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NUCLEAR DEGENERATION AS A BY-PRODUCT OF AGING AND ENVIRONMENT

Dental Science

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ABSTRACT

Background: The process of aging in the immediate toxic environment occurring in this Industrialized world is detrimental for human body. This is accelerating aging process and at the cellular level, the toxins present in environment are inducing chromosomal(micronuclei) and nuclear degenerative changes (karyorrhexis, karyolysis, pyknosis, condensed chromatin). The given study evaluates the effects of aging and environment on the chromosomal/nuclear degenerative changes, thus affecting the exfoliated cells collected from buccal mucosa.

Methods: The sample included 86 healthy subjects divided into two groups according to age: 46 women aged above 60 years and 40 women of 20-25 years of age. A questionnaire was prepared to retrieve all the data related to health and drug related history. Buccal smears were prepared and stained with both Papanicolaou and H&E stain. Hundred cells were counted from each slide to determine the number of micronuclei and other nuclear degenerative changes.

Results: The number of micronuclei and other nuclear degenerative changes were significantly higher among the elderly women ($p<0.05$) when compared with young women volunteers.

Conclusion: Aging along with environmental factors appear to be detrimental in inducing mutagenic/ genotoxic effects at the cellular level. Cytological evaluation is clearly indicative of nuclear changes evident with aging.

KEYWORDS

Aging, Mutagenic, Nuclear Degeneration, Women

INTRODUCTION:

Biological aging, is an internal physiological phenomenon of an individual, which deteriorates with time. The reparative and regenerative potential begin to slow down and eventually stop working. An increase in genomic instability due to the reduced capacity to repair damaged DNA has been coupled with aging. DNA damage builds up, attenuation of metabolic enzymatic activity and DNA-repair enzymes, together enhance the susceptibility of the cells to undergo genetic damage. Although, aging is always influenced by the inseparable external environment which acts as external mutagens.[1]

Our genome is prone to insult caused either by exogenous and endogenous agents. And, when the damage is accumulated on DNA, it leads to errors in DNA replication leading to point mutations or chromosomal rearrangements. This has proven to play a major role in aging, cancer, and neurodegenerative diseases[2](Jeppesen *et al*., **2011**).

The endogenous sources leading to DNA damage includes reactive oxygen species and exogenous sources include mutagens and radiation. All cells have various defence mechanisms to protect against these mutagens through various DNA repair pathways. The four major pathways for repairing damage to bases include- base excision repair (BER), Double strand break repair (DSBR), Nucleotide excision repair (NER), and Mismatch repair (MMR).[2](Jeppesen *et al*., **2011**).

Single stranded DNA break (SSB) are usually progressed towards repairing through Base excision repair, whereas Double stranded DNA are progressed towards Double strand break (DSB) repair pathway. Out of these, base excision repair is one of the crucial pathways in the process of DNA repair.[3](Gredilla et al., **2010**)

There are evidences present that directly link aging to the damage to nuclear DNA (nDNA) in addition to damage due to cancer, apoptosis, and cellular senescence. The efficiency of DNA repair enzymes are expected to decline with age, but this is a point of controversy till now. Though there are evidenceslinking genomic instability with the process of Aging. But, there are evidences that nDNA plays a persuasive role in aging.[4]

The present day physical environment consisting of air, water and land to natural resources like flora and fauna is deteriorating every day. This connect of our well-being with the immediate environment is indissoluble and this is reflecting in our present day world. The environment can further be artificial (industries, building, roads, automobiles) or natural. Associated health risks are majorly due to our immediate vicinity i.e., fertilizers, heating/burning, cooking/fuels, improper ventilation, industrial pollutants, road traffic etc. India is facing challenges like air, water, infrastructure-related(sewage, garbage) and pollution of the natural environment which is worsening every day. Air-pollution due to vehicular smoke and industrialization/ urbanization, is the prime challenge affecting the quality of air.[5]

The process of aging along with pollution, is enhancing nuclear degenerative process via chemical reactions. This genetic damage can further be associated with health-status, habits, lifestyle, stress and gender. Factors like smoking, drinking, long working hours, physical inactivity, type of diet, sleeping hours and psychological factors are the most crucial factors. Smoking/ drinking and fertilizers/ pesticides are adding carcinogens directly to the body and progressing the nuclear damage. The same is caused by health conditions like cancer which incorporates genetic damage.

Statistics indicate that air pollution exposure modulates the epigenetic mark. DNA Methylation was the first epigenetic mark discovered and it also has an important role in normal human development, tumorigenesis, aging, and other diseases. Outcomes like long term history of respiratory, cardiovascular conditions and mental health disorders are common to be observed in cases of Air pollution exposure- associated DNA methylation.[6]

Telomeres in DNA play a pivotal role in maintaining chromosomal and genomic stability. Due to the action of chemical and physical environmental agents, accelerated cases of shortened telomere are

observed. This usually occurs due to the oxidative stress caused by reactive oxygen species capable of modifying bases and single strand breaks in genome.[7]

Extent of genetic damage can be diagnostic in comprehending the damage caused to cells and their further progress to pre-neoplastic or neoplastic changes. The accumulation of genetic damage eventually causes cellular or nuclear morphological alterations which can be read through microscopic lens. Thus, the present study was undertaken to unravel these microscopically-evident cytological changes in the prevalence of micronuclei and other nuclear degenerative alterations.[8]

Ethical Approval

Ethical clearance for the study was obtained from the Ethical Committee of the ITS Dental College, Greater Noida. All the participants were asked to sign a written and informed consent form before taking smears.

MATERIALS AND METHODS

A cross-sectional study was conducted in 46 geriatric female population(>60years) of the rural and urban area along with the history including systemic conditions, occupation, medication etc., using a questionnaire. 40 young female volunteers (22-25 years) were considered as controls.

02 Buccal smears were prepared from each subject using wooden spatula and the subjects were asked to rinse the mouth to clear all the debris before making smears. 01 smear was kept for air-fixation and 01 was immersed in 95% ethanol for fixation for 24hours for PAP and H&E stain respectively. 100 cells were counted from each smear under oil-immersion lens and all the cellular and nuclear changes were recorded.

All the nuclear changes were recorded, namely, Micro-nuclei, Binucleation and other Degenerative nuclear changes (like karyorrhexis, condensed chromatin, pyknosis, and karyolysis).

Sarto *et al.* criteria for the identification of Micro-nuclei was applied according to which it is morphologically similar to a nucleus when they exhibited up to 1/3 of its size and were visible on the same plane.[9]

RESULT

The statistical analysis using mean and chi square test using statistical package data SPSS confirmed statistically significant differences between the elderly women and the young-aged volunteers($p>0.05$). Also, there was no significant differences in the same age group women. The frequency of micronuclei (Figure – 1) and nuclear degenerative changes (Figure 2) appeared significantly more in elderly women.

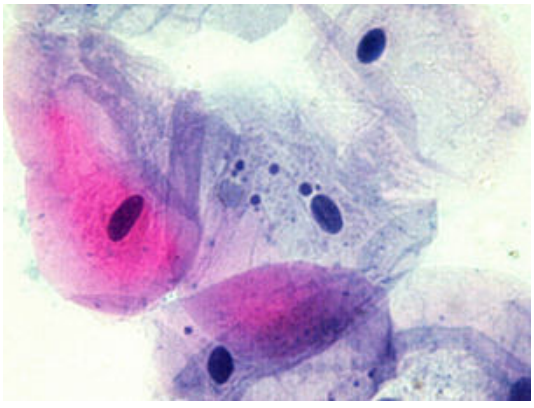

Fig. 1: Photomicrograph of Smear from Buccal Mucosa showing Micronuclei

DISCUSSION:

Amidst all the techniques available for assessing damaged cells, nuclear degeneration is one of the commonest, effective and easy to do method which can be used widely in population biomonitoring.[13] These changes have been increasingly utilized these days especially for screening and monitoring the changes, before any invasive technique is considered in a grave pathology. Besides its non-invasive nature, it comes cheap and it is less technique sensitive. These readily available nuclear cytopathologies include micronucleus, Micro-nuclei and other Degenerative nuclear changes like karyorrhexis, condensed chromatin, pyknosis, karyolysis, Broken nuclei and binucleation.[8,9]

Micronuclei consists of structures that cause break in chromosome or complete chromosomes that did not bond with spindle during cellular division, neither are included in the nucleus of daughter cells. Therefore, we can state that the micronucleus is the last remenant of chromosomal damage caused by a clastogenic or aneugenic event. [11]

The micronucleus and nuclear degenerative tests have been considered to be a significant and reliable mean for assessing the genetic damage in accordance with Tolbert *et al*. and Thomas *et al*. These protocols calculate micronuclei and degenerative nuclear alterations that are indicative of genetic changes. The hue and chroma of micronuclei morphological

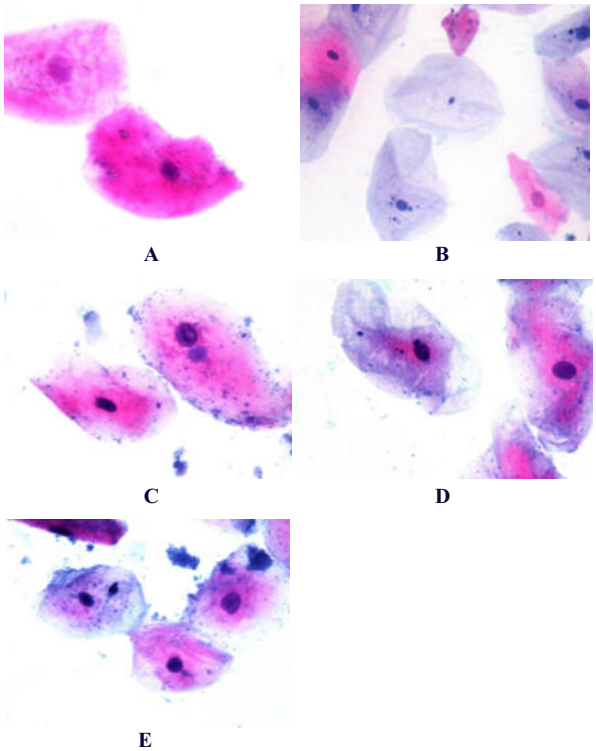

Fig. 2: Photomicrographs of cells exfoliated from Buccal Mucosa exhibiting different nuclear morphology (stained using H & E stain) (A) Karyorrhexis, (B) Pyknosis, (C) Chromatin condensation, (D) Broken Egg, (E) Binucleation

resembles the central nucleus whereas the other nuclear degenerative changes exhibit a plethora of colour variability. As described by Fenech and Bonassi, an increase in the number of micronuclei is a by-product of the cumulative effect of mutations in genes involved in DNA repair, chromosomal segregation and checkpoints of the cellular cycle; and numerical and structural alterations in chromosomes that are induced by endogenous and/or exogenous genotoxins, along with detrimental lifestyle factors.[12]

Nuclear degenerative changes observed in a cell in the process of apoptosis, e.g., karyorrhexis, condensed chromatin and pyknosis, demonstrate genotoxic effects. On the other hand, karyolysis is the morphological change which exhibits cytotoxic effect in the event of Necrosis. All these changes have been a cumulative effect of the environmental toxins, habits such as alcohol/smoking/tobacco chewing and unhealthy lifestyle.[5,12,13]

This assessment opens up a whole new worlds of application to study the mutagenic effects of smoking and/or alcoholic beverages[14], [15], as well as effects of decreasing nutrients in the diet. [16]

The effects of age on the occurrence of micronuclei and nuclear degenerative alterations recorded in the present study, clearly indicated the enhanced genetic instability changes with aging and is inseparable from the damaging environmental changes. Even though, any statistical difference couldn't be ascertained with respect to drug

history, environmental variations, and occupation, an increased number of sample size would ascertain the same.

Thus, the process of aging seems to be dependent on- 1. genetically programming, 2. Exogenous factors, and 3. endogenous factors. The enzymes involved in DNA repair become fewer and fewer in count, during the process of aging. Therefore, it increases the susceptibility to genotoxic agents. [17], [18]Kirsch Valder et al. [19] suggested that the different functions by DNA repair enzymes in the process of cellular defence systems, or reducing the efficiency of DNA repairs, can lead to accumulation of mutations that are further responsible for aging.

However, further studies with larger samples are required, given that the quantity of individuals analysed could be considered a limitation of the present study.

CONCLUSION

Age and immediate environment which includes various endogenous and exogenous harmful particles invading or affecting the human body are all accelerators of the morphological nuclear damage occurring in a cell.

COMPETING INTERESTS

The authors declare that there are no conflicts of interest.

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