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Comet assay of healthy elderly people in Thai people in Tambon Lak Hok population at Pathumthani province by Lucia program

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Abstract

The Comet assay is a sensitive and rapid method for DNA damage in individual cells. It has found wide acceptance in epidemiological and bio monitoring studies for determination of DNA damage resulting from lifestyle, occupational and environment exposure. However, there is no information regarding the influence of the Thai people in Tambon Lak Hok population at Pathumthani on the results of the assay in lymphocytes of females and males. A study was therefore undertaken among 30 elderly healthy Thai females and males volunteers to assess the effect of the pollution on Comet assay responses. During the October 2015 to October 2016 the basal levels of DNA damage in the lymphocytes as evident by LUCIA Comet parameters were observed. The mean value for the Head DNA % Tail length Tail DNA % Tail moment and Olive moment parameter for all subjects were 99.20±0.08, 0.94±0.10, 0.80±0.08, 0.07±0.02 and 0.18±0.02, respectively. In summary, the results obtained present background data that could be considered as normal values for healthy elderly people living in Metropolitan Region, and can later on serve as baseline values for further toxicological.

Keywords: Pathumthani province, Elderly people, DNA damage, Comet assay, Lucia program

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1. Introduction

The Comet assay (also known as single cell gel electrophoresis) was introduced almost 40 years ago by Ostling and Johason as a simple way to detect a straightforward and highly sensitive method for measuring the rate of DNA damage and repair at the level of individual cells. Measurement of genotoxicity as migration in agarose gels by electrophoresis has passed more than 20 years of research [1-3]. The alkaline Comet assay (single-cell gel electrophoresis) is widely used technique due to sensitivity, simplicity and inexpensiveness for results in the assessment of strand breaks (SBs) and alkali-labile sites. The alkaline version of the Comet assay is a method in which DNA lysis and electrophoresis are performed under neutral conditions and SYBR Green is used for staining of the double-stranded DNA molecules. Individual Comet images may be recorded and these images are analyzed for a variety of densitometric and geometric parameters by purpose-designed image analysis software of LUCIA program when viewed by fluorescence microscopy. [4] There are many ways to report DNA damage by the Comet assay. The tail moment is obtained by multiplication of the tail length and the percentage of fluorescence in the tail (%T); this is still very popular, although it has been criticized severely because it is not possible to picture the level of DNA damage by the value of the tail moment [5]. Also, there are several calculations of the tail moment, e.g. the Kinetic image analysis system calculates the Olive and Extent tail moments that have different values because of the method of calculation.

The aim of study was to evaluate the baseline DNA damage in the LUCIA Comet assay program of healthy elderly, selected from the Tambon Lak Hok population at Pathumthani, to investigate if there is a relation with age and gender by measuring the tail length, tail intensity and tail moment as comet assay parameters. These baseline records may contribute to future genotoxicological monitoring.

2. Materials and Experiment

2.1. Population characteristics

The present study comprised a group of 30 healthy elderly people (15 males and 15 females), between the ages of 60 and 80 years (average age, 68.00 ± 6.9 years; median age, 68 years) gathered form headman of Tambon Lak Hok at Pathumthani examinations. In this research use G*Power, which significance level of 0.05, a power of 0.95 and effect size of 1.50, to determine the sample size needed for tests of the difference between two sample means. Detailed population characteristics are presented in Table 2. The selected elderly were in a similar age range (median value for both groups were 68). The selected elderly were all from the general Tambon Lak Hok population (Pathumthani Metropolitan Region). Criterion for the selection was that all were healthy at the moment of blood sampling and interviews. In addition, none of the selected ageing for the present study has been exposed to ionizing

or non- ionizing radiation for diagnostic or therapeutic purposes during the six- month period before blood sampling. Also, none of them reported intake of medication, or the presence of known inherited genetic disorders or chronic diseases.

This study was approved by the Ethical committees of the Rangsit University and was performed in the Study of DNA Damage Level in Pathumthani Populations Group: Bases on Age. All blood donors were informed about the aim and the experimental details of the study and gave their informed consent and volunteered to donate blood samples to be used for scientific purposes. They were asked to fill in a standardized questionnaire, designed to obtain relevant details about their lifestyle and health status. No private details on subjects involved in study are or will be highlighted in public.

2.2. Blood sampling

Peripheral blood samples were taken from the veins and were collected in heparinized tubes (Becton Dickinson) under aseptic conditions in the morning hours, between 9 and 10 am. After collection, all blood samples were handled in the same manner. They were randomly coded, stored in ice, protected from light, transferred to the laboratory for hematology parameters and comet assay. The routine hematology profile – leukocytes with 8- parameter differential, erythrocytes, haemoglobin, haematocrit, mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) and thrombocytes with an automated hematology analyzer (Advia120, Siemens Diagnostic Solutions, USA). The comet assay was performed no later than 24 h after sampling. Blood samples for genotyping were stored at -20 ^{O}C until the use.

2.3. Alkaline comet (SCGE) assay

To evaluate baseline frequency of DNA damage in the ageing population the comet assay was carried out under alkaline conditions. Fully frosted slides were covered with 1% normal melting-point (NMP) agarose. After solidification, the gel was scraped off the slide. The slides were then coated with 0.6% NMP agarose. When this layer had solidified, a second layer containing 5 µl of whole blood sample mixed with 0.5% low melting-point (LMP) agarose was placed on the slides. After 10 min of solidification on ice, the slides were covered with 0.5% LMP agarose. The slides were then immersed overnight in ice- cold, freshly prepared lysis solution (2.5 M NaCl, 100 mM disodium EDTA, 10 mM Tris– HCl, 1% sodium sarcosinate, pH 10, with 1% Triton X-100 and 10% dimethyl sulfoxide), which was added immediately prior to use, to lyse the cells and to allow DNA unfolding. The slides were then placed in a horizontal gel-electrophoresis tank, facing the anode. The unit was filled with fresh electrophoresis buffer (300 mM NaOH, 1 mM disodium EDTA, pH 13.0) and the slides were placed in this alkaline buffer for 20 min to allow DNA unwinding and expression of alkali-labile sites. Denaturation and electrophoresis were performed at 4 ^{O}C under dim light. Electrophoresis was carried out for 20 min at 25 V (300 mA). After electrophoresis the slides were rinsed gently three

times with neutralization buffer (0.4 M Tris–HCl, pH 7.5) to remove excess of alkali and detergents. Each slide was stained with SYBR Green and covered with a coverslip.

2.4. Comet capture and analysis

One hundred randomly captured comets from each slide were examined at 400X magnification by use of a fluorescence microscope (Nikon, Japan) connected via a black-and-white camera to an image-analysis system (The Lucia Comet Assay program, UK). An automated image-analysis system was used to acquire images, compute the integrated intensity profile for each cell, estimate the comet cell components and evaluate the range of derived parameters. Comets were randomly captured at a constant depth of the gel, avoiding the edges of the gel, occasional dead cells, and superimposed comets or comets without distinct head ("clouds", "hedgehogs", or "ghost cells"). For each sample, 100 cells records per subject, for statistical analysis purposes. The interpretation of images is based on their classification into five classes: class 0= tail-free comet, no DNA damage index; class 1= slight damage to DNA due to the presence of a few fragments that create a single damage around the head of the comet; class 2=moderate DNA damage; class 3= extended damage; and class 4= almost completely fragmented DNA (remarkable tail length) (Figure 1). To quantify DNA damage, the following comet parameters were evaluated: tail length, tail intensity (percentage of tail DNA), and tail moment. Tail length (i.e., the length of DNA migration) is related directly to the DNA fragment size and is presented in micrometers. It was calculated from the center of the cell. Tail intensity is defined as the percentage of fluorescence migrated in the comet tail. Tail moment was calculated as (tail length × % DNA in tail)/100.

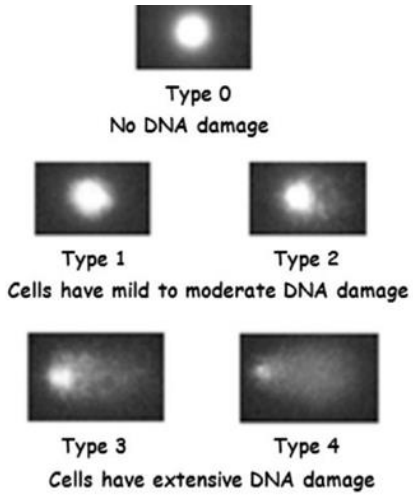


Figure 1 Class of DNA damage [7]

2.5. Statistical analysis

Statistical analysis was done by use of the Statistics software package. Basic statistics for each determined parameter were calculated separately for the male and female population. Possible difference at $P < 0.05$ for each parameter regarding the gender was assessed by use of t-test or the Mann–Whitney U test. In conducted statistical tests the statistical significance was set to $P < 0.05$.

3. Results and Discussion

This paper presents the results of the alkaline comet assay and hematology parameters obtained from healthy elderly people in the general Tambon Lak Hok population (Pathumthani

Metropolitan Region). The Lucia Comet Assay program was used to evaluate parameters for measuring baseline frequency of DNA damage. (Table 1)

Table 1 Summary of comet parameters computed by the LUCIA Comet Assay program. [7]

Parameter	Description
Head DNA %	Head DNA content as a percentage of comet DNA content
Tail length	Length of the tail in pixels
Tail DNA%	Tail DNA content as a percentage of comet DNA content
Tail moment	Tail length times Tail DNA%
Olive moment	Product of tail DNA% and the distance between the intensity-weighted centroids of head and tail

Table 2 Population characteristics (mean values± standard error of the mean)

Gender	n	Age	Age range(years)
Female	15	69.33 ± 2.122	60-86
Male	15	67.80 ± 1.461	60-82
Total	30	68.00 ± 1.274	60-86

n = number of sample size

3.1 Baseline frequency of DNA damage

With the alkaline comet-assay parameters, Head DNA %, Tail length, Tail DNA%, Tail moment, Olive moment, we evaluated the baseline frequency of DNA damage in a group of healthy elderly. The results are summarized in Table 3.

The mean value for the Head DNA % Tail length, Tail DNA%, Tail moment, Olive moment parameters for all subjects were 99.20±0.42, 0.94±0.56, 0.80±0.42, 0.07±0.09 and 0.18±0.11, respectively. Minimum value for the tail length was 0.15 while the maximum was 2.18 μm. Values for Head DNA % ranges from 98.20 to 99.79, while the range for the Olive moment was from 0.04 to 0.41. (Table 3)

Table 3 Basic statistic parameters calculated for the alkaline comet assay (mean values ± standard error of the mean of tail length, tail intensity and tail moment) parameter in peripheral blood lymphocytes of healthy elderly people depending on their gender.

Groups	n	Comet assay parameters				
		Head DNA %	Tail length	Tail DNA%	Tail moment	Olive moment
Female	15	99.11± 0.12	1.03± 0.14	0.88± 0.12	0.09± 0.03	0.20± 0.03
Male	15	99.28± 0.09	0.84± 0.15	0.71± 0.09	0.05± 0.01	0.15± 0.02
Total	30	99.20± 0.08	0.94± 0.10	0.80± 0.08	0.07± 0.02	0.18± 0.02

3.2 Hematology parameters from healthy elderly people

Female healthy elderly people compared to male healthy elderly people showed normal parameter in Table 4 ($P < 0.05$).

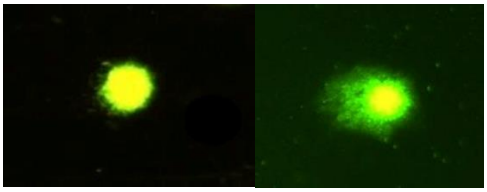
Table 4 Common hematology parameters from healthy elderly people

Parameter	Female	Male	Total
Leukocytes ($\times 10^9$/L)	7.13±0.66	8.03±0.30	7.58±0.35
Erythrocytes ($x10^{12}$/L) *	4.3±0.09	4.7±0.12	4.55±0.09
Hemoglobin (g/L)	12.14±0.30	13.12±0.42	12.63±0.27
Hematocrit (L/L) *	36.34±0.77	39.40±1.05	37.87±0.70
MCV (fl)	84.13±1.65	82.73±2.15	83.43±1.34
MCH (pg)	28.13±0.67	27.64±0.86	27.89±0.54
MCHC (g/dl)	32.73±0.64	33.38±0.23	33.05±0.34
Platelet count($\times 10^9$/L)	282.66±22.14	243.46±17.21	263.06±14.25

MCV – mean corpuscular volume; MCH - Mean corpuscular hemoglobin; MCHC- Mean corpuscular hemoglobin concentration

Results are presented as means ± standard error of the mean or medians (5th/95th percentiles) according to data distribution.

* Statistically different ($P < 0.05$; t-test or Mann-Whitney U test).



A B

Figure 2 Fluorescence microscopy images of cells to A = healthy elderly people comet cell; B = positive control

3.3 The pollution in Tambon Lak Hok population at Pathumthani province in 2016

Pathumthani is one of the central provinces of Thailand. The province is north of Bangkok and is part of the Bangkok metropolitan area. The province lies on the low alluvial flats of the Chao Phraya River that flows through the capital. Many canals (khlongs) cross the province and feed the rice paddies. There are: industry software park Thailand, Nava Nakorn Industrial Promotion Zone (1,376 acres / 5.6 km²), Bangkadi Industrial Park (470 acres / 1.9 km²), Techno Thani (a "Technology City" administered by the Ministry of Science and Technology), and a number of industrial parks in neighboring Phra Nakhon Si Ayutthaya and Nonthaburi Provinces. In 2016, the main air quality is good air quality by sulfur dioxide (SO_2), Nitrogen dioxide (NO_2) and carbon monoxide (CO) showing normal range in Table 5.

Table 5 The mean Air quality in Pathumthani in 2016 [7]

Air quality(ppm)	So_2	No_2	CO
Pathumthani	2	25	0.81
Normal Range	40	30	-

There is increasing interest in the bio-monitoring of elderly, who are considered as a specific subgroup which could be more sensitive than the average in children and adults [6], [8-9]. Elderly may have different response to environmental hazards than children due to the continued phase of inactive development, which is one of the main reasons [10]. One of the problems regarding population bio- monitoring is confounding effects of modern lifestyle. Basically, in this experiment elderly do not smoke, consume alcohol, and are not exposed to any occupational hazards, and that is why the interference with results is reduced to the minimum. Nevertheless, potentially genotoxic factors in bio-monitoring of elderly population includes indoor tobacco smoke, airborne nanoparticles, regional ozone level, food contaminants (pesticides and chemicals generated by cooking). Potential genotoxic factors can vary between Capital and Metropolitan Region, vary in ionizing and non-ionizing radiation because of air pollution, oil and coal combustion emissions [11]. The comet assay is to detect the first exposure effects of environmental and occupational hazards, and the second is to assess the risk for cancer and other diseases [12].

In the past few years, the comet assay has become important tool for assessment of cytogenetic damage in population [13]. Evaluation of baseline levels of cytogenetic damage in the general population is essential for suitable interpretation of data obtained by monitoring of population that are occupationally or accidentally exposed to known or potentially genotoxic physical and/or chemical agents. Although many bio-monitoring studies indicate that baseline indicators of genetic damage in white blood cells depend on various internal and external factors, it is not clear how an individual' s inborn genetic constitution may influence the level of such damage. Over the past years our laboratory has accumulated data on cytogenetic biomarkers of general and various exposed human population [14-15]. There is no data for the elderly population especially for elderly population in metropolitan region, whose age generally does not exceed 80 years.

The present study used the alkaline comet assay as aim to evaluate the frequency of cytogenetic damage. The relationship between DNA damage as detected by the comet assay and aging, has suggested an age- dependent accumulation of the DNA damage.

4. Conclusion

This work presents background data that could be considered as normal values for healthy elderly, and can later on serve as baseline values for

further genotoxicological monitoring studies, especially in Pathumthani. In addition, the results may also be used for future comparisons with data obtained on elderly population in other cytogenetic laboratories, and facilitate the creation of a large comet assay database for elderly. The results also confirm the usefulness of both assays as sensitive endpoints that must be further evaluated and standardized in measuring cytogenetic damage, especially in bio-monitoring of elderly. Recently progress in this direction is made by new, automated image-analysis systems such as Lucia program that are already available for techniques, which facilitate easier and faster scoring of the above-mentioned parameters. Standardization of the culture and scoring conditions are performed to minimize both inter- and intra-laboratory variations in measuring assay outcomes and to facilitate reliable comparison among laboratories. This data presented have to be considered as a starting point of baseline DNA damage in Tambon Lak Hok population at Pathumthani and should encourage further investigation with a larger sample size and including more SNPS for primary prevention and early detection of disease-associated genes by identifying high-risk individuals.

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6. References

[1] Fahim M., Ahmed A. and Hussain S. Single cell gel electrophoresis and its applications in different fields. *MOJ Bioequivalence & Bioavailability*. 2017. 4(2) : 256-259

[2] Speit G. and Hartmann A. The comet assay. In: Henderson D. S. (eds). DNA repair protocols. Methods in Molecular Biology™, vol 314. Humana Press. 2006. 275-286.

[3] Koppen G., Azqueta A., Pourrut B., Brunborg G., Collins A. R. and Langie S.A.S. The next three decades of the comet assay: a report of the 11th International Comet Assay Workshop. *Mutagenesis*. 2017. 32(3) : 397– 408.

[4] Dvoŕák M., Urbanová P. and Matejovicova M. Evaluation of computer-assisted comet assay - repeatability and reproducibility of data. *Chemicke Listy*. 2014. 108 : 1149-1152.

[5] Gyori B.M., Venkatachalam G., Thiagarajan P.S., Hsu D. and Clement M.-V. Open comet : an automated tool for comet assay image analysis. *Redox Biol*ogy. 2014. 9(2) : 457 - 465.

[6] Intranuovo G., Schiavulli N., Cavone D., Birtolo F., Cocco P. and Vimercati L. Assessment of DNA damages in lymphocytes of agricultural workers exposed to pesticides by comet assay in a cross-sectional study. *Biomarker*. 2018. 23(5) : 462-473.

[7] Air Quality and Noise Management Bureau. Thailand's air quality and sltuation

reports [Internet]. 2016 (cited 2019 Sep 9). Available from: http://air4thai.pcd.go.th/webV2/download.php

[8] Žrskovi ́c L., Djeli ́c N., Baji ́cs V., Bogavac-Stanojwi ́c N., Žukovec D., Cabarkapa A. Evaluation of DNA damage in the lymphocytes of young, elderly and Alzheimer's disease patients treated with β-estradiol in the Comet assay. *Journal of Medical Biochemistry*. 2013. 32(3) : 238-244.

[9] Garm C., Moreno-Villanueva M., Bürkle A., Petersen I., Bohr V.A. and Christensen K. Age and gender effects on DNA strand break repair in peripheral blood mononuclear cells. *Aging Cell*. 2013.12(1) : 58–66.

[10] Mergener M., Martins M.R., Antunes M.V., da Silva C.C., Lazzaretti C. and Fontanive T.O. Oxidative stress and DNA damage in older adults that do exercises regularly. *Clin Biochem*. 2009. 42(16–17) : 1,648–1,653.

[11] Piperakis S. M., Kontogianni K., Karanastasi G., Iakovidou-Kritsi Z. and Piperakis M.M. The use of comet assay in measuring DNA damage and repair efficiency in child, adult, and old age populations. *Cell Biol Toxicol*. 2009. 25(1) : 65–71.

[12] Sasaki Y.F. The power of the comet assay to detect low level genotoxicity and DNA repair factors affecting its power. *MOJ Toxicol*. 2017.3(2) : 35-42.

[13] Gunasekarana V., Victor raj G. and Chand P. A comprehensive review on clinical applications of comet assay. *J Clin Diagn Res*. 2015. 9(3) : 1-5.

[14] Zare Sakhvidi M. J., Hajaghazadeh M., Mostaghaci M., Mehrparvar A. H., Zare Sakhvidi F. and Naghshineh E. Applicability of the comet assay in evaluation of DNA damage in healthcare providers' working with antineoplastic drugs: a systematic review and meta-analysis. *Int J Occup Environ Health*. 2016. 22(1) : 52–67.

[15] Dusinska M. and Collins A.R. The comet assay in human biomonitoring: gene-environment interactions. *Mutagenesis*. 2008. 23(3) : 191–205.