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RESEARCH ARTICLE

EVALUATION OF MICRONUCLEI COUNT IN EXFOLIATED BUCCAL MUCOSAL CELLS AMONGST DIFFERENT AGE GROUPS OF NORMAL HEALTHY INDIVIDUALS: A QUANTITATIVE STUDY

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ABSTRACT

Background: Micronuclei (MN) are a small additional nucleus and are readily identifiable by light microscopy. Biologically, MN is the chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division. MN occurs due to genetic damage of the cell and the MN scoring is the indicator of the genetic damage. But, it has been shown by various studies that MN formation is not always related with genetic damages and may be developed from the physiological damage of double stranded DNA break when a cell enters from G0 to G1 phase of the cell cycle. Therefore simple presence of MN may not indicate any disease as this may be seen even in normal healthy cell. Only high MN count may be suggestive of a genetic damage. Thus MN1 counting in normal healthy individuals can be used to supervise genotoxicity, biomonitoring of diseases, screening of preneoplastic diseases and identification of high risk patients.

Objectives:

- To compare total number of micronuclei and number of cells with micronuclei in exfoliated buccal mucosal cells amongst different age groups of normal healthy individuals.
- To determine a normal range of total number of micronuclei and number of cells with micronuclei in exfoliated buccal mucosal cells amongst different age groups of normal healthy individuals in the studied population.

Methods: This study was conducted on normal healthy individuals (n=500) age ranged from 18-70 years. Based on age normal healthy individuals were categorized into 5 age groups: Group A: 18-30 years; Group B: 31-40 years; Group C: 41-50 years; Group D: 51-60 years and Group E: 61-70 years. Each age group comprised n=100 normal healthy individual. The exfoliated cytosmears prepared from oral buccal mucosa of normal healthy individuals and stained with Papanicolaou (PAP) technique. We calculated the total number of MN (TMN) and number of cells with MN (CMN) per normal healthy individual since some cells had multiple MN.

Results: The mean of TMN found were increased with increase in age and this difference was statistically significant. (p= 0.007) The mean of CMN also found were increased with increase in age. However, statistical test did not show any significant difference amongst them. (p= 0.071) In the normal healthy individuals, the normal range for TMN and CMN was 1 to 12 and 1 to 10 respectively.

Conclusions: There is an increase in total number of MN (TMN) and number of cells with MN (CMN) with increasing age.

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INTRODUCTION

Micronuclei (MN) have been defined as a microscopically visible oval or round cytoplasmic chromatin mass next to the nucleus. It consists of acentric chromosomes, chromatid fragments or whole chromosomes that have failed to be incorporated in the daughter nuclei during mitosis (Fenech, 2007). MN are formed by the substances that cause breakage of chromosomes (clastogens) as well as by agents which affect the spindle apparatus (aneugens) (Falck, 2002 and Ford, 1988). Micronuclei count or assay can be done on human erythrocytes, lymphocytes and exfoliated epithelial cells (eg. Oral, urothelial, nasal, vaginal or cervical) to obtain a measure of genome damage induced in vivo (Samanta, 2012). On exfoliated cytosmear MN can visualize using DNA specific stain such as Feulgen or ordinary routine stains such as May Grunwald Giemsa or Papanicolaou's stain and their frequency can quantified microscopically (Samanta, 2012 and Arora, 2010) MN occurs due to genetic damage of the cell and the MN counting or scoring is the indicator of the genetic damage (Jadhav, 2011).

Therefore MN scoring can be used in various clinical setting such as to supervise genotoxicity, biomonitoring of diseases, screening of preneoplastic diseases and identification of high risk patients (Samanta, 2012). According to various studies MN formation is not always related with genetic damages (Pickering, 2006 and Bartkova, 2005). DNA double strands break is a physiological phenomenon when a cell commits from G0 to G1 of the cell cycle and may occur in normal healthy cells. So MN may also be noted in normal healthy individual but the numbers of micronuclei and micronucleated cells may be less (Pickering, 2006 and Bartkova, 2005). Hence the present study was carried out to evaluate and correlate the micronuclei count in exfoliated buccal mucosal cells amongst different age groups of normal healthy individuals and also to determine a normal range of micronuclei count in exfoliated buccal mucosal cells of normal healthy individuals.

MATERIALS AND METHODS

Patient selection

This study was conducted in the Department of Oral Pathology and Microbiology, Mahatma Gandhi Mission's Dental College and Hospital, Navi Mumbai. The research procedures utilized in this study were approved by the university ethical committee. All healthy individuals where verbally and written informed about the risks and the main objectives of this research and they signed an informed consent form. The study was carried out on normal healthy individuals (n=500) and were excluded of the any individual who was taking any medicine, smokers, person who are using any mouthwash, or have received any recent dental treatment or have taken facial or oral radiographs. Individual who declared regular weekly alcohol beverage consumption, or those who worked with known carcinogens like as gas station attendants, painters, or activities which regularly expose the workers to pesticides or wood dust were also excluded from this research. The study included normal healthy individuals (n=500) age ranged from 18-70 years old and were categorized into 5 different age groups: Group A- 18-30 yrs (n=100), Group B- 31-40 (n=100), Group C- 41-50 (n=100), Group D- 51-60 (n=100) and Group E- 61-70 (n=100).

Collection of exfoliated cells: The healthy individual was examined under dental potent light to assure the healthy conditions of the oral tissues. Subjects were asked to rinse their mouth gently with tap water. Exfoliated cells of the buccal mucosa were obtained by scraping the buccal mucosa with a slightly moistened wooden spatula. For each individual, two slides were prepared by smearing the cells immediately onto the center of precleaned glass slides. Just prior to drying, the smears were fixed with commercially available spray fixative (available with the RAPIDPAPTM kit). The slides were coded and preserved in dust-free boxes.

Cytological preparation and evaluation: The smears were stained by Papanicolaou (PAP) technique using a commercially available staining kit RAPIDPAP. From two slides of each patient, 1000 cells were examined under the light microscope using 400x magnification for screening and were MN cells were located, they were examined under 1000x magnification for counting of MN. Total number of micronuclei (TMN) and number of cells with micronuclei (CMN) were counted. TMN were defined as the total number of MN per 1000 cells per individual. CMN was defined as the number of cells containing MN per 1000 cells per individual (some cells may have multiple MN).

Scoring criteria: The criteria which were developed by Fenech[1] were used for counting the MN. Screening of each slide was made in a zigzag manner from one end, toward the other end of the slide.

Fenech criteria parameters for identifying MN are as follows:

- Being less than 1/3 and more than 1/16 diameter than the main nucleus,
- Being on the same focus plane,
- Not superimposing to the main nucleus,
- Having the same colour, texture and refraction as the main nucleus,
- Having a smooth round or oval shape,
- Having limits clearly distinguishable from the main nuclear membrane if touching the main nucleus.

Only those structures fulfilling the above-mentioned criteria were recorded as MN.

Inclusion and exclusion criteria

Nuclear blebbing (MN-like structure connected with the main nucleus with a bridge) were not considered. Clumps of cells with obscured nuclear or cytoplasmic boundaries and overlapping of cells were avoided and separated or cells lying singly were preferred for counting of MN. Dead or degenerated cells (karyolysis, karyorrhexis, nuclear fragmentation), apoptotic cells and cytoplasmic fragments were excluded from evaluation.

Data entry and statistical analysis

The data obtained was entered in Microsoft excel worksheet and presented using descriptive statistics such as mean value and standard deviation. Also data was presented using appropriate graphs and tables. All collected data was entered into SPSS 16.0 (statistical package for social sciences version 16.0) worksheet. Further analysis was performed using

statistical test such as ANOVA test. A significance level of 0.05 was applied to decide the statistical significance of the hypothesis being tested.

RESULTS

The MN cells observed are shown in figure 1. The mean of total number micronuclei (TMN) and number cells with micronuclei (CMN) are shown in Table 1. The mean of TMN in age groups A, B, C, D and E were 5.33, 5.39, 5.49, 5.82 and 5.94 respectively. The mean of TMN found were increased with increase in age. (Graph 1) In comparison, the mean difference among the number of TMN in different age groups is statistically significant. (p= 0.007) ((Table 2). The mean of CMN in age groups A, B, C, D and E were 4.54, 4.57, 4.57, 4.91 and 4.95 respectively. The mean of CMN found were increased with increase in age. (Graph 1) But in comparison, the mean difference among the number of CMN in different age groups is statistically insignificant. (p=0.071) (Table 2). Normal range of TMN and CMN in exfoliated buccal mucosal cells amongst different age groups of normal healthy individuals in the studied population is shown in Table 3.

Table 1. Evaluation of mean of TMN and CMN in different age groups of normal healthy indivuals

	TMN		CMN	
	Mean	Standard Deviation	Mean	Standard Deviation
Group A (18-30 yrs)	5.33	1.30310	4.54	.09992
Group B (31-40 yrs)	5.39	1.27837	4.57	.14017
Group C (41-50 yrs)	5.49	1.67269	4.57	.15971
Group D (51-60 yrs)	5.82	1.20922	4.91	.12235
Group E (61-70 yrs)	5.94	1.64421	4.95	.15851

Table 2. Statistical correlation of the mean of TMN and CMN amongst different age groups of normal healthy individuals

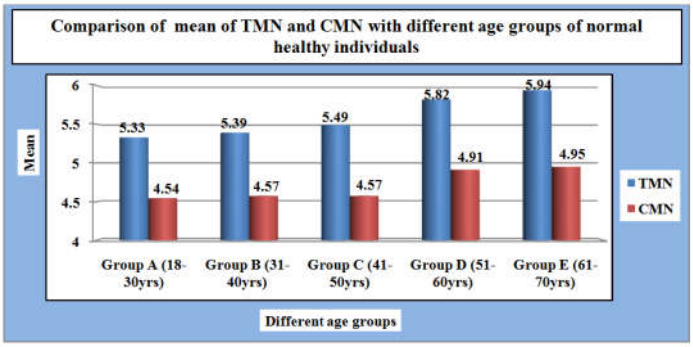
ANOVA results

		Sum of Squares	df	Mean Square	F	p-value
TMN	Between Groups	29.292	4	7.323	3.556	.007*
	Within Groups	1019.290	495	2.059		
	Total	1048.582	499			
CMN	Between Groups	16.568	4	4.142	2.175	.071
	Within Groups	942.800	495	1.905		
	Total	959.368	499			

*. The p value is significant at the 0.05 level

Table 3. Normal range of TMN and CMN amongst different age groups of normal healthy individuals

	Different age group of normal healthy individuals	Normal Range	
		Minimum	Maximum
TMN	18-30	1	8
	31-40	1	8
	41-50	1	9
	51-60	2	10
	61-70	4	12
	Total	1	12
CMN	18-30	1	8
	31-40	1	6
	41-50	1	8
	51-60	1	7
	61-70	2	10
	Total	1	10



Graph 1. Compares the mean of TMN and CMN with different age groups of normal healthy individuals. An increase in mean of TMN and CMN is noted from Group A to Group E of normal healthy individuals

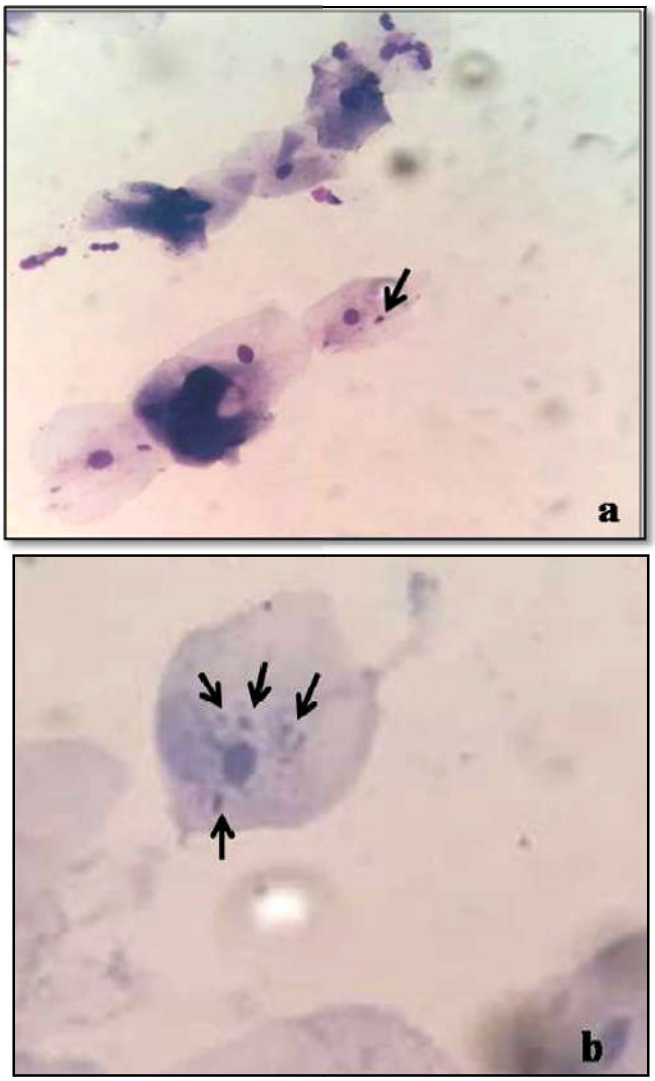


Figure 1. Photomicrograph of exfoliated buccal mucosal cell showing single (a) and multiple (b) MN (arrow) [PAP Stain].

DISCUSSION

The MN are round to oval extranuclear cytoplasmic bodies associated with chromosomal aberrations. Cells often have errors in chromosomal segregation that lead to the formation of a lagging chromosome or chromosomal parts that become lost during the anaphase stage of cell separation and are excluded from the reforming nuclei. The laggards are observed in the cytoplasm as MN. Chromatin texture and staining intensity of MN are similar to the main nucleus (Samanta, 2012 and

Fenech, 2007). The diameter of MN is less than 1/3rd of that of the main nucleus and so it can be readily differentiated from a binucleated cell. It is also different from a broken-egg cell as it does not have any connection with the main nucleus (Samanta, 2012). MN should always be distinguished from the stain deposits, bacteria, nuclear dusts, clumped cytoplasmic fragments, partial Karyorrhexis or necrotic nucleus, superimposed nuclear fragments from other cells and keratohyaline granules (Samanta, 2012). During last few decades, MN count has generally been used as a biomarker of chromosomal damage, genome instability and cancer risk (Jadhav, 2011). According to various studies MN formation is not always related with genetic damages (Pickering, 2006 and Bartkova, 2005). It may be developed from the physiological damage of double stranded DNA break when a cell enters from G0 to G1 phase of the cell cycle. Thus simple presence of MN may not indicate any disease or genetic damage as this may be seen even in normal healthy cell. Only high MN score may be suggestive of a genetic damage (Samanta, 2012). Hence the present study was carried out to evaluate and correlate the micronuclei count in exfoliated buccal mucosal cells amongst different age groups of normal healthy individuals. The present study was conducted on normal healthy individuals (n=500) age ranged from 18-70 years old and were categorized into 5 different age groups: Group A- 18-30 years (n=100), Group B- 31-40 years (n=100), Group C- 41-50 years (n=100), Group D- 51-60 years (n=100) and Group E- 61-70 years (n=100). The exfoliated cytosmears prepared from oral buccal mucosa of normal healthy individuals and stained with Papanicolaou (PAP) technique. From two slides of each patient, 1000 cells were examined under the light microscope using 400x magnification for screening and were MN cells were located, they were examined under 1000x magnification for counting of MN. We calculated the total number of MN (TMN) and number of cells with MN (CMN) per normal healthy individual since some cells had multiple MN. The criteria which were developed by Fenech (Fenech, 2007) were used for counting the MN. In our study, on statistically evaluating and correlating the mean of TMN and CMN with different age group of normal healthy individuals, we found that the mean of TMN in group A, B, C, D and E were 5.33, 5.39, 5.49, 5.82 and 5.94 respectively while the mean of CMN in group A, B, C, D and E were 4.54, 4.57, 4.57, 4.91 and 4.95 respectively. Mean values of both TMN and CMN was increasing with increase in age from Group A to Group E. Statistically the mean difference of the number of TMN in different age groups is significant. (p=0.007) While the mean difference of the number of CMN in different age groups is insignificant. (p=0.071). The normal range for TMN and CMN in the normal healthy individuals was 1 to 12 and 1 to 10 respectively.

Our result was in accordance with various studies, who found that the prevalence and frequency of spontaneous occurrence of MN in human lymphocytes increases with age (Pickering, 2006; Bartkova, 2005 and Fenech, 2007). Bolognesi C et al (Bolognesi, 1999) also observed the increase of MN frequency with age. Orta T et al found that the spontaneous MN frequency in peripheral blood lymphocytes increased first then started to decrease after 50 years of age. Konopacka A et al (2003) showed no statistical correlation between age and the spontaneous frequency of MN in buccal epithelial cells of helthy individuals. Many studies showed that MN frequency did not vary with age of healthy person (Casartelli, 2000; Karahalil, 1999; Lucero, 2000). Bukvic N et al indicated that the age related increase in sex chromosome loss correlates with the increased level of MN formation. Ageing is associated with changes in chromosomal structure and function (Bolognesi, 1999). The increase in spontaneous chromosomal instability with age, as reflected in the higher basal level of MN frequency, is associated with an accumulation of DNA damage due to a progressive impairment of overall DNA repair capacity (Casartelli, 2000). The present study is the first study to determine the normal range and to correlate both TMN and CMN in exfoliated buccal mucosal cells amongst different age groups of normal healthy individuals. In our study on normal healthy individuals, we found that the mean numbers of TMN and CMN was increased with increase in age which indicated the role of age on baseline frequency of micronuclei. However, more research work on a much larger sample size would further authenticate our observation.

Conclusion

In conclusion, MN count in normal healthy individuals is a potential biomarker to screen genotoxicity, biomonitoring of various diseases, detection of malignancies and preneoplastic conditions. Also, confirms the role of age on baseline frequency of micronuclei. This parameter has to be considered in the study design and analysis of biomonitoring studies aimed at evaluating exposure to genotoxic agents.

Conflict of Interest: There are no conflicts of interest.

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