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BIOCHEMICAL ASSESSMENT OF SERUM LEVELS OF INTERLEUKIN 1β AND INTERLEUKIN 6 IN ORAL LEUKOPLAKIA – A CROSS-SECTIONAL STUDY

Dentistry

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ABSTRACT

Objectives: The study intended to assess serum levels of IL 1β & IL-6 in patients with Leukoplakia and to identify whether there is any correlation between the degree of dysplasia and serum levels of IL 1β & IL-6 in Leukoplakia patients. **Materials And Methods:** The study group comprised 75 individuals divided into 3 groups GROUP A with 25 Homogenous Leukoplakia patients, GROUP B with 25 Non- Homogenous leukoplakia, GROUP C with 25 healthy individuals as controls. Serum levels of IL-1β and IL-6 were measured using enzyme-linked immunosorbent assay kits in all subjects. **Results:** There were significant differences in the serum IL-1β and IL-6 levels among the three groups and there was correlation between the dysplastic features and the levels of serum IL-1β in leukoplakia patients. **Conclusion:** Proinflammatory cytokines IL-1β and IL-6 levels were elevated in leukoplakia, and correlation was present between dysplastic features and IL-1β levels, which can be used as prognostic tool for screening of malignant transformation in leukoplakia.

KEYWORDS

Homogenous Leukoplakia, Non-homogenous Leukoplakia, Interleukin 1β, Interleukin 6, ELISA.

INTRODUCTION

A potentially malignant disorder is the risk of malignancy being present in a lesion or condition either at the time of initial diagnosis or at a future date. The most common potentially malignant disorder of the oral cavity is 'LEUKOPLAKIA'. In 2012, Vanderwaal (Brouns E, Baart JA et al.) proposed a new definition which seems more appropriate as it includes the histological exclusion "A predominantly white lesion or plaque of questionable behaviour having excluded, clinically and histopathologically, any other definable white disease or disorder[1]". The most recent definition, formulated at a World Health Organization is "A predominantly white, non-wipable lesion of the oral mucosa having excluded clinically, histopathologically or by the use of other diagnostic aids other, well-defined predominantly white lesions".[2]

Cytokines have also been linked with increased tumor growth and metastasis and could contribute to the pathogenesis of the disease. According to Virchow's hypothesis, "inflammatory infiltrate might be the origin of dysplasia in sites of chronic inflammation, which includes lymphocytes, macrophages and dendritic cells. The emergence of cytokines as a marker for detecting malignancies shows the importance of inflammation-mediated carcinogenesis in oral premalignant disorders. Of the above cytokines, IL -1βand IL-6 levels are predominantly increased in patients with Leukoplakia[2].

The aim of the study was to measure the molecular markers in the serum which could potentially aid as a screening tool for the early risk assessment or potential of the leukoplakia to get transformed into oral cancer. The study intended to determine whether there was an increase in levels of IL-1β, IL-6 in patients with Leukoplakia and if there was any correlation exists between Interleukin levels and degree of dysplasia in the patients with Oral Leukoplakia

MATERIALS AND METHODS

Study Design:

For this study, the ethical clearance was obtained from the institutional ethical committee [IECC/OMR/DISS/12-18/03] and written informed consent was obtained from the patients. The procedures followed were following the ethical standards of the Helsinki Declaration of 1975, as revised in 2000.

The patients willing to participate in the study were selected along with healthy controls willing to participate in the study. Patients having other lesions in the mouth, suffering from chronic diseases&/or immune mediated diseases were excluded from the study. The patients were divided into two groups and group of controls. All the patients for the study were recruited from the Department of Oral Medicine & Radiology from JANUARY 2019 to JANUARY 2020. Patients were divided into three groups (each group had 25 patients). Detailed case history recordings & thorough clinical examination was done for each patient**.** An anamnestic questionnaire was administered to the patient with information about age, gender, drug history, medical history, allergic history followed by evaluation of status of the oral cavity.

The present study included 50 leukoplakia patients [with an age range of 28 - 60 years with 37 males and 13 females divided into two groups. GROUP A included Homogenous leukoplakia patients and GROUP B Non-homogenous leukoplakia patients and 25 healthy controls as GROUP C [with an age range of 25 – 56 years with 13 male and 12 females]

Blood Sample Collection

5ml of the venous blood sample was collected from each subject (study group and control group) with disposable syringe under aseptic conditions through the median cubital vein's venipuncture. The serum samples were subjected to centrifugation at 3000 rpm for 15 minutes. The collected samples were stored at -20^0 c degrees until analysed for INTERLEUKIN levels.

Serum Interleukin Levels Estimation

According to the manufacturer's instructions for human ELISA kit (Krishngen biosystems- IL-1β and IL-6 ELISA KIT) for IL-1β and IL-6, standard, sample, Human IL Biotin Conjugated antibody was added to the wells followed by addition of HRP conjugate to form an immune complex. Unbound HRP conjugate was removed by washing with buffer sample after incubation.

TMB substrate was added resulting in development of blue colour during the incubation period. The reaction was stopped after addition of the stop solution with the development of yellow colour. Absorbance was read at 450 nm within 30 minutes of stopping reaction by using ELISA reader [Rayto RT-2100C]. The concentration of the interleukins of sample is directly proportional to the yellow colour developed in well and positively correlated.

Fig 1 Homogenous Leukoplakia **Fig 2** Non- Homogenous Leukoplakia

Statistical Analysis:
In this study, the data was entered into MS-Excel and statistical analysis was done by using IBM SPSS Version 22.0. For categorical variables, the data values were represented as number and percentages. To test the association between the groups, ANOVA one way test was used. For continuous variables, the data values were shown as mean and standard deviation. To test the mean difference between two groups, Student´s t-test [Paired sample t-test & Independent sample t-test] was used. All the p values less than 0.05 were considered as statistically significant. Serum IL-1β and IL-6 levels were estimated using the ELISA kit as per the manufacturer's instructions.

RESULTS

The study population in GROUP A consisted of 19 males and 6 females with mean age of 51.37±5.23 and GROUP B consisted of 18 males and 7 females with a mean age of 53.39±4.91, in GROUP C there were 17 males and 8 females with a mean age of 48.82±4.90. Levels of serum IL-1β, IL-6 were statistically significantly higher in GROUP A and GROUP B than in GROUP C.

Table 1 shows The mean difference of IL-1β , IL-6 levels between GROUP A and GROUP B ; GROUP C and GROUP B; GROUP C and GROUP A and the p-value is 0.000, which is statistically significant.

Table 1: Mean Comparison Of IL 1 β Levels Among GROUP A, Group B, Group C.

		Interleukin 1β levels		Interleukin 6 levels	
Group	**N**	**Mean ± Std. Deviation**	**P-value**	**Mean ± Std. Deviation**	**P-value**
Group A	25	20.79±2.58	0.000	15.24 ± 2.14	0.000
Group B	25	24.76 ± 2.70		16.35 ± 2.98	
Group C	25	16.75 ± 2.53		5.79 ± 1.32	

Table 2 shows Mean comparison of Interleukin 1 βlevels of Histopathological features in GROUP A and GROUP B which is statistically significant

Table 2: Mean Comparison Of IL 1 β Levels Of Histopathological Features In Group A And Group B

Histopathological features	Group A		Group B		P value
	Mean	Std. Deviation	Mean	Std. Deviation	
No	18.80	0.68	21.20	0.00	--
Mild	19.96	1.84	23.01	1.33	0.000 S
Moderate	24.38	1.21	27.56	1.42	0.000 S

Table 3 shows Mean comparison of Interleukin 6 levels of Histopathological features in GROUP A and GROUP B which is statistically not significant

Table 3: Mean Comparison Of IL 6 Levels Of Histopathological Features In Group A And Group B

Histopathological features	Group A		Group B		P value
	Mean	Std. Deviation	Mean	Std. Deviation	
No	14.32	2.77	17.80	0.00	--
Mild	15.26	2.19	15.99	2.73	0.448 NS
Moderate	15.93	1.41	16.72	3.50	0.612 NS

DISCUSSION

Oral Leukoplakia is the most common of all the OPMDs, with a global prevalence range of 0.5% to 3.46%[13]. Aetiology is multifactorial, but the tobacco in both forms, i.e., smoking, and smokeless seems to be associated with most of the lesions. In India, the prevalence of Leukoplakia among betel quid chewers with tobacco ranged from 0.4 to 1.8% and among betel quid chewers without tobacco ranged from 0.3 to 0.7%. Oral Leukoplakia is usually seen in middle-aged people, and its prevalence is higher with age. Males are more commonly affected than females, probably due to the greater prevalence of males' tobacco use[14]. The studies done by Pindborg et al[7]. and Christian et al. showed that tobacco in any form could predispose to OL. The following frequently linked risk factor is alcohol, but alcohol as an independent etiological factor in oral Leukoplakia development is questionable. Although alcohol by itself not a significant risk factor for oral Leukoplakia, it produces synergistic effects when combined with the habit of chewing tobacco or smoking.

Leukoplakia clinically presents as homogeneous and nonhomogeneous forms. **Homogeneous Leukoplakia** is defined as a predominantly white lesion of uniform flat and thin appearance that may exhibit shallow cracked mud appearance or a smooth, wrinkled, or corrugated surface with a consistent texture throughout. This type is usually asymptomatic. **Non-homogeneous Leukoplakia** has been defined as a predominantly white or white-and-red lesion (erythroleukoplakia) that may be either irregularly flat, nodular (speckled Leukoplakia) or exophytic (exophytic or verrucous Leukoplakia). These types of Leukoplakia are often associated with mild complaints of localized discomfort[16]. Most lesions occur on the buccal mucosa and commissures (25.3% and 37.5%, respectively), with approximately 8% each on tongue, hard palate, and alveolar ridge and 1.3% on the soft palate[17].

The prevalence of epithelial dysplasia in oral Leukoplakia ranges from 5% to 25%.[18] Dysplastic features are more frequent in non-homogeneous than in homogeneous Leukoplakia, and dysplasia is probably the histopathological expression of molecular and genomic alterations in a field of keratinocytes[19,20]. The histopathological pattern of oral Leukoplakia is variable, ranging from atrophy of the epithelium to hyperplasia, either with or without dysplasia. The epithelial dysplasia exhibits various degrees of severity like mild, moderate, and severe. The presence of dysplasia is a marker of the malignant potential of Leukoplakia, and the risk of an individual leukoplakic lesion to progress to carcinoma increases with the increase of the grade of the epithelial dysplasia[21]. If the Leukoplakia exhibits dysplastic features, there is a increased chance of malignant transformation. If the lesions with dysplastic and non-dysplastic features are considered, the malignant transformation percentage ranges between 0.13% to 17.5%[22].

Cytokines are intercellular signalling proteins that regulate growth, angiogenesis cellular proliferation, and tissue repair[24]. They show a significant role in immune responses to infection, injury, and inflammation. The identification of suitable biomarkers has given newer options for early detection and risk assessment of oral cancer. However, most of these are identified either in, cell lines, or biopsy specimens, making it challenging for largescale screening. Measurement of molecular markers in serum could potentially aid in the development of a practical screening tool[25]. The levels of specific proangiogenic, inflammatory cytokines in serum, and tissue specimens of patients with oral cancer are elevated. IL 1β and IL-6 are essential serum biomarkers for predicting therapeutic responses and survival in advanced-stage, head and neck cancer. The important inflammatory cytokines such as IL-1β, IL-6, and IL-8 are upregulated in melanomas[23].

IL-1β is a proinflammatory cytokine released upon infection, cellular injury, or antigenic challenge[5]. It stimulates the induction of adhesion molecules and other mediators that facilitate and amplify the inflammatory response. It is released upon activation of a multiprotein innate immune pathogen-sensing complex called the inflammasome. Inflammasomes can be induced to various stimuli, including microbial components (e.g., bacterial toxins) and environmental particulates (e.g., silica and asbestos).

IL-6 is an NF-κB dependent cytokine produced by inflammatory cells as well as tumour cells. It binds to a heterodimeric receptor, the common cytokine receptor signal-transducing subunit gp130. IL-6 receptor engagement leads to activation of the JAK family of tyrosine kinases, stimulating multiple pathways involving MAPKs, PI3Ks, STATs, and other signalling proteins[25].

Various subcomponents of these pathways have been implicated in influencing the carcinogenic cascade in various cancers including OSCC and premalignant oral lesions[6].The increase in these cytokines in serum might indicate the potential precancerous conditions into oral cancer. These cytokines may be improperly produced in the precancerous lesion, which leads to induced growth, invasion, disruption of tumour suppress ion and immune status[11].

This study evaluated the levels of IL-1β, IL-6 in patients with Leukoplakia and found that correlation exists between Interleukin levels and degree of dysplasia in the patients with Oral Leukoplakia.

Interleukin 1β and 6 levels

There is a significant correlation between IL-1β and IL-6 levels in homogenous and non-homogenous groups than controls. The interleukin levels were higher in a homogenous group and non-homogenous group when compared with controls. These findings were close to the study done by Jasdeep et al[11]. who demonstrated that serum and salivary IL-6, TNF-α, and IL-8 were significantly increased in oral Leukoplakia, submucous fibrosis and lichen planus in contrast to normal healthy subjects. Ron N. Apte et al[8]. determined that IL-1β is essential for already existing malignant cells' invasiveness by promoting angiogenesis and other aspects of malignancy. His findings were in concordance with our results. Arellano-Garcia et al[27]. reported significantly higher salivary IL-1β concentrations in patients with oral cancer compared to healthy controls. Katakura et al[28]. reported higher salivary IL-1β and IL-6 concentrations in oral cancer patients compared to healthy controls. The higher concentrations of salivary cytokines might result from a lesion with epithelial discontinuity and surrounding inflammation, not more related to cancer.The study done by Bagan et al[10]. showed that Salivary and serum IL-6 levels are altered in patients with PVL, with more extensive verrucous areas being associated with the highest IL-6 levels. This could be a significant tool for monitoring patients with PVL, their progression to more advanced stages and their recurrences. Boras et al[29]. reported significantly higher salivary IL-6 concentrations in oral cancer patients while St. John et al[26]. found no significant differences in salivary IL-6 in oral cancer patients compared to healthy controls. Despite the heterogeneity of the results, one can conclude that patients with oral cancer have altered cytokine production, reflecting the concentrations of at least one proinflammatory cytokine in saliva. In our study, we considered the serum levels than the salivary concentration.

Histopathological Features:

There was a significant correlation between IL-1β levels and histopathological features in both homogenous and non-homogenous Leukoplakia. The IL-6 levels and histopathological features were not significant between the homogenous and non-homogenous group. These results are in contrary to the study done by Rhodus et al[30]., who demonstrated that IL-6 was increased in mild, moderate, and severe dysplasia compared to controls. The other study was done by Jasdeep et al[11]., who demonstrated that serum and salivary IL-6, TNF-α, and IL-8 were statistically significantly higher in severe dysplastic features in precancerous lesions than mild dysplasia.

CONCLUSION

Our results indicate that Leukoplakia patients had increased levels of a serum IL-1β and IL-6 compared to the control group. The correlation between the histopathological grading and interleukin levels showed a positive association. The IL-1β level was found to be increased from mild to moderate level of dysplasia. But this association was not found with the IL-6 levels. Our study showed a strong association with IL-1β levels when compared to that of IL-6 levels.These findings suggest that pro-inflammatory cytokines have an essential role in the prognosis of oral Leukoplakia, and serum levels of interleukins could be a possible indicator of the lesion's malignant potential. As our study's sample size is small, future studies should consider investigating the association of IL1β and IL-6 with the degree of dysplasia in Leukoplakia and other cytokines in multivariate models with sample size relatively large, taking into consideration the influence of demographic variables.

Conflicts Of Interest: There are no conflicts of interest.

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