Long Term Tai Chi Exercise Reduced DNA Damage and Increased Lymphocyte Apoptosis and Proliferation in Older Adults

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SUMMARY
Effect of Tai Chi exercise on the level of DNA damage using the comet assay, lymphocyte viability and frequency of sister chromatid exchange (SCE) were determined in adults aged above 45. Tai Chi participants of 7 years (n=35), showed higher level of normal DNA and lower level of mild and severely damaged DNA as compared to the sedentary subjects (n=35). The former are suggested to have effective DNA repair mechanism as their frequency of SCE was markedly lower. Higher lymphocyte apoptosis and proliferation found in the Tai Chi participants also indicated that the exercise promotes renewal and regeneration of lymphocytes.

KEY WORDS:
Tai Chi, DNA, Lymphocyte, Chromosome

INTRODUCTION
Chronic diseases that commonly affect older adults contribute to disability, diminished quality of life and increased cost of basic and long term health care. The onset of diseases in aging could be attributed to age-related immune dysfunction because of excessive apoptosis of lymphocytes observed in aged humans. Also the conversion of activation or proliferation signals into cell death may cause the loss of proliferative potential of lymphocytes which is associated with immune senescence. Increased sensitivity of lymphocytes to this death signal may play an important role in the pathogenesis of lymphopenia during the aging process. Despite the much recent advancement in medical care, perhaps the most universal and effective treatment and prevention for chronic illness and disability in aging is physical exercise. However, older adults have been suggested to exercise cautiously by selecting the appropriate mode and intensity of exercise to minimize the possibility of exercise-induced oxidative stress while getting the full benefits of exercise.

At rest the human body produces reactive oxygen species (ROS) continuously but in healthy individuals these ROS are produced at levels well within the capacity of the body's antioxidant defense system. During aging and certain exercises, a reduction in the body's antioxidant defense system can produce an imbalance of this redox condition and cause oxidative stress. Increased oxidative stress elicits the production and accumulation of oxidative damage to biological compounds such as lipid, protein and DNA resulting in the cellular senescence observed in aging. Recent studies suggested that adaptations to regular exercise through the hormesis phenomena, especially in older individuals can help reduce oxidative stress by enhancing antioxidant capacity that is usually reduced in aging.

Besides oxidative stress, structural chromosomal rearrangements in cells could also contribute to the process of aging. Unstable chromosome aberrations cause the loss of dividing cells while stable chromosome aberrations do not affect genetic imbalance but accumulates with age. Therefore, the frequency of stable chromosome aberration has been suggested to be a useful biomarker for the aging process in humans. Examples of stable chromosomal aberrations are sister chromatid exchanges (SCE) and translocations. SCE in lymphocytes are indicators of pre-existing in vivo DNA damage, which remains, unrepaired in two replicative cycles.

Despite the knowledge that aging influenced lymphocyte viability and chromosomal changes, information concerning the effect of exercise intervention on the aging lymphocyte and chromosome is limited. This study was done to determine the effect of Tai Chi, which has been established as an exercise of moderate intensity and suitable for the elderly on DNA damage, lymphocyte apoptosis and proliferation and frequency of SCE in older adults. Increased knowledge on the interaction between exercise-induced oxidative damage, lymphocyte viability and chromosomal aberration is important to understand the underlying mechanisms of health related benefits of exercise in the aging process.

MATERIALS AND METHODS
Blood samples
The protocol for this study was approved by the Ethics Committee of Universiti Kebangsaan Malaysia. A total of 35 healthy Chinese Tai Chi participants aged above 45 years, who performed the 18 step Tai Chi exercise at least three times a week for more than a year, were enrolled for the study. Age, weight, height, gender and race matched control subjects, who have not been practicing any form of exercise regularly for more than once a week for the past three months were selected from the community.
Questionnaires were used to determine the subjects’ exercise habits and dietary intake. Both groups of subjects were subjected to a complete general physical examination which included weight, height and body mass index (BMI). Routine biochemical and hematological analyses that included liver function test (LFT), renal profile (RP), lipid profiles (LP), full blood count (FBC), uric acid, calcium and fasting blood sugar were also done in all subjects. Subjects were excluded based on any positive history of cardiovascular disease, respiratory disease, diabetes, hypertension and cancer; presence of pathologic values in blood clinical parameters and consumption of supplements or pharmaceutical products with antioxidant properties.

Subjects were asked to fast for 12 hours and refrain from performing any form of exercise for 24 hours preceding blood sampling. Peripheral venous blood (6 ml) was collected from all subjects into sterile heparinized tubes.

Alkaline microgel electrophoresis (comet assay)
Determination of DNA damage was carried out using the comet assay according to the method of Singh et al.  
Briefly, slides were prepared by adding 5 µl fresh whole blood with 80 µl 0.6% low-melting-point agarose (LMA) and sandwiched between a layer of 0.6% normal-melting-point agarose (NMA) on a frosted slide. Slides were then immersed in a concentrated salt solution for 1 hour at 4°C to release the DNA. Later, the slides were placed into an alkaline solution for 20 minutes at 4°C to denature and unwind the DNA. Electrophoresis was then conducted at 4°C for 20 minutes using 25 V and 300 mA. After electrophoresis, Tris buffer (0.4 M Tris-base) was added dropwise to the slides to neutralize excess alkali. Finally, the DNA was stained with ethidium bromide (20 µg/ml) and analysed using a fluorescence microscope. A total of 500 peripheral blood mononuclear cells were analysed for each subject and DNA was categorised into normal, mildly damaged and severely damaged based on microscopic observation of the tail length of each cell (Figure I).

SCE analysis
Lymphocyte culture was prepared according to the method by Cloustan 16. Within two hours of drawing of peripheral blood, 1 ml whole blood was added to 10 ml of complete culture medium (CCM) supplemented with 2% phytohemagglutinin (PHA). Cultures were incubated in a 37°C CO₂ incubator for 72 hours. For the determination of SCE, 100 µl BrdU (0.01 M) was added into each culture flask after 24 hours of incubation. An hour prior to harvesting, the cultures were incubated with colcemid (10 µg/ml). Lymphocyte cells were harvested by swelling them in 0.075 M potassium chloride and then fixed three times in 3:1 (v/v) methanol/glacial acetic acid. Well spread metaphase cells were prepared on a slide and aged overnight in a 56°C oven.

Analyses were done according to the method of Benn and Tantravahi 17 where slides were firstly immersed into Hoechst solution (0.5 µg/ml) for 30 minutes and then exposed to UV for 2.5 hours in the presence of saline sodium citrate (SSC). Giemsa dye solution was used to stain the slides. For each subject, 100 well spread metaphases containing 46 chromosomes in their second division were analysed for the frequency of SCE.

Lymphocyte culture
Heparinized blood was diluted 2-fold with RPMI 1640 medium (Flowlab, Australia) and lymphocytes were isolated by Ficoll-Hypaque density sedimentation. Lymphocyte suspensions were prepared by adding 1 ml CCM (final volume, 1 ml of RPMI 1640 medium containing 15% AB serum, 100 µg/ml streptomycin and 100 U/ml penicillin) into the supernatant containing lymphocyte cells.

Apoptosis assays
A total of 100 µl peripheral blood lymphocytes (1x10⁶ cells/ml) were cultured for 24 hours in a 96-well flat-bottomed microculture plate with CCM at 37°C in an atmosphere of 5% CO₂ and 90% relative humidity. After 24 hours, cells were harvested by centrifugation and then fixed with 80% methanol. Quantitative determination of apoptotic cell death was then performed using the ssDNA ELISA kit (Chemicon, USA).

Proliferation assays
A total of 100 µl peripheral blood lymphocytes (2x10⁵ cells/ml) were cultured for 72 hours in a 37°C CO₂ incubator. The lymphocytes were cultured in a 96-well flat-bottomed microculture plates with CCM containing PHA at concentrations of 0.5%, 1.0% and 2.0% respectively to determine the lymphocyte mitogenic response. Maximum proliferation capability of lymphocytes was quantified by determining the uptake of 5-bromo-2-deoxyuridine (BrdU) added 18 hours before harvesting the cells and tested with the ELISA Proliferation BrdU kit (Roche, Switzerland).

Statistical analysis
Results obtained were expressed as mean ± SEM. Statistical significance was taken at p<0.05. All data analysis was performed using SPSS version 11.0. The independent t-test was used to ascertain the differences observed between the two groups of subjects.

RESULTS
Results from a preliminary screening questionnaire showed that the sedentary and Tai Chi subjects enrolled in this study were either retirees or housewives. Both groups of subjects were matched for age and gender. Preliminary assessment of dietary habit using a food frequency questionnaire also reviewed that the two groups of subjects had no significant differences in their nutrient intakes. Although the Tai Chi participants were significantly shorter (p<0.05) as compared to the sedentary subjects, both groups were not significantly different in their BMI (Table I). Tai Chi participants selected for the study practiced the exercise five times a week for a mean duration of 6.9 ± 0.6 years. Results of the physical examination revealed that the Tai Chi participants had lower heart rate (p<0.05) as compared to the sedentary subjects (Table II). In all the participants, results of routine biochemical and hematological analyses were found to be within the normal range. Blood biochemistry tests also showed that the Tai Chi participants had higher plasma HDL (p<0.05) and creatinine (p<0.05) concentrations than the sedentary subjects (Table II).
The level of DNA damage seen on each cell was determined based on the degree of tail length (Figure 1). The percentage of normal DNA was significantly higher (p<0.05) in the Tai Chi participants than the sedentary subjects. The Tai Chi participants also had lower levels of mild (p<0.05) and severely (p<0.05) damaged DNA as compared to the sedentary subjects (Table III).

The frequency of SCE in the Tai Chi participants and controls are shown in Figure 2. Under the microscope, chromosomes with SCE were stained in two shades of dark and light purple on their chromatids and arranged in a mosaic pattern. The frequency of SCE for each subject was determined by counting the number of chromosomes with SCE in complete metaphases which contain 46 chromosomes. When compared with the sedentary subjects, the Tai Chi participants showed a lower frequency of SCE (p<0.05) in cultured peripheral lymphocytes at resting conditions (Table III).

The percentage of cell apoptosis was significantly higher (p<0.05) in the Tai Chi participants than the sedentary subjects (Table III). This indicated that the Tai Chi subjects experienced a higher level of lymphocyte cell death at resting conditions when compared to the sedentary controls. The absolute absorbance values of lymphocyte proliferation test were read using the ELISA reader. The highest absorbance value from three concentrations of PHA was selected as the maximum proliferation ability of lymphocyte for each subject. The mean proliferation ability of the Tai Chi participants was significantly (p<0.05) higher than the sedentary subjects.

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### Table I: Demographic data of sedentary and Tai Chi participants

<table>
<thead>
<tr>
<th></th>
<th>Sedentary (n=35)</th>
<th>Tai Chi participant (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Females</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.0 ± 1.1</td>
<td>58.5 ± 0.9</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.6 ± 1.5</td>
<td>58.7 ± 1.4</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.58 ± 1.1</td>
<td>1.55 ± 0.7*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6 ± 0.5</td>
<td>24.5 ± 0.6</td>
</tr>
</tbody>
</table>

BMI = body mass index  
* significant difference between Tai Chi participants and sedentary subjects

### Table II: Physical examination and blood test data of sedentary and Tai Chi participants

<table>
<thead>
<tr>
<th>Test</th>
<th>Sedentary (n=35)</th>
<th>Tai Chi participant (n=35)</th>
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</thead>
<tbody>
<tr>
<td>Physical examination:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>71 ± 1</td>
<td>66 ± 1*</td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>138 ± 4</td>
<td>141 ± 3</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>85 ± 2</td>
<td>82 ± 2</td>
</tr>
<tr>
<td>Blood biochemistry test:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.40 ± 0.23</td>
<td>5.54 ± 0.14</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.36 ± 0.12</td>
<td>1.28 ± 0.07</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.41 ± 0.20</td>
<td>3.66 ± 0.12</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.41 ± 0.08</td>
<td>1.61 ± 0.06*</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>139.48 ± 0.51</td>
<td>140.35 ± 0.39</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>4.22 ± 0.07</td>
<td>4.08 ± 0.05</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>103.66 ± 0.59</td>
<td>103.82 ± 0.46</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>4.66 ± 0.22</td>
<td>4.38 ± 0.18</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>66.20 ± 1.50</td>
<td>74.97 ± 2.99*</td>
</tr>
</tbody>
</table>

LDL = low density lipoprotein, HDL = high density lipoprotein  
* significant difference between Tai Chi participants and sedentary subjects

### Table III: Comparison between the levels of DNA damage, frequency of SCE, lymphocyte apoptosis and proliferation between sedentary subjects and Tai Chi participants

<table>
<thead>
<tr>
<th></th>
<th>Sedentary (n=35)</th>
<th>Tai Chi (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comet Assay (%DNA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>84.7 ± 2.4</td>
<td>90.8 ± 0.9*</td>
</tr>
<tr>
<td>Mildly damaged</td>
<td>14.5 ± 2.4</td>
<td>8.0 ± 0.7*</td>
</tr>
<tr>
<td>Severely damaged</td>
<td>14.5 ± 2.4</td>
<td>8.0 ± 0.6*</td>
</tr>
<tr>
<td>SCE (frequency/metaphase)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apoptosis</td>
<td>5.7 ± 0.3</td>
<td>4.6 ± 0.4*</td>
</tr>
<tr>
<td>(% apoptotic cells)</td>
<td>7.7 ± 1.0</td>
<td>11 ± 0.8*</td>
</tr>
<tr>
<td>Proliferation (absolute absorbance)</td>
<td>1.2 ± 0.1</td>
<td>1.5 ± 0.1*</td>
</tr>
</tbody>
</table>

* significant difference between Tai Chi participants and sedentary subjects
DISCUSSION

Results obtained from our study have shown that long term regular Tai Chi exercise helped to reduce the heart rate of older adults. This is similar to the results of previous studies, which have reported that adaptation to regular exercise helped to reduce heart rate \(^1\). Besides improvement in strength of heart muscles \(^1\), this effect has been suggested to be due to more efficient blood utilization in the tissues as a result of increased density of capillaries in the skeletal muscles \(^2\). The reduction of heart rate among the older adults is beneficial as it helps to reduce their cardiac workload \(^2\).

Similar to other forms of exercise \(^3,^4\), adaptation to regular Tai Chi has also been found to increase the level of HDL among the older adults. The increase in HDL is crucial as it helps to prevent coronary artery diseases by transporting excess cholesterol into the biliary system for decomposition\(^5\). Besides impeding the accumulation of cholesterol in peripheral tissues, regular Tai Chi exercise is also suggested to help increase the muscle mass of older adults as increased creatinine levels have been found in the Tai Chi participants as compared to their sedentary counterparts. The concentration of creatinine have been reported to reflect the muscle mass of the body as creatinine is formed spontaneously from creatine, where its concentration correlates significantly with muscle mass\(^6\).

Regardless of the exercise protocol used, increases in DNA damage have been reported, generating consensus that exercise does induce DNA damage\(^7\). Some of these studies have employed the comet assay method to investigate the effect of exercise on oxidative DNA damage\(^8\). A recent study reported that 14 weeks of progressive resistance exercise training resulted in a significant reduction in oxidative damage to DNA in older adults. Based on this investigation, it was suggested that exercise training may be an effective means of improving electron flux in the mitochondria and up-regulating DNA repair mechanisms\(^9\). Reduced DNA damage may also be attributed to increased clearance of damaged cells, redistribution of damaged cells and up-regulation of cellular proteolytic enzymes as a result of adaptive response to regular training\(^10\). In the present study, we found that practicing Tai Chi exercise for about six years

Fig. 1: Comet assay images of DNA at different degree of damages (ethidium bromide stain, 200X).
Score 1 = normal DNA, score 2 & 3 = mildly damaged DNA, score 4 & 5 = severely damaged DNA.

Fig. 2: Representative example of chromosomes with SCE in a metaphase stained with giemsa.
Reduced oxidative DNA damage in older adults. Results of our investigation are in line with these previous findings reporting that exercise helped to stimulate DNA repair mechanisms because the lower level of DNA damage observed in the Tai Chi subjects was accompanied by a reduction in the frequency of SCE, which is produced from the misrepair of DNA occurring during the repair process.

Stable chromosome aberrations were found to accumulate with age as compared to unstable chromosome aberrations but lifestyle factors have been suggested to contribute to the accumulation of cytogenetic damages. Exercise to exhaustion has been reported to have no influence on the frequency of SCE in human lymphocytes. However, similar to the results of our study, previous researchers have also found that the frequency of SCE in the peripheral blood lymphocytes are lower in healthy Tai Chi practitioners as compared to their sedentary counterparts at resting conditions. If SCE represents a form of DNA misrepair as has been suggested earlier, our findings would indicate that long term Tai Chi exercise increases the proficiency of DNA repair mechanism.

Long term Tai Chi exercise was also found to increase lymphocyte apoptosis and proliferation in our subjects. The high percentage of apoptotic cell death among the Tai Chi participants even at rest is in agreement with a study which suggested that apoptosis signal persist for 24 hours after a bout of exercise. Older adults have been suggested to be more susceptible to apoptosis because of enhanced production and reduced elimination of ROS in the aging process. ROS may induce cell apoptosis through the mitochondria regulated pathway where it reduces the potential between the membranes of mitochondria and causes the release of pro-apoptotic molecules that activates the caspase cascade of the cell apoptosis pathway. As lymphocyte apoptosis was found to be elevated in the Tai Chi participants, ROS produced during each session of regular Tai Chi exercise might have helped to increase apoptosis signal in the older adults even at rest. However, the amount of ROS generated by the exercise is suggested to be relatively low but sufficient to stimulate apoptosis as no visible oxidative DNA damage was found in the Tai Chi participants. It has been suggested that regulated lymphocyte apoptosis is crucial for the coordination of immune response and lymphocyte population.

Previously, lymphocyte apoptosis has been documented immediately after exercise and was suggested to be the cause of lymphopenia and reduced immunity. Another study reported that lymphocyte apoptosis may also contribute to the regulation of immune response after exhaustive exercise. However, whether the mechanism could be regarded as beneficial for deletion of autoreactive cells or suppression of the immune response was unclear. Despite the higher level of lymphocyte apoptosis among our Tai Chi subjects, the exercise was not deemed to cause lymphopenia as there was a significant elevation of lymphocyte proliferation as compared to the control subjects. Elevated lymphocyte proliferation is beneficial as it helps to restore the decreased immune response, which usually accompanies the aging process.

The increased apoptosis and proliferation of lymphocytes with Tai Chi exercise may also help to prevent replicative senescence in aging. In an *in vitro* model of cellular aging, T cells, which were stimulated to divide repeatedly, were found to reach an irreversible state of growth arrest known as replicative senescence. It was suggested that the diminished capacity of cells to undergo apoptosis caused progressive accumulation of these T cells in aging, chronic infection and autoimmune diseases.

In conclusion, long term Tai Chi exercise stimulated apoptosis and proliferation of lymphocytes, even at rest, possibly through mild enhancement of oxidative stress in the older adults. The increased occurrence of these processes may help to prevent replicative senescence by renewal and regeneration of old lymphocytes. Tai Chi exercise is also beneficial in improving the proficiency of DNA repair mechanisms as evidenced by diminished frequency of SCE in the Tai Chi participants.

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