Frequencies of nuclear anomalies in buccal mucosal cells of vegetarian and non-vegetarian subjects

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ABSTRACT *The Buccal Micronucleus Cytome (BMCyt) in exfoliated buccal epithelial cells is a minimally invasive method for assessing chromosomal instability, DNA damage and cell death. It has been widely used in occupational and lifestyle related studies. For the present study 63 male subjects were taken out of which 32 were vegetarian and 31 subjects were non-vegetarian matched with respect to their age, sex and dietary habits. The result of micronucleus assay depicted significantly ($p<0.05$) increase in the mean frequencies of BN and KL in non-vegetarian subjects as compared to vegetarians.*

Keywords: *Miconucleus, MN, BN, KL.*

Introduction:

The Buccal Micronucleus Cytome (BMCyt) assay also called as micronucleus (MN) assay in exfoliated buccal epithelial cells is a minimally invasive method for assessing chromosomal instability, DNA damage and cell death (Thomas *et al*., 2009). This method is increasingly being used in molecular epidemiologic studies investigating the impact of nutrition, life-style factors, genotoxin exposure (Thomas *et al*. 2009). Holland *et al*. (2008) proposed that the micronucleus (MN) assay in exfoliated buccal cells is a useful and minimally invasive method for monitoring genetic damage in humans. Micronuclei appear in cytoplasm of daughter cells as small additional nuclei and contain whole chromosomes or acentromeric chromosome fragments (Fenech *and Bonassi*, 2011). It has been widely used in occupational and lifestyle related studies (Rosin, 1992; Nersesian, 1996). MN assay has also been successfully applied to identify dietary factors that have a significant effect on genome stability (Kimura *et al*., 2004). It has been suggested that an increased frequency of chromosome breaks was an initial event in assessing oncogene risk, thus these alterations may play a crucial role in carcinogenesis (Tucker and Preston, 1996; Bonassi *et al*., 2000). Higher frequency of micronuclei reflects chromosomal damage and may thus provide a marker for cancer risk (Hagmar *et al*., 1998; Bonassi *et al*., 2000). The level of genotoxic damage in vegetarians and non-vegetarians as determined by the frequency of micronuclei has previously been studied by various researchers (Verhagen *et al*., 1996; Fenech and Renaldi 1995; Tucket *et al*., 1993; Kazimirova *et al*., 2006; Abramsson-Zetterberg *et al*., 2008). The present study was carried out to evaluate the mean frequeny of micronucleus (MN) and other nuclear anomalies viz. binucleate cell (BN), broken egg (BE), karyolysis (KL) and karyorrhexis (KH) in vegetarian and non-vegetarian subjects.

Materials and methods:

For the present study 63 male subjects were taken out of which 32 were vegetarian and 31 subjects were non-vegetarian matched with respect to their age, sex and dietary habits. Subjects were selected from different areas of Haryana. The mean age of vegetarian was of 53.875±2.878 years, very close to that of non-vegetarian subjects which was 50.968±2.717 years. Prior to sampling an informed consent was taken from each individual. Ethical clearance was obtained from Institutional Ethics Committee, Kurukushetra University, Kurukshetra.

The standard technique of Tolbert *et al.* (1992) was followed for micronucleus assay. Buccal epithelial cells were obtained gently from the inner cheek of the subjects with the help of moistened steel spatula. Then the cells were smeared on to pre-cleaned microscopic glass slides.

Slides were stained with 2% Aceto-orecin (HIMEDIA, acetic acid RM5564, orcein RM277) for 20 minutes at 40°C and then washed in ethanol and distilled water, respectively for two to three times. After that slides were counter-stained with 0.1% Fast green solution (HIMEDIA RM 4266) for 12 minutes and rinsed in ethanol and distilled water respectively. Then the slides were air dried.

For each subject 1000 cells were scored for micronucleus and other nuclear anomalies under Olympus CX-41 trinocular microscope at 1000 X magnification. The criterion of Tolbert *et al.* (1992) [9] was followed for scanning buccal epithelial cells for micronuclei and other nuclear anomalies. In order to be considered as micronucleus the suspected nucleus is required to meet the following criteria : (a) smooth, rounded, perimeter suggestive of membrane; (b) less than third the diameter of the main nucleus (c) staining intensity similar to nucleus; (d) same focal plane as that of main nucleus. In addition to MN other nuclear anomalies were also reported like binuleate (BN); the presence of two similar nuclei with in a cell, broken egg (BE); nuclei that appear to be broken but still connected to the main nuclei with a thin nucleoplasmic bridge, karyorrhexis (KH); nuclear disintegration involving loss of integrity of the nucleus and karyolysis (KL); complete nuclear dissolution, ghost-like image of the nucleus remains.

Statistical analysis was done using ANOVA test.

Results:

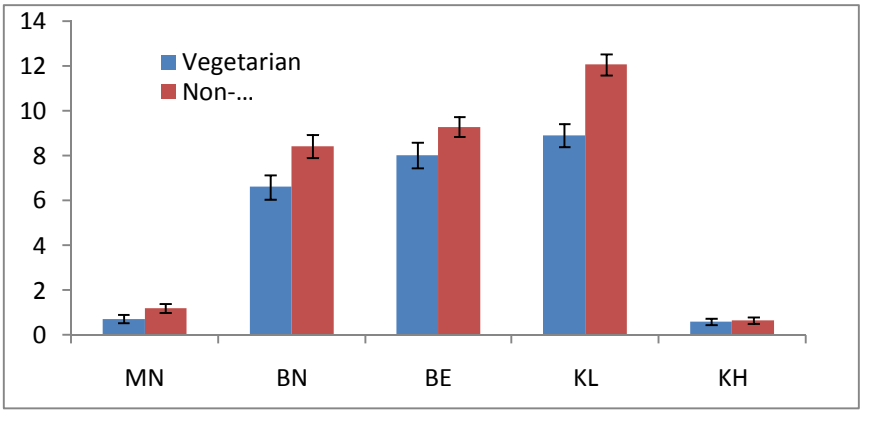
Micronucleus and other anomalies were assessed with the help of micronuclei assay in buccal mucosal epithelial cells. The result of micronucleus assay depicted significantly ($p<0.05$) increase in the mean frequencies of BN and KL in non-vegetarian subjects as compared to vegetarians (Fig.1).The mean value of MN and BE were also slightly higher than vegetarian subjects but the difference was not significant ($p>0.05$). The mean frequencies of micronuclei and other nuclear anomalies such as binucleate (BN), broken egg (BE), karyolysis (KL) and karyorrhexis (KH) in vegetarian and non-vegetarian subjects are shown in Table 1.

Table 1: Mean frequency of nuclear anomalies in vegetarian and non-vegetarian subjects.

Sr. No.	Nuclear Anomalies	Subjects	Mean±S.E.
1	MN	Vegetarian	0.719±0.186
		Non-vegetarian	1.193±0.209
2	BN	Vegetarian	6.594±0.560
		Non-vegetarian	8.419±0.514
3	BE	Vegetarian	8.031±0.579*
		Non-vegetarian	9.290±0.440*
4	KL	Vegetarian	8.906±0.516
		Non-vegetarian	12.064±0.466*
5	KH	Vegetarian	0.594±0.141*
		Non-vegetarian	0.645±0.135

*Significant ($P<0.05$; 2-tailed) (Independent sample t test)

Fig.1 Nuclear anomalies in vegetarian and non-vegetarian subjects.



Discussion:

Food constituents and nutrients can prevent or contribute to the genotoxicity (Fenech and Renaldi, 1995). Several micronutrients have been identified as being cruceal in reducing or protecting against the DNA damage. Micronutrients act as co-factors for enzymes that required in DNA repair or maintenance (Thomas *et al*., 2011). Kotova *et al.* (2014) proposed that non-vegetarian have higher frequency of micronuclei as compared to vegetarian which indicates higher level of DNA damage in non-vegetarians. While Fenech (1995) not supported that non-vegetarian have higher genetic damage as compared to vegetarian. In the present investigation we have observed higher frequencies of nuclear anomalies in non-vegetarian subjects as compared to vegetarian which indicated higher level of DNA damage in non-vegetarian subjects as compared to vegetarians. Kotova *et al*. (2014) proposed that the vegetarian diet might be beneficial in lowering genomic instability in healthy individuals. From the finding of the present study it can be suggested that non-vegetarian subjects have higher level of DNA damage as compared to vegetarian subjects.

Conflict Of Interest*:* There are no conflicts of interest.

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