Original Article

Indian Journal of Genetics and Molecular Research
Volume 3 Number 1, January – June 2014

Age-related Alteration in the Frequency of Nuclear Anomalies in Exfoliated Buccal Mucosa of Healthy Population of Haryana

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Abstract

The basic mechanism of ageing refers to the instability in the organization and expression of the genetic material, which induces DNA strand breaks, chromosomal loss and nuclear anomalies. Subsequent loss of DNA repair mechanism with age also causes irreversible damage to the genetic material. The present study is an attempt to understand the effect of ageing on the incidences of micronuclei in the exfoliated buccal epithelial cells of healthy individuals of Haryana using micronucleus assay. The study was conducted on 150 healthy individuals of four age groups with mean ages of 18.45±0.31, 28.25±0.87, 46.42±1.00 and 66.37±0.79 years respectively. The mean frequencies of MN, BN, BE, KL and KH were found to be significantly different in all the age groups ($p<0.05$). There was a significantly positive correlation observed between age and nuclear anomalies ($p<0.01$) demonstrating increase in the chromosomal damage with increasing age.

Keywords: Ageing; DNA damage; Chromosome; Micronucleus test.

Introduction

Ageing in humans refers to a multidimensional process of physiological and social changes. The process comprises structural alterations as well as functional decline in the body systems with a consequential mutilation of homeostasis and increased susceptibility to age-related diseases, eventually leading to death.[1] The mechanism of ageing is not clearly understood but effect of ageing seems to be a genetically programmed process[2] combined with genetic alterations induced by some endogenous or exogenous factors.[3] With age there is increased production of highly reactive free radicals which induce structural alterations in the body tissues, proteins as well as DNA. Further the telomere shortening and reduced DNA repair capability in aged individuals causing the increase in irreversible DNA damage and accumulation of mutations with time[4-5] further causing DNA strand breaks, formation of adducts[6], chromosomal breakage and various chromosomal anomalies[7-8] which increase the risk of cancer and other age-associated degenerative diseases.[9]

Many earlier studies have shown that cytogenetic damage accumulates with age due to repetitive mutations and subsequent loss of DNA repair mechanism.[10-12] The age dependent increase in chromosomal damage has been detected for sister-chromatid exchange, chromosomal aberrations and increased micronuclei frequency.[13] The aberration in chromosome number and structure were the first anomalies observed to be associated with age.[7-8] It has been

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reported earlier that aging and cancer both comprise the chromosome loss and mal-segregation of chromosomes which are probably due to defects in spindle formation, centromere or as a consequence of under-condensation of chromosomes before metaphase.[14-16] The micronucleus assay with exfoliated buccal epithelial cells is a less invasive, cost effective technique and an important biomarker tool to assess DNA damage in large populations in which formation of anomalous nuclei or cell is the end point to spot damage.[6,17]

The formation of micronuclei from either acentric chromosome fragment or a whole lagging chromosome occurs as a result of chromosome breakage due to unrepaired or mis-repaired DNA strand breaks or mal-segregation of the chromosomes due to mitotic malfunction.[18] Besides micronuclei other nuclear anomalies are also observed like binucleate cell (BN), broken egg (BE), karyolysis (KL) and karyorrhexis (KH). The main objective of the present study was to analyze incidences of nuclear anomalies in the exfoliated buccal epithelial cells of people with different age groups.

Materials and methods

150 healthy subjects of different age groups, belonging to both genders, matched with respect to their lifestyle, were selected randomly for the present study from different areas of Haryana. These were categorized into four age groups: group 1 (comprising <20 years old individuals), group 2 (21-40 years old individuals), group 3 (41-60 years old individuals) and group 4 (61-80 years old individuals). A questionnaire was filled up to collect the details about their age, sex, dietary habits, smoking and drinking habits and medical history. Only those subjects were selected who had no smoking or drinking habits and were medically fit and healthy. Before 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*[19] was followed for micronucleus assay with slight variations. Exfoliated buccal epithelial cells were scrapped gently from the inner cheek of the subjects with a moistened wooden spatula. Then the cells were smeared on to the pre-cleaned microscopic glass slides. Two slides were prepared for each individual. The samples were stained with 2% aceto-orcein stain (HIMEDIA, acetic acid RM5564, orcein RM277). Counterstaining was done with 0.1% fast green solution (HIMEDIA RM 4266). Slides were observed under Olympus CX-41 trinocular microscope at 1000 X magnification. At least 1000 cells were scanned for each subject, and the cells with cytogenetic anomalies were scored. The criterion of Tolbert *et al*[19] was followed for scanning cells for micronuclei and other nuclear anomalies. The suspected nucleus is required to meet the following criteria in order to be considered as micronucleus : (a) rounded, smooth perimeter suggestive of membrane; (b) less than third the diameter of the main nucleus, but large enough to discriminate shape and color; (c) staining intensity similar to that of nucleus; (d) same focal plane as nucleus. In addition to MN other nuclear anomalies were also studied like BN; the presence of two similar nuclei with in a cell, BE; nuclei that appear to be broken but still connected to main nuclei with a thin nucleoplasmic bridge, KH; nuclear disintegration involving loss of integrity of the nucleus and KL; complete nuclear dissolution, in which aceto-orcein negative, ghost-like image of the nucleus remains. In addition, some other phenomenon occurring in the cells such as pycnosis and condensed chromatin, were also observed during scoring of the anomalies. However, these were not counted in the anomalies. Pycnosis and condensed chromatin are considered as a part of normal epithelial cell differentiation and maturation.

Statistical analysis was done using ANOVA followed by Duncan's multiple range tests.

Correlation between various parameters was studied using Pearson correlation analysis with the help of SPSS v16.

Results

Out of 150 subjects (mean age 43.01 ± 1.44 years) 87 were males and 63 females. All of them were non-smoker and non-alcoholic. The mean ages of all the four groups were 18.45±0.31, 28.25±0.87, 46.42±1.00 and 66.37±0.79 years, respectively (Table 1). The mean frequencies of micronuclei (MN), binucleate (BN), broken egg (BE), karyolysis (KL) and karyorrhexis (KH) are illustrated in table 1. The comparison of mean frequencies of nuclear anomalies in different age groups is shown in figure 1. All the nuclear anomalies were found to be significantly different at 0.01 levels (ANOVA) in different age groups. When further comparison was made between the groups using Duncan's multiple range test, MN frequency was observed to be significantly increasing ($p<0.05$) in the age groups 2, 3 and 4 with age ranges of 21-40, 41-60 and 61-80, respectively. No significant difference was observed between group 1 and group 2. When comparison was made between all the age groups for BN and BE frequency, significant difference ($p<0.05$) was observed at the cut point of 40 i.e. at 40 years of age. Though no significant difference was observed for karyolysis and karyorrhexis frequency between adjacent age groups but both KL and KH frequencies of age group 1 was found to

Table 1: Indices of nuclear anomalies in different age groups

Age gp.	N	Age range	Age	MN	BN	BE	KL	KH
1	22	=20	18.45±0.31	0.23 ± 0.11^{C}	4.04 ± 0.82^{B}	1.00 ± 0.35^{B}	4.77 ± 0.81^{C}	0.86 ± 0.28^{B}
2	35	21-40	28.25±0.87	0.77 ± 0.15^{C}	4.48 ± 0.84^{B}	1.62 ± 0.37^{B}	6.97 ± 0.86^{BC}	0.65 ± 0.22^{B}
3	56	41-60	46.42±1.00	3.10 ± 0.33^{B}	9.30 ± 0.95^{A}	5.00 ± 0.53^{A}	9.28 ± 1.08^{AB}	1.55 ± 0.22^{B}
4	37	60-80	66.37±0.79	4.86 ± 0.49^{A}	10.05 ± 1.39^{A}	5.08 ± 0.62^{A}	12.51 ± 1.34^{A}	2.70 ± 0.48^{A}

Values for age, MN, BN, BE, KL and KH are expressed in Mean± S.E.
Values are significant at $p<0.05$ for ANOVA further modified for Duncan's multiple range test.
Values with different alphabets are significantly different from each other at $p<0.05$ along a column.
MN- micronucleus, BN- binucleate, BE- broken egg, KL- karyolysis, KH- karyorrhexis

Figure 1: Comparison of frequency of nuclear anomalies in different age groups