

Study of Superoxide Dismutase (SOD) Activity Among Betel Quid Chewers of Indian Population

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Abstract

Introduction: Betel quid (BQ) chewing, a habit practiced in Eastern and North Eastern part of India, has known to be associated with cancer of the oral or buccal cavity. BQ is also one of the common mood elevating substances among Indian population. The BQ is a mixture of areca nut (Areca catechu), catechu (Acacia catechu) and slaked lime (calcium oxide and calcium hydroxide) wrapped in a betel leaf (Piper betel). BQ products have been classified by the International Agency for Research on Cancer (IARC) as group I human carcinogens. Superoxide Dismutase (SOD), one of the major enzymatic antioxidant defence system, responsible for scavenging free radicals. Antioxidant enzymes catalyse decomposition of Reactive Oxygen Species (ROS). Overall balance between production and removal of ROS may be more important in various cancers including OSCC (Oral squamous cell carcinoma) or oral cancer.

Methods: In this study subjects were screened from Department of Oral and Maxillofacial surgery & E.N.T. of Ramakrishna Mission Seva Pratishthan Hospital (RKMS), Kolkata and different areas of West Bengal and North Eastern states of India. Quantitative in vitro determination of superoxide dismutase activities in whole blood were estimated manually with 0.05 ml whole blood. The samples were assayed by UV-Visible Spectrophotometer (SPECORD 50 PLUS) at a wavelength of 420 nm.

Results: Most of the subjects had betel quid chewing habit. Superoxide dismutase values are lower in healthy control than Cancer cases and Pre cancer with betel quid chewing habit, which is statistically significant.

Conclusion: Reactive oxygen species (ROS) are generated due to slaked lime, one of the important constituents of betel quid which can modulate the oral pathology and promote carcinogenesis.

Key Words: Superoxide Dismutase, ROS, Betel quid, Oral squamous cell carcinoma.

Introduction

A molecular fragment which contains an odd number of unpaired electrons in the valence shell known as radical which is capable of existing freely (i.e. free). They may interact with cellular macromolecules such as DNA, protein, lipid and carbohydrate to initiate inflammatory, toxic or carcinogenic processes. Well-known risk factors are consumption

of smokeless tobacco mainly betel quid (BQ) with areca nut, which result in increased free radicals production. Reactive oxygen species (ROS), which are produced following single electron reductions of molecular oxygen. Antioxidant enzymes (Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT)) catalyse decomposition of ROS. Redox modulation is mostly observed by distinctive changes in the enzyme activities systems in oxidative stress. As a result of these, the

stability between production and removal of ROS may be more important in various cancers including Oral squamous cell carcinoma (OSCC) (Yokoe H, et al; 2009).

The pH (changes mainly during betel quid chewing) of body fluids due to smokeless tobacco consumption affects the formation and stabilization of free radicals. The alkaline conditions observed in betel nut chewing are reported to favour the formation of free radicals. In multi stage process of carcinogenesis (by bringing out a continuous endogenous damage to cellular DNA), these Reactive oxygen metabolites (ROMs) such as superoxide anion, hydroxyl radical, hydrogen peroxide, malondialdehyde and nitric oxide are directly involved. (Patel et al; 2005).

The superoxide dismutase (SODs) are the first and most important line of antioxidant enzyme defense systems against ROS (Chang et al.1988, Keller et al.1991, Crapo et al.1992; Liou et al.1993). Cancer development

can be described by three stages: initiation, promotion and progression, and ROS can act in all these stages of carcinogenesis in the cell by mutagenesis, cytotoxicity, and changes in gene expression (Klaunig and Kamendulis 2004). Calcium hydroxide content of lime in the presence of the areca nut (One of the major component of betel quid) is primarily responsible for the formation of ROS that might cause oxidative damage in the DNA of buccal mucosa cells of betel quid chewers, leading to OSCC (Nair et al; 1990).

Materials & Methods:

(A) Screening of Subjects:

I) Camp in Eastern India, II) Camp in North East India and III) patients attending ENT and Maxillofacial department, RKMS hospital, Kolkata, India.

I) **Eastern India Camp:** - 220 subjects were screened at a camp held in Bankura, Atghara and Purba Midnapur of West Bengal. Out of them, more than 60% were betel quid chewers.

II) **North East India Camp:** - 56 subjects were screened at a camp held in Karimganj, Assam. Out of them, 58% were betel quid chewers.

III) **RKMSP Hospital, Kolkata:** 2885 cases attending in one year at E.N.T OPD and Oral Maxillofacial OPD of RKMS Hospital, Kolkata. 35 Patient were selected for our study. More than 68% were betel quid chewers. Out of 35, 40% cases had pre-cancerous lesion, 37% cases had squamous cell carcinoma, 29% cases had pre-cancerous condition.

Every subjects filled up their consent form for sample collection.

(B) Methods:

i) Detailed history was taken from all cases by filling up questionnaire and all cases gave the

Results:

written consent form, and this study are followed the Institutional Ethical Committee's

guidelines.

❖ Inclusion criteria

- Patients clinically and histopathologically diagnosed with oral submucous fibrosis, oral leukoplakia, and OSCC.
- Cases mainly chewed betel quid more than 2 – 3 / day.
- Cases who agreed for the hematological examination.
- Normal subjects without any oral lesions and systemic diseases.

❖ Exclusion criteria

- Cases below the age of 20 and above 75 years.
- Cases suffering from any systemic diseases like diabetes, hypertension, cardiovascular diseases, renal dysfunction, chromosomal disorder, neurological problems and liver disorders

ii) Superoxide Dismutase Assay:

This method based on (Woolliams et al.1983) employs Xanthine and Xanthine Oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The Superoxide Dismutase (SOD) activity was then measured by the degree of inhibition of this reaction. Quantitative in vitro determination of SOD activities in whole blood were estimated with 0.5ml heparinized whole blood manually. The whole blood was centrifuged at 3000 rpm for 10 minutes followed by aspiration of plasma to obtain the erythrocytes which were washed with 0.9% NaCl solution. The erythrocytes were made up to 2 ml with cold redistilled water followed by incubation at 4°C. The lysate was assayed by UV-Visible Spectrophotometer (SPECORD 50 PLUS) at the wavelength of 420 nm. Appropriate negative and positive control were maintained with each batch of estimation.

PLACE	NO	AGE GROUP (in years)						Addiction			No BQ Addiction
		Below 30	31-40	41-50	51-60	61-70	Above 70	Smoking	Alcohol	Betel Quid	
NORTH EAST INDIA CAMP 1. Assam, Karimganj	56	1	2	12	24	11	6	9	6	33	23
EASTERN INDIA CAMP 1) Bankura, Dhulai West Bengal	34	5	20	8	1	0	0	16	14	19	15
2) East Midnapur, Bibhisapur. West Bengal	46	22	13	3	6	2	0	28	29	36	10
3) North 24 Pgs, Atghara. West Bengal	89	28	18	21	15	6	1	27	3	56	33
4) Narrah, Bankura West Bengal	51	8	13	12	8	6	4	14	5	22	29
RKMSP Hospital, Kolkata	35	2	7	8	11	7	0	20	8	24	11

TOTAL	311	66	73	64	65	32	11	114	65	190	121
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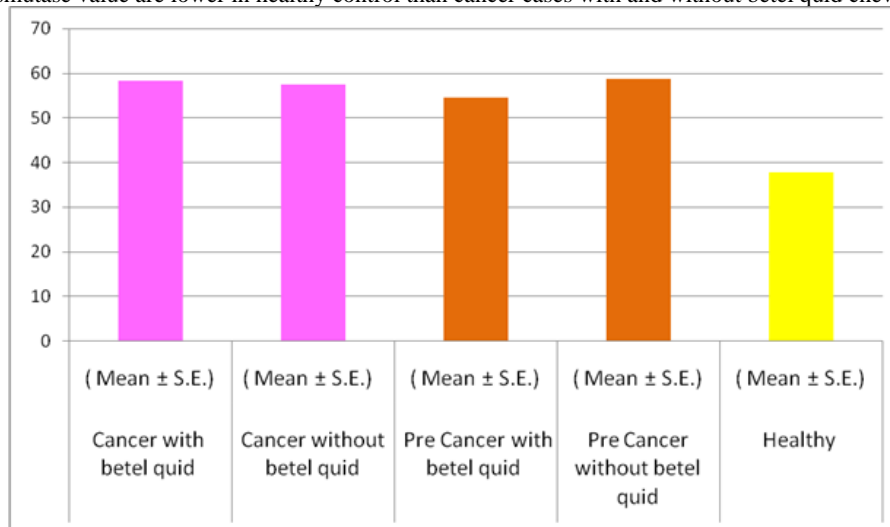
Table 1: Detailed history of subjects of different areas

Note: Most of them are betel quid chewers and percentages of male cases are higher in age of 51-60 years.

Cancer with betel quid (Mean ± S.E.)	Cancer without betel quid (Mean ± S.E.)	Pre Cancer with betel quid (Mean ± S.E.)	Pre Cancer without betel quid (Mean ± S.E.)	Healthy Control (Mean ± S.E.)
52.26 ± 6.99	51.54 ± 6.42	52.61 ± 2.3	52.67 ± 6.39	36.9 ± 1.2

Table 2: Superoxide dismutase activity among of studied cases and healthy control

Inference: Super oxide dismutase value are lower in healthy control than cancer cases with and without betel quid chewers (p < 0.0001*).



Discussion:

In this Study Superoxide dismutase activity of cancer cases showed about higher as compared to healthy individuals.

Conclusion:

In this study, Super oxide dismutase values are lower in healthy control than cancer cases with and without betel quid chewers which is statistically significant (p < 0.0001*).

Ethics approval and consent to participate

This study was approved by the institutional ethical committee on October 2013. No. ECR/150/Inst/WB/2013 issued under Rule 122DD of the Drugs & Cosmetics Rules 1945.

Competing interests

The authors declare that they have no competing interests.

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