Effects of age and gender on peripheral lymphocyte micronucleus

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Introduction

Aging in humans appears to be associated with genetic instability. The cytokinesis-blocked micronucleus assay (CBMN) is a comprehensive method for measuring chromosome breakage, DNA misrepair, chromosome loss, non-disjunction, necrosis, apoptosis and cytostasis. Age and gender are the most important demographic variables affecting the micronucleus (MN) index and studies report frequencies in females being greater than those in males by a factor of 1.2 to 1.6 depending on the age group. It has been shown that a higher MN frequency directly corresponds to a decreased efficiency of DNA repair and increased genome instability.

Aim of the Study

Investigate an association between age and gender and the synergistic effect of both upon MN in peripheral lymphocytes in a sample of individual without any occupational exposure.

Methodology

The study was carried out in Portugal in a sample of 85 subjects without any occupational exposition. The evaluation of genotoxic effects was conducted by applying CBMN in peripheral blood lymphocytes. Heparinized whole-blood samples were obtained, with informed consent, from unrelated individuals, men and women, stratified according to their age: 20-30, 31-40 and 41-55 years old. Lymphocytes were isolated using Ficoll-Paque gradient and placed in RPMI 1640 culture medium with L-glutamine and red phenol added with 10% inactivated fetal calf serum, 50 ug/ml streptomycin + 50U/mL penicillin, and 10 ug/mL phytohaemagglutinin. Duplicate cultures from each subject were incubated at 37ºC in a humidified 5% CO2 incubator for 44h, and cytochalasin-b 6 ug/mL was added to the cultures in order to prevent cytokinesis. After a 28h incubation, cells were spun onto microscope slides using a cytocentrifuge. Smears were air-dried and double stained with May-Grünwald-Giemsa and mounted with Entellan. The frequencies of binucleated cells with MN were determined analyzing 1000 lymphocytes from 2 slides for each subject.

Results

The sample was constituted by 54 women and 31 men, with age mean of 32.42 ± 8.1 years old. Concerning to the analysis of gender, females have higher mean of MN in lymphocytes (0.81 0.229) than males (0.71 0.255) – Table 1.

Table 1 - Descriptive statistics by gender of MN in lymphocytes means (mean mean standard error, range)

Gender	N	Mean MN lymphocytes ± S.E. (range)
Females	54	0.81±0.229 (0-7)
Males	31	0.71±0.255 (0-6)

p>0.05 Mann-Whitney test

The analysis of age showed that there was no consistent trend regarding the variation of MN with age - Table 2.

Table 2 - Descriptive statistics by age categories of MN in lymphocytes means (mean mean standard error, range)

Age	N	Mean MN lymphocytes ± S.E. (range)
20-30	36	0.47±0.157 (0-3)
31-40	35	1.14±0.326 (0-7)
>41	14	0.86±0.501 (0-6)

The analysis of an association between gender, age as single variables and frequencies of MN by binary logistic regression was not statistical significant (p>0.05). The interaction between age and gender in determining the frequencies of MN in lymphocytes was investigated and found to be not significant (Kruskal-Wallis, p>0.05).

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Conclusions

The mean of MN was slightly higher in women than in men but not statistically significant. In general, and in conformization with other studies, women appear to reach a threshold of genome instability faster then men. Results about age showed that the age category that show highest mean of MN was 31-40. That result can be explained by the size of the sample of the last category that is approximately half of the others.