

Study of Glutathione Peroxidase (GP_x) 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. Glutathione peroxidase (GPx), one of the major enzymatic antioxidant defence system, responsible for scavenging free radicals. Antioxidant enzymes catalyze decomposition of 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 glutathione peroxidase 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 340nm.

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

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

KEYWORDS: Glutathione peroxidase, Betel quid, Oral cancer

INTRODUCTION

Antioxidants are the first line of defense against free radical damage and are essential for maintaining optimum health and well-being. Glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) are the three major enzymatic antioxidant defense systems responsible for scavenging free radicals [1]. Glutathione peroxidase (GPx) is a selenocysteine – dependent enzyme and also important hydrogen peroxide (H₂O₂) scavenging enzyme. GPx is well known first line defense anti-oxidants. These antioxidant enzymes catalyze decomposition of “reactive oxygen species” (ROS). Considerable evidence suggests that antioxidant enzymes act to inhibit both initiation and promotion of carcinogenesis. The low activities of these enzymes play a key role in progression of lesion or condition [2].

ROS are formed in the cells of aerobic organisms, and can initiate autocatalytic reactions so that molecules to which

they react are themselves converted into free radicals to propagate the chain of damage. Cytochrome P450 has also been proposed as a source of ROS since on its induction, superoxide anion and hydrogen peroxide production [3]. Calcium hydroxide content of lime in the presence of the areca nut is primarily responsible for the formation of ROS that might cause oxidative damage in the DNA of buccal mucosa cells of betel quid chewers [4].

The main ingredients used in quid are areca nut, catechu, betel leaf, slaked lime. In 1985, International Agency for Research on Cancer (IARC) monograph on betel quid reported that there was sufficient evidence for carcinogenicity to humans for betel quid containing tobacco as Group 1 carcinogen, but betel quid without tobacco reported as Group 3 carcinogen [5].

Recently 2003, IARC monograph declared chewing of betel quid, by itself, to be a Group 1 human carcinogen and the areca nut is also fall under those human carcinogen [6]. The Indian population chew BQ as a mood elevator from ancient period of time.

Materials & Methods:

A. Screening of Subjects:-

1. Camp in Eastern India, II) Camp in North East India and III) patients attending Maxillofacial and ENT department of RKMS hospital.
2. Eastern India camp: - 220 subjects were screened at a camp held in Bankura, Purba Midnapur, Atghara. West Bengal. Out of whom 133 were betel quid chewers.
3. North East camp: - 56 subjects were screened at a camp held in Karimganj, Assam. Out of whom 33 were betel quid chewers.
4. RKMS Hospital: - 2885 cases attending in one year at E.N.T OPD and Oral Maxillofacial OPD of RKMS. 35 Patient were selected for our study. 24 cases were betel quid chewers. Out of 35, 14 cases had pre cancerous lesion, 13 cases had squamous cell carcinoma, 8 cases had pre cancerous condition.

An informed consent was taken from all the subjects.

B. Methods:

1. Detailed history were taken from all cases by filling up questionnaire and all cases gave their written consent prior to the participation, and the study procedures followed the guidelines of the Institutional Ethical Committee.
2. Glutathione peroxidase Assay:- This method was based on Paglia and Valentine (1967) [7]. Glutathione peroxidase catalyzes the Oxidation of Glutathione (GSH) by Cumene Hydroperoxide. In the presence of Glutathione reductase and NADPH the oxidized Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340nm was measured. Quantitative in vitro determination of glutathione peroxidase activities in whole blood were estimated manually with 0.05 ml whole blood. Blank was run for each sample. The samples were assayed by UV-Visible Spectrophotometer (SPECORD 50 PLUS) at a wavelength of 340nm. Appropriate negative and positive controls were maintained with each batch of estimation.

RESULTS:

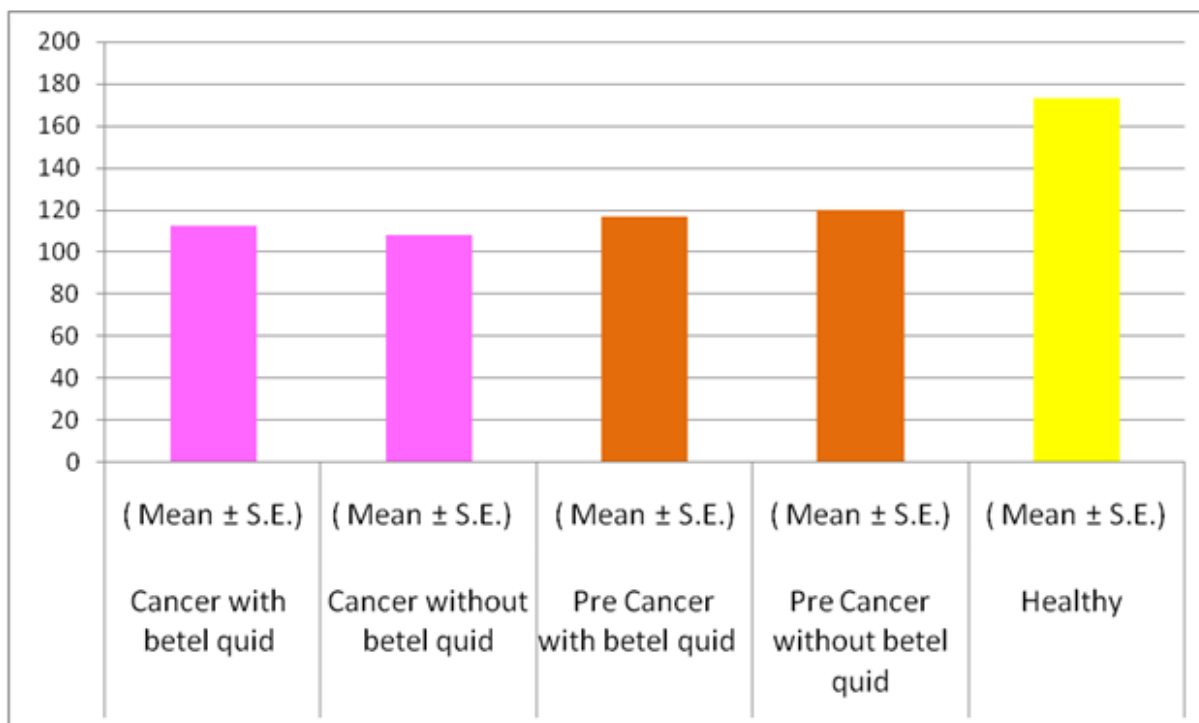
Table 1: Detailed history of subjects of different areas

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
RKMS 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

Note: Some cases had more than one addiction

Table 2: Glutathione peroxidase activity among of studied cases and healthy control.

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.)
99.57 ± 13.54	97.65 ± 10.83	111.15 ± 1.79	108.09 ± 12.4	172.39 ± 1.41



Inference: Glutathione peroxidase values are higher in healthy control than cancer cases with and without betel quid chewers. Glutathione peroxidase value are higher in Pre cancer cases than cancer cases who chew betel quid ($p < 0.0001^*$).

Discussion:

GPx plays an important role in the defence mechanisms of mammals against damage by catalyzing the reduction of H_2O_2 [8]. The low levels of antioxidants in the blood of oral cancer patients may be due to their increased utilization to scavenge lipid peroxides as well as their sequestration by tumor cells and also growth advantage on tumor cells. GPx levels were low suggesting that most cancer cell types couldn't detoxify hydrogen peroxide [9].

In this study, a statistically significantly decrease levels of mean GPx in cancer and pre cancer cases with betel quid chewing habit than the control group. This suggests that lower antioxidant enzymes activity in oral cancer patients might be due to the depletion of the antioxidant defense system that occurs as the consequence of overwhelming free radicals by the elevated levels of lipid peroxides.

Conclusion:

Free radical associated damages leads to an imbalance between pro-oxidant and anti-oxidant states. This imbalance plays an important causative role in carcinogenesis. Cases are more prone to oxidative stress due to increasing load of free radicals in body due to chemical carcinogens present in tobacco [10]. In this study, oral cancer group showed a statistically significantly decrease levels of mean GPx when compared to the control group and also the lowest levels amongst the study groups 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.

Consent for publication

Not applicable.

Availability of supporting data

All relevant data and materials can be obtained from the first author and also corresponding author and approved by second author.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

AA carried out the enzymatic studies, drafted, revised the manuscript. MD participated in the design of the study and helped to revise the manuscript. AA performed the statistical analysis. All authors read and approved the final manuscript.

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