivary expression of IL10 may be due to the fundamental differences in the composition of saliva in cancer patients **[40]**. Furthermore, the different sites of the cancerous lesions in the mentioned studies could be a cause of contradiction. In oral cavity carcinomas, the neoplastic cells are closely associated with the salivary fluid. This is distinct from other locations such as the larynx and pharynx **[41]**. Moreover, oral environmental factors, including periodontal disease and oral microbial flora, can lead to detection of protein artifacts, making a difficult comparison between individuals on basis of salivary composition. Additionally, serum cytokines may be degraded by enzymes or mucin-like proteins which are found in the serum. Finally, a significant correlation was found among IL-10 tissue expression and its serum levels in OSCC patients.

This could reflect the same way of regulation of this cytokine in different parts of the body.

CONCLUSIONS

Interleukin-10 can be detected by Elisa in OSCC. The intensity of its expression is directly proportional to the histopathological grading of the disease. Moreover, the levels of IL-10 in, serum are correlated with various parameters. This could reflect the same way of regulation of this cytokine in different parts of the body.

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