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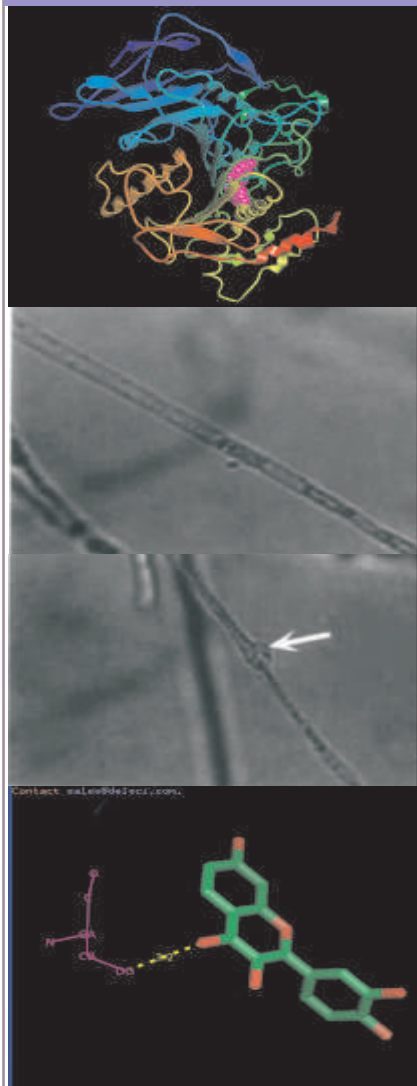
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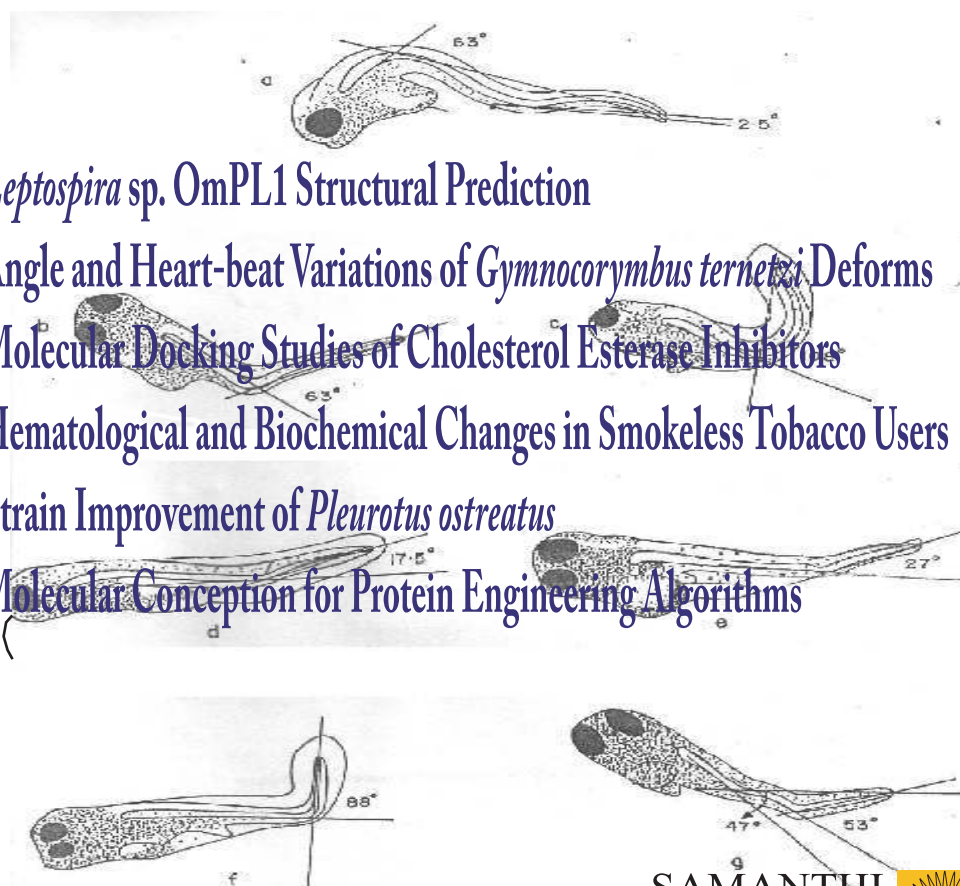
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# Hematological and Biochemical Changes in Smokeless Tobacco users from South India

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## Abstract

A mixture of betel leaf, areca nut and tobacco chewing is used in many parts of India. The aim of our study is to assess the hematological and biochemical changes of mixture of betel leaf, areca nut and tobacco users. The present study subject comprises of 120 healthy individuals which include 40 betel leaf, areca nut and tobacco chewers (chewers), 40 smokers and 40 controls with the mean age of  $35.43 \pm 9.07$ ,  $31.52 \pm 4.90$  and  $32.98 \pm 6.49$  years respectively. A significant ( $p < 0.05$ ) decrease in the level of haemoglobin ( $14.16 \pm 1.02$ ) and increase in the level of ESR ( $13.40 \pm 0.63$ ) and TLC ( $9471.06 \pm 1192.04$ ) were observed in chewers. There was a significant ( $p < 0.05$ ) difference in mean cholesterol levels among control and chewers. The level of serum enzymes Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) was found to be increased in chewers when compared to smokers.

**Keywords:** Hematological values; Smokers; Smokeless tobacco

## Introduction

A number of diseases are associated with environmental factor, life style and food habits. It is estimated that about 600 million people chew areca nut mixed with large proportion of tobacco with it (Nelson *et al.*, 1999). Betel quid chewing is an ancient practice in many parts of India. Betel quid consists of betel leaf, areca nut and slaked lime, to which tobacco is commonly added (Warnakulasuriya *et al.*, 2002). The most common way in India is to use half a leaf, 1 medium or 2 small sized betel leaf, smear them with slaked lime along with pieces of areca nut. Only ripe areca nut is used, usually after curing (generally by roasting or boiling in water). Betel quid can prepare plain is sweet. Some times cardamom and often tobacco are added to the plain variety. Habitual users generally include tobacco, which can be raw and unprocessed or processed with a mixture of spices and often sweetened with unrefined sugar or artificial sweetener and flavoured (Bhonsle *et al.*, 1992).

Betel leaf contains large amounts of carcinogens called safrole, which is readily metabolised and excreted in urine (Chang *et al.*, 2002). Betel quid and areca nut chewing leads to oral sub-mucous fibrosis, a painful disabling and potentially precancerous condition of the oral mucosa. Betel quid chewing is a major risk factor for cancer in mouth, pharyngeal cavity and upper digestive tract (Dava *et al.*, 1992; Sudha *et al.*, 2009).

There were only less attention in betel quid, areca nut and tobacco chewers and their hematological and biochemical parameters. Blood is a part of the circulatory system of the body and it has several functions. Much valuable information can be readily obtained from blood parameters. A wide variety of disease and other dysfunctions may show

signs or symptoms of a hematological diseases like eosinophilia, anemia and are highly associated with different food habits, life style and environmental hazards (Connellan, 1993).

Polymorphonuclear neutrophils are very important in the body's acute inflammatory process, while lymphocytes, macrophages and monocytes are mononuclear phagocytic cell, are indispensable in the body immune system and chronic inflammatory response (Govan, 1995). Eosinophils responds to chemotactic substances produced by mast cell when introduced by the presence of persistent antigen-antibody complexes such as chronic parasitic, dermatological and allergic conditions while basophils, whose granules contain a number of preformed mediators of the inflammatory response including histamine and chondroitin sulphate, also stimulate leukotriene and other mediators upon stimulations (Rubin and Faber, 1998). Betel quid, areca nut chewing has extensively studied in many part of world (Gupta *et al.*, 2004). Chewing a mixture of betel quid, areca nut and tobacco is a complex behavior and is poorly studied the purpose of this study was to comparatively estimate the hematological and biochemical parameters of smokers, betel quid chewers (smokeless tobacco) with their respective controls.

## Materials and methods

The present study is to comparatively evaluate the hematological and biochemical changes in smokers and mixture of betel leaf, areca nut and tobacco chewers from rural areas of Coimbatore city, South India. The study subjects comprised 120 healthy age matched individuals, which include 40 smokers 40 betel quid chewers and 40 controls. Participants were informed about the study and asked to sign an informed consent

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form and to complete a standardized questionnaire to obtain necessary data on lifestyles and personal factors (age, working period, health, etc.). None of these study groups showed significant differences with regard to lifestyle and personal factors. Five milliliters of venous blood was collected from each participant after overnight fasting and transferred into EDTA coated tubes (Sharma and Awasti, 2001). The blood was then centrifuged at 3,500 rpm for half an hour to obtain serum. The EDTA blood samples were used for analyzing Hb, ESR, DC and TLC (Win robe *et al.*, 1965; Linee and Ringsrud 1992; Varley, 1991).

The fasting blood sample was collected and allowed to stand for 30 min and serum was separated. The serum was kept at 4°C for analysis. Serum TC, HDL, LDL and VLDL-cholesterol were determined by enzymatic method (Behera *et al.*, 2003).

### Statistical Analysis

All statistical analysis was performed using SPSS 11.0. *P* values <0.05 were considered significant. Values are expressed in mean ± S.E.

## Results

The general characteristics of the study population are presented in Table 1. The group under study was analyzed based on the age and no differences were observed between the study groups. The mean level of Hb content ESR and TLC of experimental and control groups were represented in Table 2. There was a statistically significant (*p*<0.05) decrease in the value of Hb and increase in the mean values ESR, TLC and Platelet were obtained in a mixture of betel leaf, areca nut and tobacco chewers when compared to smokers.

Study group	N = 120	Age range	Mean age
Controls	40	2049	32.98 ± 6.49
Smokers	40	1845	31.52 ± 4.90
Chewers	40	2551	35.43 ± 9.07

Table 1 Characterization of participants

[Control:- Non smokers/Non chewers; Smokers:- Person smoking more than ten cigarettes per day; Chewers:- Person with Mixture of betel quid with areca nut and tobacco chewing habit].

Parameters	Controls (n=40) (Mean ± S.E.)	Smokers (n=40) (Mean ± S.E.)	Chewers (n=40) (Mean ± S.E.)
Hb (g/dl)	14.21 ± 0.64	14.57 ± 1.21	14.16 ± 1.02
ESR (mm/hr)	10.89 ± 0.23	13.12 ± 0.54	13.40 ± 0.63 d
TLC (10 <sup>6</sup> /L)	9263.75 ± 1010.20	9432.01 ± 1123.04 a	9471.06 ± 1192.04
Neutrophils (%)	54.69 ± 1.71	59.32 ± 2.86 b	53.67 ± 2.08
Lymphocytes (%)	32.18 ± 0.73	28.65 ± 0.53 b	35.25 ± 0.84 d
Eosinophils (%)	3.30 ± 0.43	2.52 ± 0.34 a	2.60 ± 0.51 c
Monocytes (%)	7.38 ± 0.05	8.09 ± 0.21 b	6.16 ± 0.15
Basophils (%)	1.03 ± 0.03	0.74 ± 0.02 a	0.86 ± 0.02
Platelet (K/L)	238.29 ± 22.31	206.47 ± 19.48 b	225.29 ± 21.52

Table 2. Hematological results of study population

[All values are given as mean ± S.E. Significant difference between study groups; a: *p*< 0.01, smokers and chewers, b: *p*< 0.05 smokers and chewers, c: *p*< 0.01, chewers and controls, d: *p*< 0.05 chewers and controls].

The mean levels of differential leukocyte count were represented in Table 2. The percentage of Lymphocytes (35.25 ± 0.84), Eosinophils (2.60 ± 0.51) and Basophils (0.86 ± 0.02) were significantly increased in chewers, and the percentage of Neutrophils (53.67 ± 2.08) and Monocytes (6.16 ± 0.15) were decreased in chewers than smokers. The level of Triglyceride, TC, HDL-C, LDL-C and VLDL-C in smokers and chewer groups were compared with control group and represented in Table 3. There was only less significant difference between the smokers and chewers group (*p*<0.01).

Parameters (mg/dl)	Controls (n=40) (Mean ± S.E.)	Smokers (n=40) (Mean ± S.E.)	Chewers (n=40) (Mean ± S.E.)
Triglycerides	103.40 ± 14.21	109.76 ± 16.84 a	107.70 ± 17.24 d
Total cholesterol	177.05 ± 20.08	181.64 ± 20.85	182.27 ± 23.42 d
HDL C	49.8 ± 7.09	44.09 ± 6.28 a	45.03 ± 6.73 d
LDL C	99.40 ± 10.20	103.29 ± 10.29	104.07 ± 10.32 d
VLDL - C	22.60 ± 1.52	24.09 ± 1.76	23.52 ± 1.62 c
ALT (IU/L)	18.79 ± 1.36	22.03 ± 1.13 a	24.21 ± 1.18 d
AST (IU/L)	26.58 ± 1.25	30.98 ± 4.74 b	33.72 ± 5.13 d

Table 3 Mean levels of Biochemical results of study population.

[All values are given as mean ± S.E. Significant difference between study groups; a: *p*< 0.01, smokers and chewers, b: *p*< 0.05 smokers and chewers, c: *p*< 0.01, chewers and controls, d: *p*< 0.05 chewers and controls].

## Discussion

The habit of chewing tobacco is increasing because of its free availability, cheaper cost and increasing education about well stabilized hazards of smoking. Studies have confirmed that use of smokeless tobacco is as harmful as smoked tobacco (Gajalakshmi *et al.*, 2003). Gajalakshmi *et al.* (2003) conducted a large case-control study in Chennai and reported that tobacco is a major risk factor for mortality. ESR has been found to be higher in patients with lung inflammatory changes and respiratory diseases of smokers (Abou Taleb *et al.*, 1995; Kumar and Clark 1998; Berner, 1983; Weiss *et al.*, 1995). High TLC count represents a primary disorder of leukocyte production or may reflect a secondary response to some disease process or toxins (Friedman and Fireman, 1991). The peripheral blood leukocyte count is a marker of inflammatory activity and ongoing tissue inflammation from whatever underlying cause. It might be viewed as a bio-marker of inflammatory response. Longitudinal studies have linked elevations of the peripheral blood leukocyte count to increased mortality from decreased pulmonary function, ischemic heart disease and cancer (Grimm *et al.*, 1985; Bartecchi *et al.*, 1994). The higher level of Cholesterol and Triglyceride in tobacco chewers may be attributed to tobacco induced stimulation on metabolism of free fatty acids in peripheral tissue (Bartecchi *et al.*, 1994). Removal of major risk factor such as mixture of betel quid, areca nut and tobacco chewing could increase healthy life expectancy in every region of the world. The strong and intriguing relation between the use of betel quid and tobacco chewing was found to be public health hazards.

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