

Inverse Correlation of Serum Lactate Dehydrogenase and Haemoglobin Levels in Patients with Oral Submucous Fibrosis

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Serum lactate dehydrogenase (LDH) is a marker of cellular necrosis and is elevated in oral potentially malignant disorders, Oral Submucous Fibrosis (OSMF). Serum LDH levels have been used as a valuable biochemical marker in diagnosis of OSMF. To evaluate and compare the levels of serum LDH and haemoglobin in OSMF with normal healthy individuals. Two mL of venous blood was collected by under aseptic conditions and serum was separated from the normal healthy control subjects (Group 1, n= 22) and the OSMF (clinically and histopathologically confirmed cases) patients (Group 2, n= 22). Quantitative estimation of serum LDH and haemoglobin was performed. Significant rise was observed in the serum LDH levels (518.14 ± 48.62 IU/L) while the mean Hb levels (11.3 ± 1.14) were decreased in patients with OSMF in comparison with normal healthy individuals (LDH: 189.77 ± 28.30 IU/L, Hb: 12.94 ± 0.98 gm%) (P value < 0.0001, < 0.0001). Serum LDH and Hb estimation would serve as a valuable biochemical marker in the diagnosis of OSMF.

Keywords: Haemoglobin; Lactate dehydrogenase; Oral Sub-Mucous Fibrosis.

Betel quid / pan chewing had been a part of Indian culture and religious customs since ages and is a socially accepted practice in India. However, commercial smokeless tobacco (SLT) products containing areca nut (pan masala with tobacco, gutka, mawa, etc.) were introduced in India only in the early 1970s. According to the Global Adult Tobacco Survey (GATS)- India, 2016-17, SLT / chewing forms are the most common form of

tobacco used among millions of Indians especially among those aged e⁷ 15 years with the average age of initiation at 17.9 years. Also, the prevalence of SLT use is more common in men (29.6%) than women (12.8%) and overall (21.4%). Among the different states and union territories, SLT use in Tamil Nadu is 10.6%.¹As per the WHO report, one in four adults and one in ten school students (13-15 years) in India use SLT and are at grave risk

due to their addiction. Higher usage of SLT could be linked to the addictive property of nicotine. Though the peak venous levels are similar, nicotine absorption is slower among SLT users than among smokers. Nevertheless, blood nicotine levels off much more slowly among SLT users compared to levels that falls rapidly after smoking.

Oral Cancer is an important public health problem in India, with increasing incidence each year. Owing to the increasing burden, cancer prevention has become the most significant public health challenge of the 21st century. Around 40% of cancer cases are preventable by reducing exposure to risk factors of which, tobacco use is reported to be a modifiable risk factor.²Tobacco use includes both smoking and SLT (betel quid with areca nut/tobacco). Nearly 90% of oral cancers are associated with tobacco usage, and e'' 50% are associated with the use of SLT. Among the potentially malignant disorders, Oral Submucous Fibrosis (OSMF) is of major concern owing to its high risk of malignant transformation, marked rigidity and trismus.

OSMF was first described by Schwartz in 1952 and he coined the term Atrophica Idiopathica Oris. OSMF is a chronic, resistant disease characterized by juxta-epithelial inflammatory reaction and progressive fibroelastic changes in the lamina propria layer, along with epithelial atrophy leading to trismus and difficulty in mouth opening. OSMF is the most common potentially malignant disorders in South Asia and is associated with significant morbidity.^{3,4}The rate of malignant transformation of OSMF to oral cancer ranges from 2.6% to 7.6% with higher rate of transformation expected over a long period of follow up.⁵⁻⁷

OSMF is considered as an Asian version of sideropenic dysphagia where in chronic iron deficiency leads to mucosal susceptibility to irritants especially the alkaloids in areca nut products. The alkaloids of areca nut act as initiating factors causing a juxta-epithelial inflammatory reaction.⁸Arecoline increases the production of collagen, in addition, tannins present in areca nut inhibit collagenases thereby reduce collagen degradation and result in a more stable collagen structure that leads to increased fibrosis. The combined effect of tannin and arecoline of areca nut act as contributing factors in the pathogenesis of OSMF.⁹Areca nuts (more so in commercial

SLTs) contain high copper concentrations that are released during prolonged chewing and it stimulates collagen synthesis in oral fibroblasts and is implicated in the pathogenesis of OSMF.¹⁰

Detection of biochemical markers play a vital role in diagnosis and prognosis of head and neck cancer. Serum Lactate dehydrogenase is reported to be a reliable diagnostic marker in premalignant and malignant lesions of the oral cavity.^{11,12}The enzyme LDH is a ubiquitous enzyme which catalysis the reaction of lactate to pyruvate during anaerobic glycolysis. Malignant transformation leads to genomic changes which leads to changes in LDH activity. Also, haemoglobin levels are reported to be decreased in subjects with OSMF.¹³This study was designed to assess and compare the levels of serum LDH and haemoglobin levels between healthy individuals and the OSMF patients.

MATERIALS AND METHODS

The study protocol was reviewed and approved by the Institutional Human ethical committee, SBDCH. Subjects who report to the department of oral pathology, SBDCH were screened for OSMF. The demographic details, details of habits including, smoking/chewing of betel quid with or without tobacco/alcohol consumption were also recorded. Those who fulfilled the following inclusion criteria (age group of 20-75 years, both the sexes, with habits (tobacco and/or smokeless tobacco, chewing betel quid, areca nut), clinically diagnosed (burning sensation, restricted mouth opening, fibrous bands palpable in the blanched buccal mucosa) and histopathologically confirmed cases of OSMF (Juxta-epithelial hyalinization, epithelium markedly atrophic with loss of rete pegs, subepithelial band of chronic inflammatory cells, thickened/ dense collagen fibres,) were recruited for the study. Those subjects with systemic illnesses that are known to increase serum LDH levels were excluded. Study group comprised of controls (Group 1) consisting of normal healthy subjects without habits (n=22) and Group 2 including patients with clinically and histopathologically confirmed cases of OSMF (n=22). Biopsy was performed at the Department of oral pathology, SBDCH. The histopathological grading of OSMF was performed as per Khanna *et al.*, 1995.¹⁴

Two mL of venous blood was collected by under aseptic conditions from the normal healthy control subjects (Group 1, n= 22) and the OSMF patients (Group 2, n= 22). Blood was allowed to clot and serum was separated and aliquoted in 1.5 mL microfuge tubes and stored at -20°C until use. Serum LDH estimation was carried out using a calorimetric assay which is based on the following principle, LDH, an oxidoreductase catalyses the conversion of lactate to pyruvate with the simultaneous reduction of NAD to NADH that results in a proportional increase in absorbance due to the reduction of NAD. Estimation of serum haemoglobin was performed by Sahli's method. We analysed and correlated the levels of serum LDH and haemoglobin levels between healthy individuals and the OSMF patients.

Statistical analysis

Unpaired Student's *t* test was applied to determine the statistical significance of the mean LDH levels and mean Hb levels between the groups.

RESULTS

The normal values of serum LDH in adult males and adult females are 135-225 U/L and 35-214 U/L respectively. A significant rise was observed in the serum LDH levels in patients with OSMF (518.14 ± 48.62 IU/L) in comparison with normal healthy individuals (189.77 ± 28.30 IU/L). Comparison of the mean Hb levels of the OSMF patients (11.3 ± 1.14) with normal subjects (12.94 ± 0.98) showed a statistically significant difference with a *p* value < 0.0001 .

DISCUSSION

India bears the highest burden of oral cancer globally, due to high prevalence of SLT use. Incidence of OSMF has increased in the recent decades in India. The oral health consequences of the use of SLT as suggested by GATS-INDIA, 2010 indicate that SLT users are at high risk of premalignant oral lesions especially OSMF.¹⁵ OSMF is reported to be a high-risk precancerous condition caused by using areca nut in SLT products such as pan, gutka, and mawa. Areca nut and tobacco chewing plays a combined effect on the etiology of OSMF. Also, the morbidity rate is

expected to increase 1.5 million annually by 2020.

Various studies have documented that serum LDH as a reliable biochemical marker of tissue break down in malignant and premalignant conditions such as OSMF.^{16,17} Increased LDH levels are due to genetic changes during malignant transformation, increased mitotic index and increased lactic acid production by the dysplastic cells due to altered glycolytic pathway in hypoxic conditions.¹⁸ Our results are in concurrence with the findings of Mishra *et al.*, 2018, Swamy *et al.*, 2016, Rathore *et al.*, 2015, Anuradha *et al.*, 1998, who reported increased serum LDH in patients with OSMF compared to the normal healthy controls.^{16,18-20} Increased hypoxia is documented to play a major role in malignant transformation and progression of OSMF. Hypoxia induced upregulation of HIF-1 alpha at both protein and mRNA levels occurs in OSF and well correlates with epithelial dysplasia.²¹

The results of our study indicate that mean Hb levels were significantly lower among the OSMF patients compared to the healthy control subjects. This is in line with the findings of Thakur *et al.*, 2017, Bhardwaj *et al.*, 2016, Karthik *et al.*, 2012, Taneja *et al.*, 2007.^{13,22-24} Lower Hb levels in OSMF subjects could be a result of the SLT usage practice, as arecoline present in areca nut act as an initiator and an irritant that induces oro-epithelial inflammatory reaction that results in burning sensation, vesiculation and ulceration of oral mucosa.²⁵ OSMF has a high rate of morbidity as a result of stiffness of oral mucosa, limited mouth opening, tongue protrusion, restricted mouth opening which leads to difficulty in the intake of food, chewing deglutition thereby marked reduction in the consumption of normal diet that further progresses to nutritional deficiencies especially the trace elements, iron. This could be associated with the reduced haemoglobin levels and concomitant anaemia. It is suggested that the lack of iron in the oral tissues could result in decreased vascularity and thereby alter the percolation of esters of arecoline that induce fibrogenesis and the formation of fibrotic bands, characteristic of OSMF. Also, increased utilization of iron for the hydroxylation of proline and lysine in collagen synthesis may lead to decreased serum iron levels in OSMF patients.²⁴

Of the various carcinogens in SLT,

Tobacco specific Nitrosoamines (TSNAs) which arise from nitrosation during the process of drying tobacco leaves is the most potent carcinogen and arecoline, an areca-nut specific carcinogen exhibit a combined effect on the etiology of OSMF. Previous Indian reports have documented a strong association between SLT use (which includes betel quid containing areca nut / tobacco) and cancers of the oral cavity (Odds Ratios of 3 to 22) and pharynx (Odds Ratios of 2 to 4). Nevertheless, the risk of oesophageal cancer is higher among those who use SLT with areca nut and (Odds Ratios of 2 to 7) compared to the use of plain tobacco (Odds Ratio=4.9).¹⁵

Available evidences suggest that mortality and morbidity due to consumption of smokeless tobacco (SLT) is very high in India. The mortality rate increases with the transformation of OSMF into OSCC, the risk of which is 7-30%. The increased risk of malignant transformation could be attributed to, the easy availability and affordability of tobacco and areca nut products and more importantly the lack of awareness regarding the increased risk of oral premalignant lesions and their progression to malignancy. This had led to the development various Tobacco cessation strategies that include Tobacco Cessation Clinics (TCCs), mass media campaigns, targeted campaigns at work places, and community-based programmes. Despite these efforts, there is only a slight decline in SLT use among Indians (25.9% in 2009-10 to 21.4% in 2016-17)^{1,15}, SLT usage is still quite common among the disadvantaged, labourers in the unorganized sector and people living in rural areas. The increased use of SLT among youth is of great concern as it puts young people at risk of OSMF and hence initiation of cancers at an early age. Also as indicated by other authors, effective management of OSMF in high risk groups should include iron supplements and a proper balanced diet that could curtail the further progression/transformation into oral cancers.²⁶

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