Original Article

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Evaluation of Genetic Damage in Lymphocytes of Healthy Population of Haryana Using Comet Assay

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Abstract

Ageing is a multifaceted process in which bodily structures and functions undergo a negative deviation from the optimum. It occurs as the result of genetic alterations that repair DNA, reduce oxidative damage or reduce apoptosis. Ageing is among the largest known risk factors for most human diseases. The present study was planned to evaluate the genetic damage in healthy individuals of Haryana using comet assay. The relationship between genetic damage and age in healthy individuals was also assessed. The study was conducted on 90 randomly selected healthy subjects belonging to two age groups: <50 years (mean age = 43.833±0.789) and $\overset{24}{\underset{12}{}}$50 years (mean age = 60.222±0.885). Significantly (P<0.001) higher Tail DNA (%), Tail length (µm), Tail moment, Olive moment and Tail area were found in individuals with $\overset{24}{\underset{12}{}}$50 years of age as compared to those belonging to <50 years of age. Tail DNA (%), Tail length (µm), Tail moment and Olive moment showed a highly significant (P<0.001) positive Pearson correlation with age. Significant (P<0.01) positive Pearson correlation was found for integral intensity with respect to age. In conclusion, the accumulation of deleterious genetic damage may be one of the causes responsible for ageing.

Keywords: Ageing; Comet assay; Genetic damage.

Introduction

Ageing is an incessant, intricate and dynamic process that begins with birth and ends with death process. It is a multifactorial and multidimensional process of physical, psychological and social change. It occurs at a cellular level as a result of interaction between genetic and environmental factors. Ageing leads to aggregation of diverse detrimental changes occurring in cells and tissues with increasing age, that is responsible for increased risk of disease and death. [1] Ageing has been reported to be negatively related to ability to respond to stress and positively associated with homeostatic imbalance, ultimately leading to death. [2]

The number of people aged 65 years or older is projected to grow from an estimated 524 million (8% of the world's population) in 2010 to nearly 1.5 billion (16% of the world's population) in 2050, with most of the increase in developing countries. India's older population (those over 65 years age) will likely swell to 227 million by 2050 from 60 million in 2010. Driven by falling fertility rates and remarkable increases in life expectancy, population aging will continue, even accelerate. [3]

The advancing age related oxidative stress ultimately results in imbalance between the antioxidant defences and free radical production. [4] Higher levels of free radicals in aged individuals cause oxidative modifications in DNA, protein and lipid molecules. [5] Many studies have reported elevated levels of both oxidant damaged DNA and proteins in aged organisms. [6-7] Our genome is susceptible to genetic damage by various exogenous and endogenous damaging agents. Garm *et al.* [8] found that ability to respond and repair double strand breaks decreases with advancing age. DNA damage accumulates with age. [9] Increased DNA damage may provoke cellular signalling pathways, such as apoptosis, that result in a faster exhaustion of stem cells, which in turn contributes to accelerated ageing. [10]

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The comet assay is considered fast, non-invasive and suitable test to study the genetic damage in human biomonitoring studies. It is inexpensive and sensitive technique for qualitative and quantitative assessment of DNA damage in peripheral blood lymphocytes and requires little biological sample (10,000- 50,000 cells). [11-13] The purpose of the present study was to investigate the genetic damage in healthy individuals using comet assay by evaluating various comet parameters i.e. Head DNA (%), Tail DNA (%), Integral intensity, Head radius (μm), Tail length (μm), Tail moment, Olive moment, Head area (μm^2) and Tail area (μm^2). Additionally, we investigated the association between the genetic damage and age in healthy subjects.

Materials and Methods

A group of randomly selected 90 healthy volunteers (53 males and 37 females) was recruited. These were categorized into two age groups i.e. <50 years and $\frac{24}{12}$50 years. Individuals with history of chronic disorders, infection, cancer, diabetes, hypertension were excluded. The study was approved by Institutional Ethics Committee, Kurukshetra University, Kurukshetra and all the volunteers gave written consent to participate. A questionaaire regarding medical history, sociodemographic characters (age, race and education), physical activity, occupation, dietary and lifestyle habits was filled up for each subject. They were of mixed social class and occupation (housewives, teachers, administrative employees, blue collar workers and unemployed).

Blood samples were collected early in morning (9am - 11am) in K_2-EDTA coated tubes by venipuncture method. After collection, the samples were coded, stored on ice and transported to laboratory. Samples were processed as quickly as possible (usually within 3-4 hours following the blood sampling).

The comet assay was carried out under alkaline conditions, as described by Ahuja and Saran. [14] Two frosted microscopic slides per individual were prepared. Each was covered with 150-200 μl of 1% normal melting point agarose and allowed to solidify at room temperature. The slides were then coated with mixture of 20 μl blood and 100 μl of 0.5% low melting point agarose and covered with coverslip. After 5-10 minutes of solidification at 4^0C, coverslips were gently slid off. Later, third layer of 180-200 μl of 1% normal melting point agarose was added and covered immediately with coverslip. After solidification step, coverslips were removed and slides were immersed in ice cold freshly prepared lysing solution overnight at 4^0C to allow DNA unfolding. The slides were then placed side by side in horizontal gel electrophoresis tank filled with electrophoretic buffer for 30 minutes to allow DNA unwinding and expression of alkali labile sites. Electrophoresis was carried out 25V, 300mA for next 30 minutes. Slides were then stained with ethidium bromide. All the steps were carried out in dark room to avoid additional DNA damage. Slides were examined using Olympus CX 41 trinocular research microscope at 100X. A total of 100 cells were scored for each individual (50 from each of two replicate slides). Lucia Comet Assay software (Nikon) was used for image analysis. Nine computed measurement of images were used as indices of genetic damage i.e. Head DNA (%), Tail DNA (%), Integral intensity, Head radius (μm), Tail length (μm), Tail moment, Olive moment, Head area (μm^2) and Tail area (μm^2).

Statistical analysis was carried out using SPSS v 16.0. The continuous data was summarized as mean and standard error (S.E.). A comparison between the groups was done by independent sample t test. Pearson correlation coefficients were calculated to evaluate the relationship between age and genetic damage.

Results

The general characteristics of the subjects i.e. sex, age, diastolic and systolic blood pressure are presented in Table 1. Out of 90 subjects, 36 belonged to <50 years age group (mean age = 43.833±0.789) and 54 belonged to $\frac{24}{12}$50 years age group (mean age = 60.222±0.885). Genetic damage parameters in relation to age (years) in healthy subjects are illustrated in Table 2. Tail DNA (%), Tail length (μm), Tail moment, Olive moment and Tail area (μm^2) were found to be significantly ($P<0.001$) higher in individuals with e$\frac{24}{12}$50 years of age as compared to those belonging to <50 years of age. Head DNA (%) showed highly significant ($P<0.001$) decrease from individuals aged $\frac{24}{12}$50 years (88.358±0.571) to <50 years (93.893±0.576).

Figure 1 shows the Pearson correlation for various comet parameters with respect to age (years) in peripheral blood lymphocytes of healthy individuals. Tail DNA (%), Tail length (μm), Tail moment and Olive moment presented a highly significant ($P<0.001$) positive correlation with age. Integral intensity also showed a significant ($P<0.01$) positive Pearson correlation with respect to age, depicting a linear increase in the per cent of DNA with advancing age. While Head DNA (%) was found to have a highly

Table 1 General characteristics of the healthy subjects.

S.No.	Characteristic	Healthy subjects	
		<50	≥50
1	n	36	54
2	Average age (years)	43.833±0.789***	60.222±0.885***
3	Sex		
	Male	25 (69.4%)	28 (51.9%)
	Female	11 (30.6%)	26 (48.1%)
4	Smoking habits		
	Non-smoker	27 (75%)	46 (85.2%)
	Smoker	9 (25%)	8 (14.8%)
5	Drinking habits		
	Non-drinker	28 (77.8%)	38 (70.4%)
	Drinker	8 (22.2%)	16 (29.6%)
6	Dietary habits		
	Vegetarian	18 (50.0%)	43 (79.6%)
	Non-vegetarian	18 (50.0%)	11 (20.4%)

***Significant (P<0.001; 2-tailed) (Independent sample t test)

Table 2 Assessment of genetic damage (Mean±S.E.) with respect to the age (years) in healthy subjects (n=90).

Comet Parameters	Age (years)	
	<50 (36)	≥50 (54)
Head DNA (%)	93.893±0.576***	88.358±0.571***
Tail DNA (%)	6.108±0.560***	11.642±0.571***
Integral Intensity	5.041±0.319	5.410±0.329
Head Radius (μm)	17.642±0.741	16.959±0.658
Tail Length (μm)	3.712±0.284***	6.042±0.341***
Tail Moment	0.310±0.052***	0.972±0.095***
Olive Moment	0.904±0.098***	1.766±0.119***
Head Area (μm^2)	770.450±42.487	737.290±42.029
Tail Area (μm^2)	99.305±7.733***	161.060±10.274***

***Significant (P<0.001; 2-tailed) (Independent sample t test)

Table 3 Pearson correlation coefficients for different comet parameters observed with respect to age (years) in healthy subjects.

	Age	P-value
Head DNA (%)	-0.599	P<0.001
Tail DNA (%)	0.599	P<0.001
Integral intensity	0.296	P<0.01
Head radius (μm)	0.037	P>0.05
Tail length (μm)	0.511	P<0.001
Tail moment	0.576	P<0.001
Olive moment	0.562	P<0.001
Head area (μm^2)	0.067	P<0.05
Tail area (μm^2)	0.496	P<0.001

significant (P<0.001) negative correlation with respect to age in subjects.

Discussion

Age is a major factor that causes variation in the level of genetic damage in healthy individuals. Several age related studies have reported that ageing leads to degradation of genetic material in all living species including the highest intellectual form of beings, namely humans. In the current study, Tail DNA (%), Tail length (μm), Tail moment, Olive moment and Tail area (μm^2) were found to be significantly (P<0.001) increased in individuals with advancing age that corresponds to the damages accumulated at the individual strand levels. Singh *et al.* [15] observed significantly more common presence of highly damaged cells among aged individuals and DNA repair competence among a small subpopulation of lymphocytes declined with age. Significantly increased level of Tail DNA (%) was reported in leukocytes of elderly healthy individuals as revealed by alkaline comet assay. Singh *et al.* [16] analyzed the basal levels of DNA single-strand breaks and alkali-labile sites in individual human peripheral blood lymphocytes and found five-fold

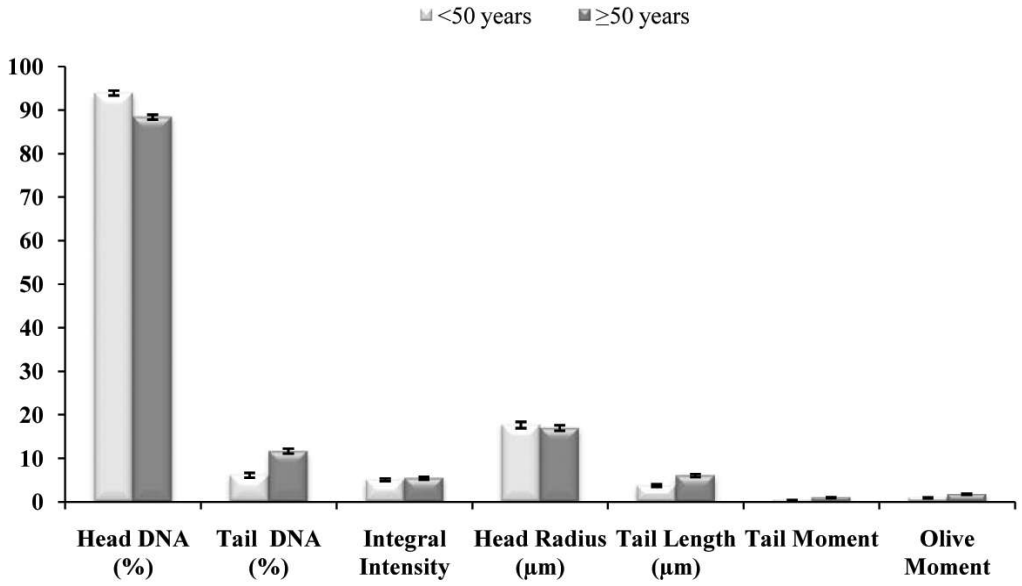

Fig.1 Comet parameters observed with respect to age (years) in healthy subjects.

increase in old (more than 60 years) than young (less than 60 years) male donors. Significantly higher (approximately 1.5 times) mean value of DNA migration length was observed in the old rats at the basal level in hepatocytes of male F344 rats. [17] Also, in a study with 80 individuals from Greece showed that men at the age of 55–60 years had an average of 14.5% more DNA damage than men at the age of 20–25 years. [18] Sarto *et al.* [19] analysed the sister chromatid exchange in human lymphocytes and the importance of several factors that affected the frequency of sister chromatid exchange and concluded that the effect of age was more severe than that of smoking, with regard to genetic damage of lymphocytes. Swain and Rao [20] observed a marked increase in DNA damage (single stranded breaks and double stranded breaks) with increasing age in isolated neurons and astrocytes in rat brain with the help of comet assay. On the other hand, Betti *et al.* [21] did not detect any age-related effect on the level of DNA damage in the peripheral blood lymphocytes from healthy individuals.

In the present study, comet parameters correlated significantly with the increasing age. These findings are in agreement with previous work which reviewed that there is a positive correlation between age and the level of DNA damage as observed by comet assay. [22] In a study, age was found to correlate with increasing percentage of sperm with highly damaged DNA. [23] Balachandar *et al.* [24] reported that age and gender were strongly associated with the frequency of genetic damage. Manikantan *et al.* [25] studied the genetic damage in peripheral blood lymphocytes of photocopiers and reported significant association between level of DNA damage and age. Soares *et al.* [26] analyzed 4676 participants included in 36 studies and found a highly significant ($P<0.001$) correlation between age and DNA damage. Contrary to the findings of the present study, Olinski *et al.* [27] found no association of genetic damage with age. No influence of age was observed on apoptotic DNA levels in blood leukocytes of patients with T2DM and thyroid dysfunctionism. [28]

In conclusion, our results show age related increase in genetic damage which might be caused due to the accumulation of deleterious genetic damage. Significant correlation was observed between comet parameters and advancing age. Thus, the accumulation of deleterious genetic damage may be one of the causes responsible for ageing.

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Disclosure

The authors declare no conflict of interests.

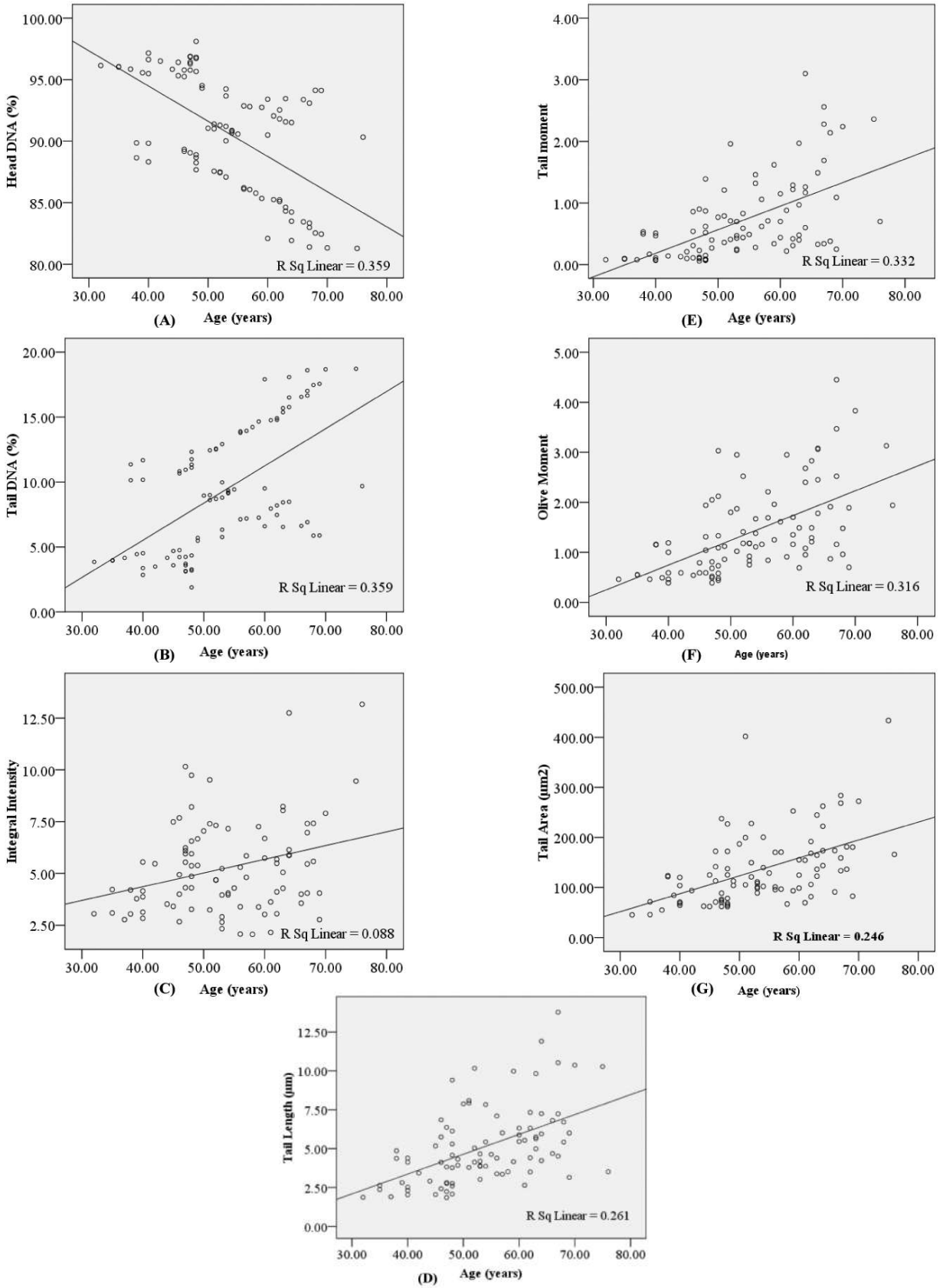

Fig. 2 Correlation for (A) Head DNA (%),(B) Tail DNA (%), (C) Integral intensity, (D) Tail length (µm), (E) Tail moment, (F) Olive moment and (G) Tail area (μm^2) with respect to age (years) in healthy subjects.

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