



Effect of Black Tea on Micronuclei (Oral Cancer Biomarker) Among Indian Population

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Abstract: Introduction Tea is the most widely consumed beverage worldwide and important agricultural product. Being rich in natural antioxidants, tea is used in the management of different types of cancers including oral cavity. Micronuclei (MN) act as a biomarker for oral cancer. These are small, extra nuclear bodies that are formed during mitosis from lagging chromosomes. The micronucleus test is used as a tool for genotoxicity. In this present study subjects were screened from Department of E. N. T. & Oral and Maxillofacial surgery of RKMS hospital, Kolkata and different areas of Eastern and North Eastern states of India. Exfoliated cell were examined from buccal mucosa for MN. Percentage of MN was low after black tea supplementation. We can concluded that betel quid has an immense role in changing the oral pathology and tea has chemo preventive property.

Keywords: Black Tea, Cancer, Polyphenols, Micronuclei, Betel Quid

1. Introduction

Tea is one of the most important beverages of the world, it is cultivated in at least 30 countries around the world. Tea beverage is an infusion of the dried leaves of *Camellia sinensis*, a member of Theaceae family. Tea stood mythologically as a beverage with positive health effects in Far-East. The traditional use of tea prompted scientific research employing modern research methodology in China, Japan, and US on the influence of tea consumption on human health, since mid-sixties. The ancient use of tea as a beverage has been practiced by mankind since anywhere between 500-5000 years ago [1]. India is the largest black tea producing country in the world. In India a collaborative research programme was launched between Tea Research Association (TRA) and the Indian Institute of Chemical Biology (IICB) in 1990s with the primary objective to evaluate the pharmacotherapeutics of black tea in totality i.e. as is consumed. The fresh tea leaves contain four major catechins as colorless water soluble compounds epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Most of the green tea catechins, during the manufacture of black tea, are oxidized and converted into orange or brown products known as theaflavins (TF) and thearubigins (TR). Theaflavins consist of two catechin

molecules joined together, and account for about 10% of the converted catechins, whereas the thearubigins are more complex flavonoid molecules, whose structural chemistry are still unknown, and may account for up to 70% of flavonoids in black tea. The anti-carcinogenic activities of tea polyphenols are generally believed to be related to their antioxidative properties. Tea may affect the metabolism of carcinogens by induction or inhibition of various cytochrome P450s, but the practical importance of this mechanism is not known.

Black tea and its two polyphenols (TF and TR) were investigated against chemically induced genetic damage as measured by chromosome aberrations and sister chromatid exchanges in mice [2]. Tea consumption has attracted much attention for the potential cancer preventive effect for a long time. There are lots of studies about the effects of the tea extracts on cancer in both vitro and in vivo [3, 4]. Being rich in natural antioxidants, tea is used in the management of different types of cancers including oral cavity. Oral cancer is the sixth most common human cancer [5], representing 3% of all types of cancer. They are located in the oral cavity in 48% of cases, and 90% of these are oral squamous cell carcinoma [6]. They are sometimes preceded by precancerous lesions, such as Leukoplakia and Erythroplakia. More than 300,000 new cases of oral squamous cell carcinoma are diagnosed annually [7]. The most common site for intraoral carcinoma is

the tongue, which accounts for around 40% of all cases in the oral cavity proper. Tongue cancers most frequently occur on the posterior-lateral border and ventral surfaces of the tongue. The floor of the mouth is the second most common intraoral location. Less common sites include the gingival, buccal mucosa, labial mucosa, and hard plate. Cancer is the eventual outcome of the transformation of normal cells by DNA-reactive, genotoxic carcinogens and growth promotion of mutated cells by enhancing factors [8]. Cancer is the product of interaction of genetic factors and environmental exposures like ionizing radiation, smoking, specific infectious agents, and dietary factors, which develops over a long time and goes through many stages. To evaluate the genotoxic risks / effects in tobacco users on buccal mucosa, DNA damages can be assessed by chromosomal aberrations, sister chromatid exchanges and MN test. Oral cytology is becoming increasingly important in the early diagnosis of oral cancers, as a procedure for obtaining cell samples that can then be analyzed by sophisticated diagnostic techniques [9].

Evaluation of the genotoxic risks in tobacco users and alcoholics on buccal mucosa, observed as DNA damages can be assessed by exfoliative cytology such as micronuclei test. Micronuclei are chromatin containing bodies that represent fragments or even whole chromosomes that were not incorporated into a daughter cell nucleus at mitosis [10], have been used as biomarkers for the assessment of DNA damages. The study aims to show that this micronucleus assay can be done as a screening test in a large population to find out the development of cancer of the oral cavity at an early stage. Casartelli et al. [11] concluded that the gradual increase in MN counts from normal mucosal to precancerous lesions to carcinoma suggested a link of this biomarker with neoplastic progression.

2. Materials & Methods

2.1. Screening of Subjects

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

I. 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.

II. North East camp: - 56 subjects were screened at a camp held in Karimganj, Assam. Out of whom 33 were betel quid chewers.

III. RKMS Hospital: - 2885 cases attending in one year at E. N. T OPD and Oral Maxillofacial OPD of RKMS Hospital had other complications like auditory, nasal, throat & facial problem. 35 Patients 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.

Detailed history were taken from all cases by filling up questionnaire.

2.2. Micronuclei Study

The subjects were asked to rinse their mouths with water and a premoistened wooden spatula was used to sample cells from the oral mucosa. The spatula was applied to a precleaned microscope slide. Smears were air dried and fixed in 80% methanol. Slides were stained by the Giemsa solution and the MN frequency was scored using the criteria described by Sarto et al. (1990) and Tolbert et al. (1992). The same person scored 1000 cells blindly in each case to determine the MN percentage.

2.3. Follow up Study

Black tea was supplemented to oral cancer cases and betel quid chewers.

250 gm of black tea was given to each subject. The subjects were advised to drink 3 cup of tea brewed with approximately 2.5 gm in 100 ml of water. Subjects were asked not to add milk in it or not to boil it for long time.

It was advised to keep tea liquor inside the oral cavity for 1–2 mins and then drink it.

The polyphenol content of the supplied tea was 28 ± 1.86 / 100 gm of dry tea.

Tea was supplemented to total 190 cases who had betel quid chewing habit.

Follow up of the cases was done after 6 months.

3. Results

Table 1. Detailed history of subjects of different areas.

PLACE	NO	AGE GROUP (in years)						Addiction			No BQ Addiction	Tea Drinker	Non Tea Drinker
		Below 30	31-40	41-50	51-60	61-70	Above 70	Smoking	Alcohol	Betel Quid			
NORTH EAST CAMP	56	1	2	12	24	11	6	9	6	33	23	40	16
1. Assam, Karimganj	34	5	20	8	1	0	0	16	14	19	15	34	0
EASTERN INDIA CAMP	46	22	13	3	6	2	0	28	29	36	10	40	6
1) Bankura, Dhulai	89	28	18	21	15	6	1	27	3	56	33	73	16
2) East Midnapur, Bibhisampur	51	8	13	12	8	6	4	14	5	22	29	49	2
3) North 24 Pgs, Atghara	35	2	7	8	11	7	0	20	8	24	11	29	6
4) Narrah, Bankura	311	66	73	64	65	32	11	114	65	190	121	265	46
RKMS													
TOTAL													

Note: Some cases had more than one addiction

Table 2. Mean Percentage of Micronuclei before and after supplementation of black tea.

Before supplementation of Black Tea (Mean \pm SE)	After supplementation of Black Tea (Mean \pm SE)
13.86 \pm 2.70	3.05 \pm 0.59

Note: Mean percentage of micronuclei is higher in cases and after supplementation of black tea micronuclei percentage are lower than before.

4. Discussion

Micronuclei are the small extra nuclei which are formed in metaphase and anaphase stage. The presence of micronuclei reflects a genotoxic and carcinogenic exposure. In every camps, percentage of micronuclei present in betel quid chewers are higher than the normal. Tea, for the presence of polyphenols has the ability to reduce the micronuclei. In our studied population percentage of micronuclei becomes lower after supplementation of tea. So we conclude that black tea is actually effective for oral cancer patients as it has the ability to decrease the micronuclei (which is a potent marker of oral cancer).

5. Conclusion

Betel quid has an immense role in changing the oral pathology and developing oral cancer. In this present study it has been found that the micronuclei percentage can be used as a oral cancer biomarker, which becomes lower after supplementation of tea than previous one.

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