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A PROFILE OF SERUM-BASED BIOMARKERS IN PATIENTS WITH ORAL LICHEN PLANUS: A CROSS-SECTIONAL ANALYTICAL STUDY FROM A TERTIARY CARE HOSPITAL IN EASTERN INDIA

Biochemistry

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ABSTRACT

Introduction: Oral lichen planus (OLP) is a chronic disorder affecting the immune system and in severe cases likely to progress towards malignant outcome (oral squamous cell carcinoma). Effective diagnosis and adequate therapeutic management largely dependent on diagnostic techniques which involves several biomarkers which are sensitive as well specific to the condition. This present study is aimed in identify such potential biomarkers. **Methodology:** We conducted a cross-sectional and analytical study with 48 cases of Oral lichen planus who were recruited following the set inclusion & exclusion criteria from Department of Dermatology, R.G. Kar Medical College & Hospital and in parallel 48 apparently healthy age & sex matched normal individuals were selected as controls. Serum extracted from both the population was subjected to analysis of the selected biomarkers, namely, iron, calcium, magnesium, phosphate, folic acid, vitamin B12, homocysteine and vitamin D3, estimated by manufacturer recommended methods. The IFN-γ +874 T/A polymorphism was analysed by polymerase chain reaction (PCR) with sequence-specific primers (SSPs) according to the amplification refractory mutation system–polymerase chain reaction (ARMS-PCR). The data obtained was subjected to statistical analyses by using Graph Pad Prism, (v 8.4.3). In all statistical analysis p value of <0.05 was considered to be statistically significant. **Results:** It was found that levels of serum magnesium (p = 0.0192), homocysteine (p<0.0001) were significantly lower in cases of OLP as compared to controls. IFN-gamma polymorphism in Oral Lichen Planus cases showed significant negative correlation with serum calcium levels (p value 0.0173, r =-0.3791). **Conclusion:** Markers reflective of nutritional status, mainly serum calcium, magnesium and homocysteine exhibiting significantly lower levels in patients of OLP as compared to their heathy counterparts, may require dietary supplementation in such patients.

KEYWORDS

INTRODUCTION

Oral potentially malignant disorders (OPMD)s is a group of mucosal disorders which may precede the diagnosis of oral squamous cell carcinoma (OSCC). According to the World Health Organization (WHO), Oral Lichen Planus (OLP) is included in these potentially malignant disorders or (OMPDs). Recently Cancer development in oral lichen planus patients is reviewed by Gonzalez-Moles et al., (2019) [1]

Any malignancy can be prevented by early diagnosis and it is always preferable to be done by some easy screening tools. Our goal is screening of OLP, a premalignant or OPMD, by measuring some serum biomarkers. Some studies, mostly in the eastern and western Asia, show correlation between serum biomarkers and oral lichen planus but till date no such studies are found in India.

Oral lichen planus (OLP) is a relatively common inflammatory mucocutaneous T cell mediated chronic inflammatory oral disease. This chronic inflammatory disorder mainly affects stratified squamous epithelia. Oral lesions in OLP being chronic in nature, they rarely undergo spontaneous remission. So, the lesions are potentially premalignant and are often a source of morbidity.

OLP develops most commonly in the fifth or sixth decade of life, and it occurs in women more than twice as often as in men, but patients of all ages may develop this disorder. Oral lichen planus lesions are usually bilateral and symmetrical. As well as lesions are well recognized and have distinct clinical features and characteristic distribution.

Although Oral lichen planus (OLP) is a chronic disorder with the primary role of immune system (Eisen et al., 2005), stress, anxiety and other factors in relation with immune system may also be triggering factors of this disease.[2] For proper functioning of immune response, adequate intake of micronutrient is mandatory prerequisite. Micronutrient deficiencies suppress immune functions by affecting the innate, cellular, and humoral immunity systems (affecting the physical barriers in skin/mucosa), and they also dysregulate the balanced host natural defence response.

It has been observed that deficiency of some micronutrients is closely associated with some common mucosal diseases and oral lichen planus is one of them.[3] Many studies of OLP cases show significantly lower serum levels of Hb, iron, folate and vitamin B12 and of high serum level of 1st October 2022 and continued till 31st March 2024 homocysteine. [4,5]

Considering the role of immune system in the aetiology of OLP and the effect of vitamin D on immune system, the present study was conducted to compare the levels of vitamin D in OLP patients and healthy controls.[6]

Vitamin D, calcium and phosphate are bone biomarkers. Calcium and phosphate metabolism are related to each other. Till date no such studies had been done which measured phosphate level in OLP patients. But limited studies have been conducted on the role of vitamin D as an associated factor of OLP. There is an important role of Magnesium (Mg) in cellular membrane stabilization and neural conduction and enzyme's function. Various studies showed controversies regarding the Magnesium level in OLP patients.

OLP occurs through both antigen-specific and non-specific mechanisms. inflammatory response in OLP is mainly characterized by the accumulation and expansion of T helper 1 (Th1) lymphocytes. IFN-γ is considered to be the principal cytokines produced by Th1 cells. [7,8] Till date, no studies have been performed in Indian population to evaluate whether the IFN-γ gene polymorphisms are associated with OLP susceptibility.

Objectives

To assess the serum levels of Iron, Folic acid, Vitamin B 12, Homocysteine, Calcium, Magnesium, Phosphate and Vitamin D in untreated cases of Oral lichen planus in comparison to the levels of the biomarkers in age and sex matched healthy individuals as control.

To detect presence of gene polymorphism of Interferon -γ (874A/T) in the study population and to see how the serum levels of above said biomarkers correlate with the above polymorphism.

MATERIALS & METHODS

Study Design & Setting

It was a cross-sectional analytical study. The study was conducted in the Department of Biochemistry after getting approval from the West Bengal University of Health Sciences and the Institutional Ethics Committee, in collaboration with the Department of Dermatology of R.G.KAR Medical College and Hospital, Kolkata. The study was conducted for a period of 18 months, from 1st October 2022 upto 31st March 2024.

Study Population

39 Diagnosed cases of oral lichen planus from outpatient Department of Dermatology of R G Kar Medical College and Hospital were recruited after obtaining informed consent. Age and sex matched apparently healthy adults were considered as controls.

Exclusion Criteria

- Lichen planus involving other muco-cutaneous sites.
- Diagnosed case of Anaemia
- History of any oral addiction
- Chronic debilitating diseases like chronic kidney disease, degenerative bone disorder
- Pregnant and lactating mother
- <18 and >65 years age group

Sample Size

39 cases of Oral lichen planus were selected depending on total patients attended in the desired period and estimated number of samples was calculated by the below mentioned formula

$$N=\frac{Z^2 \times p \times (1-p)}{l^2}$$

Z =standard deviate from normal tables (1.96)
p = prevalence (2.6%) [9]
l = absolute precision (5%)
N = 38.89 ≈ 39

Methodology

i. Estimation of biochemical parameters: 10 ml whole blood collected from cases and controls under fully aseptic precautions. Serum was separated and 25 hydroxy Vit D, folate, vitamin B12 and homocysteine levels were assayed by chemiluminescence technique, in ADIVA Centaur CP & ADIVA Centaur XP. Calcium, magnesium, phosphate and iron levels were assayed in Konelab 60i, by manufactured assigned methods.

ii. Detection of genetic polymorphism: DNA was isolated by Genomic DNA isolation kit QIAmp DNA Mini and Blood Mini Kit (QIAGEN) and primers used were primers designed for detection of polymorphism (Integrated DNA technologies, United States. The IFN-γ +874 T/A polymorphism was analysed by polymerase chain reaction (PCR) with sequence-specific primers (SSPs) according to the amplification refractory mutation system–polymerase chain reaction (ARMS-PCR).

Briefly, the ARMS-PCR genotyping of the +874 T/A polymorphism was performed as follows: the 20 μL PCR reaction mixture contained 0.2 μM of the five primers. 25 mM KCl, 1.2 μL of MgCl2, 200 μM dNTPs, 0.5 U of Taq polymerase, and 200 ng of genomic DNA. Thermal condition was as follows: 3 min at 94 °C followed by 10 cycles of 60 sec at 94 °C, 60 sec at 62 °C, 60 sec at 72 °C, and 25 cycles of 60 sec at 94 °C, 60 sec at 58 °C, and 60 sec at 72 °C, with a final extension for 5 min at 72 °C. **(Fig 1)**

The amplified products were electrophoresed on a 2% agarose gel containing ethidium bromide. The presence of a control band with an allele-specific band of the expected size in a lane was considered as evidence for the allele, whereas the presence of a control band without an allele-specific band indicated the absence of that allele.

iii. Statistical Analysis: Data were analysed by Graph Pad Prism version 8.4.3. D'Agostino & Pearson omnibus normality test was done to assess if the data was following Gaussian distribution or not. Statistical significance was evaluated by 'p' value ≤0.05. Comparison between serum levels of parameters in cases of Oral lichen planus and controls were analysed by paired t test or a Mann-Whitney test as applicable. Spearman rank order correlation coefficients and Pearson Rank Order Correlation coefficient were used to test correlations between IFN- gamma polymorphism in OLP patients and the biochemical markers, under study.

RESULTS

I. Demographic particulars of OLP cases: 48 patient of oral lichen planus with age and sex matched healthy controls was recruited There was higher female preponderance in patient population with 62.5 % being females. It was also seen that majority of recruited patients, about 50% were 30-50 years age group. Females in the 4th-6th decade of life were commonly affected (F: M 1.5: 1) mainly in peri-menopausal females (10.91%) compared to premenopausal females (0.5–2%). **(Table 1)**

With respect to the IFN gamma polymorphism, the frequencies of genotypes and alleles of IFN-γ (874A/T) polymorphism differed in 39 OLP patients. **(Table 2).** TT and AT polymorphism were mainly predominant in OLP patients.

II. Comparison of serum parameters between cases and their age and sex matched controls: The mean blood concentrations of all the different biochemical parameters, under study in the 48 cases of OLP and in 48 age- and sex-matched healthy control participants, are presented in **Table 3**. Our study revealed serum magnesium levels in cases (mean ± SD; 1.87 ± 0.6755 mg/dl) to be significantly lower **(Fig 2A)** than that of controls (mean ± SD; 2.18 ± 0.6327 mg/dl). Serum Homocysteine levels was also significantly lower **(Fig 2B)** in patients of OLP (Mean± SD; 8.997 ±13.55 μmol/L) than in controls (Mean± SD; 13.65±7.107 μmol/L)

III. Correlation of each serum parameters with IFN-gamma polymorphism in Oral Lichen Planus cases: Correlation of each serum parameters with IFN-gamma polymorphism in Oral Lichen Planus cases were performed. Serum calcium exhibited significant negative correlation with IFN-gamma polymorphism (p value 0.0173 *, r = -0.3791) **Fig 3A.**

DISCUSSSION

Oral lichen planus is a relatively common chronic inflammatory non communicable skin disorder which predominantly affects the stratified squamous epithelium. It affects about 2.6% of the population worldwide.

Our study was designed and aimed at finding a new set of biological markers which will help in early diagnosis and treatment in clinically diagnosed cases of Oral lichen planus.

In our study we had recruited 48 patient of oral lichen planus with age and sex matched healthy controls. Study shows OLP has an age and gender predilection and females in the 4th-6th decade of life are commonly affected. For detection of interferon-gamma polymorphism 39 patients of OLP was recruited and among them correlation was assessed between different parameters and IFN-gamma polymorphism. Due to inadequate sample and refusal for drawing ~10ml of blood, only 39 cases were recruited for study gene polymorphism.

Jolly and Nobile studied haematological abnormality and found folic acid deficiency (< 3 ng/mL) in five of 34 OLP patients.12 Challacombe studied haematological abnormalities in 103 OLP patients and his study showed anaemia, low iron, low folate, and low vitamin B12 levels in OLP patients. [10] According to J.Y.-F. Chang et al. [11] GPCA+/EOLP patients had significantly lower mean Hb, serum Iron, decreased vitamin B12 and folic acid levels which resulted in high serum homocysteine level in oral lichen planus. But in our study, we found that there was no significant change in serum levels of Iron, folate, Vit B12 in patients of OLP and the controls. Correlation of serum levels of above said parameters with IFN-gamma polymorphism in Oral Lichen Planus cases were done. No significant

correlation was obtained between the IFN-γ and these parameters. In our present study serum levels of Homocysteine in patients of OLP was significantly low than the controls (p value= <0.0001****). In the present study as there was no change in the levels of serum vitamin B12 and folate so the level of homocysteine did not increase rather significantly lower in the cases than the healthy controls.

In the present study there was no change in the serum levels of Calcium in patient of OLP and controls. (p value=0.9799) Correlation of serum calcium with IFN-gamma polymorphism in Oral Lichen Planus cases shows significant correlation between the two parameters (p value 0.0173 *, r =-0.3791)

No study was found regarding serum phosphate level changes in OLP patients and in present study there was no change in the serum levels of Phosphate in patient of OLP and controls. (p value=0.6720) as well as the serum phosphate levels showed no significant correlation with IFN-gamma polymorphism Sadeghi et al. found a statistically significant lower serum vitamin D concentration in OLP patients. [12] Družijanić et al. also found a statistically significant ($p < 0.05$) lower serum vitamin D concentration in the erosive OLP compared to the non-erosive OLP. in OLP case (p value 0.9854, r =- 0.003031). In our present study there was no change in the serum levels of Vitamin D in patient of OLP and controls. (p value=0.5880) as well as the serum vitamin D levels showed no significant correlation with IFN-gamma polymorphism in Oral Lichen Planus case ($p = 0.9808$, $r = 0.003981$).

Hosthor et al. assessed serum levels of Mg in oral submucous fibrosis (OSMF) and squamous cell carcinoma (SCC) patients and level of Mg found decreased significantly in both groups of patients. [13] Our study assessed serum magnesium (Mg) content in OLP patients. The results showed a significant decrease in serum Mg level in patients compared to healthy controls (p value= 0.0192 *). However, correlation of Serum Magnesium with IFN-gamma polymorphism in Oral Lichen Planus cases was done. Spearman Rank Order Correlation test was applied. No significant correlation was obtained between the two parameters (p value 0.3963, r =-0.1416). Magnesium plays an important role in immune response as well as inflammation. Reduction in Mg level may be an etiologic factor in development of OLP.

CONCLUSION

The serum levels of magnesium were found to be significantly lowered in OLP cases than age and sex matched healthy controls and serum homocysteine levels were also found to be significantly lowered in cases than in the controls. Regarding correlation between IFN-γ polymorphism and the serum biomarkers significant correlation was observed with the IFN-γ polymorphism and serum calcium levels. Biomarkers like magnesium which were found to be significantly lower in cases of OLP, can be of benefit to such patients if given as dietary supplements. Low Homocysteine level in OLP may be protective regarding cardiovascular disease.

Tables:

Table1: Distribution of Age among patients of OLP and their sex matched controls

Age in years	Cases(N=48)	Controls(N=48)
18-30	10(21%)	26(54.2%)
31-50	24(50%)	17(35.4%)
>51	14(29%)	5(10.4%)

Table 2: Genotype frequencies of IFN-γ (874A/T) polymorphism in OLP patients

Genotype	OLP(N=39)	%
AA	5	12.8%
AT	17	43.6%
TT	17	43.6%
AT+TT	34	87.2%

Table 3: Comparison of serum biomarker levels between cases of Oral lichen planus and age & sex matched healthy adults

Patient with OLP (N = 48)			Healthy participants (N=48)		
Parameters	Mean ± SD	Range	Mean ± SD	Range	p value
Calcium(mg/dl)	9.21±1.262	6.5-11.4	9.22±0.7811	7.5-11.0	0.9799
Magnesium(mg/dl)	1.87±0.6755	1.0-3.3	2.18±0.6327	1.0-3.2	0.0192*
Phosphate (mg/dl)	3.6±0.8056	2.3-5.6	3.6±0.8282	2.3-5.5	0.6720
Vit D (ng/ml)	24.24±10.90	7.0-53.7	23.18±7.931	8.6-46.60	0.5880
Iron (µg/dl)	26.32± 23.26	6.0-142.0	20.81±12.77	6.0-60.0	0.192
Vit B12 (pg/ml)	245.9±173.8	35.0-779.0	213.0±148.3	34.0-880.0	0.2936
Folate (ng/ml)	8.522±7.291	0.41-26.0	6.741±4.791	1.2-24.0	0.5905
Homocysteine (µmol/L)	8.997 ±13.55	0.12-65.0	13.65±7.107	1.18-31.30	<0.0001****

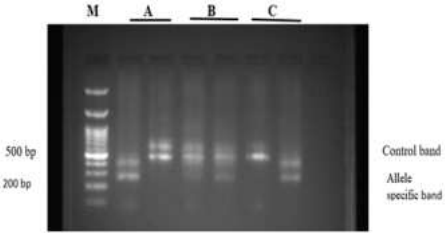


Fig 1. Agarose gel electrophoresis of ARMS-PCR products. Genotyping for the IFN-γ +874 A/T polymorphism. Lane M: DNA marker; lanes A: +874 A/T genotype; lanes B: +874 T/T genotype; lanes C: +874 A/A genotype

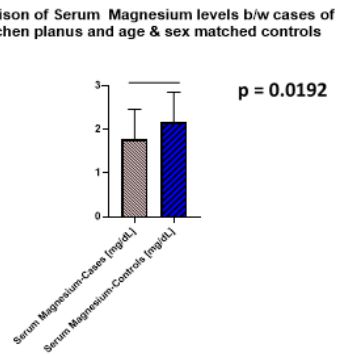


Fig 2A

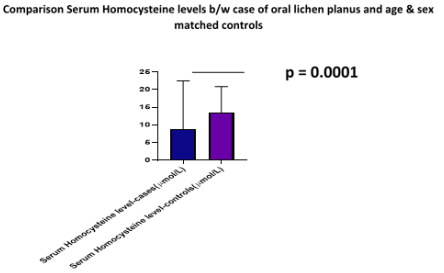
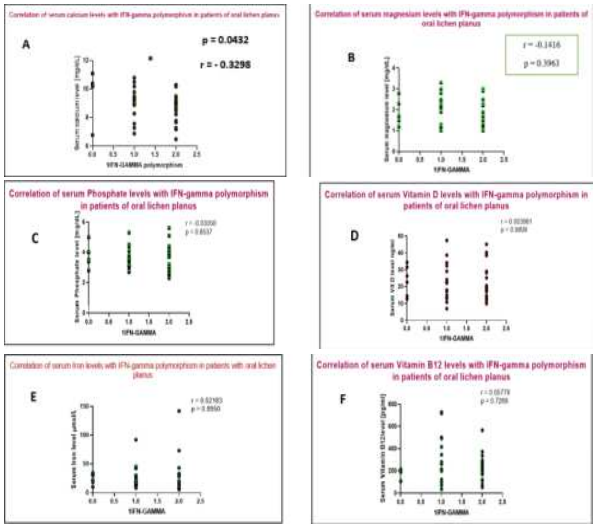


Fig 2B



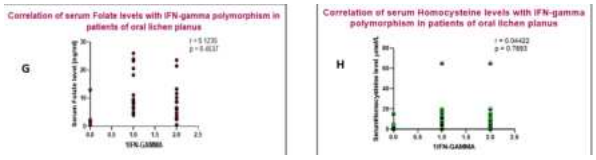


Fig 3: Scatter diagram showing correlation of Serum biomarkers with IFN-gamma polymorphism in Oral Lichen Planus cases. Serum calcium level showed significant and negative correlation with IFN-gamma polymorphism **(Fig 3A)**

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