IS THERE A SIMILARITY BETWEEN DNA DAMAGE IN ADULTS WITH CHRONIC ALCOHOLISM AND COMMUNITY-DWELLING HEALTHY OLDER ADULTS?

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Abstract — Aims: Daily alcohol consumption and ageing have been linked with DNA damage, leading to the hypothesis that chronic alcoholism causes DNA damage similar to that which occurs with ageing. Likewise, it has been suggested that chronic alcoholism is the cause of accelerated or premature ageing. The objective of this study was to evaluate the frequency and magnitude of DNA damage among adults with chronic alcoholism and healthy older adults residing in Mexico City. Methods: A cross-sectional and comparative study was carried out in a sample of 53 chronic alcoholics of 25–44 years of age (without alcohol ingestion in the past 30 days) without additional diseases, 26 healthy subjects >60 years of age, and 25 healthy adults of 25–44 years of age without alcohol addiction, all residents of Mexico City during the past 10 years. DNA damage was evaluated by single-cell gel electrophoresis technique (Comet assay). Results: Our results showed a similar percentage of DNA damage between healthy elderly subjects and chronic alcoholics (62 vs 55%, P >0.05), although average DNA migration was greater in alcoholics than in the elderly (78.1 ± 33.2 vs 58.6 ± 26.2, P = 0.09). However, the percentage of subjects with more than six damaged cells was higher in the older adults subjects group than in the group chronic alcoholics (19 vs 35%, P = 0.16). Conclusions: Data suggest that DNA damage is not similar in young subjects with chronic alcoholism that which occurs with ageing.

INTRODUCTION

Chronic and excessive consumption of alcoholic beverages provokes membrane lipoperoxidation due to triglyceride accumulation in hepatocytes, increasing fat in the cell that serves as substrate for its oxidation and thus forms free radicals (FR) (Ueshima et al., 1993; Sherman and Williams, 1994; Achord, 1995; Lieber, 1997, Lamarche et al., 2003). Likewise, this favours protein degradation and DNA oxidation in which acetaldehyde, the product of ethanol metabolism, is able to form links with free sulfhydryl and amino groups (denominated Schiff bases), provoking toxic effects, inducing crossovers and oxidation in DNA, as well as formation of adducts with proteins (Behrens et al., 1988; Tamame-González and Toranzo-Martínez, 1988; Nelson et al., 1992; Brooks, 1997). In such regard, acetaldehyde joins with hepatic glutathione, establishing hemiacetyl links with the L-cysteine amino acid that forms part of the glutathione molecule. In this sense, the cytotoxicity of the acetaldehyde is very similar to the free-radical theory proposed to explain cell ageing (Sillanaukee et al., 1992; Wehr et al., 1993). Also it was pointed that ageing is the product of the accumulation of oxidative damage in organs and systems due to the biochemical processes of normal aerobic metabolism (Harman, 1981; Kristal and Yu, 1992; Rusting, 1992).

On the other hand, it has been pointed out that oxidative stress (OxS) associated with excessive FR production is the cause of cell damage similar to that which takes place with ageing (Harman, 1992); similarly, it has been demonstrated that chronic and excessive consumption of alcoholic beverages provokes OxS and consequently liver damage, cardiovascular diseases, and cancer (Ryan, 1982; Holden et al., 1988; Varga, 1991; Trujillo and Espinosa, 1992; Poli, 1993; Brooks and Theruvathu, 2005). Likewise, it has been proposed that chronic alcoholism is the cause of accelerated or premature ageing, evidence of which can be observed through DNA measurement (Noonberg et al., 1985; Carrano and Natarajan, 1988; Singh et al., 1988, 1991a,b; Gedik et al., 1992; McKelvey-Martin et al., 1993; Anderson et al., 1994; Ross et al., 1995). For this reason, the objective of the present study was to evaluate the similarity between DNA damage in chronic alcoholic subjects and in healthy elderly individuals.

MATERIALS AND METHODS

Design and subjects
A cross-sectional and comparative study was carried out in a sample of 53 adults with chronic alcoholism, with a mean age of 36 ± 5.4 years, and a mean consumption of ethanol 383.23 ± 21.0 g/d during the past 10 years (without alcohol ingestion in the past 30 days) and without additional diseases, 26 community-dwelling healthy older adults >60 years of age, with a mean age of 70 ± 7.8 years and 25 healthy adults of 25–44 years of age, with a mean age of 32 ± 4.5 years, without alcohol addiction. All subjects had lived in Mexico City for the past 10 years at the time of the study and showed normal haemoglobin, hematocrit, leukocytes, glucose, urea, creatinine, urate, albumin, cholesterol, triglycerides, and cholesterol high-density lipoproteins (HDLC).

The subjects agreed to participate in the study after giving their informed consent. The Ethics Committee of the Universidad Nacional Autónoma de México (UNAM), Zaragoza Campus, approved the research protocol for the study, in accordance with the Helsinki Declaration.
**Nutritional measurement**

Subjects of the three groups were Well-nourished: (i) body mass index (BMI; weight in kilograms divided by the square of height in metres) of 22.1–27 kg/m², (ii) caloric intake > 2000 cal (8368 J) per day, and serum albumin > 35 g/l, (iii) proteins and vitamins were between cut-off points of RDA (Institute of Medicine, 2003). The intake of calories, proteins and vitamins was measured by dietary recalls with standardized software (Hernandez-Avila et al., 1998).

**Blood and biochemical analyses**

Blood samples were collected after a 12-h fasting period by venipuncture procedure and placed in vacutainer, siliconized test tubes containing no additives and a separating gel. Heparin was the anticoagulant agent used. Blood samples containing heparin were analysed using complete haemoglobin test protocol (including haemoglobin, hematocrit, and leucocyte counts). The serum obtained from samples containing no anticoagulant agent was subjected to the following tests: glucose, urea, creatinine, urate, albumin, cholesterol, triglycerides, HDL, total bilirubin, and conjugated bilirubin concentrations; alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), and pseudocholinesterase (CHS) activities. All reagents employed in biochemical tests were obtained from Randox Laboratories, Ltd (Crumlin, Co., Antrim, UK). Cut-off points for reference values were determined at the Gerontologic Clinical Research Laboratory of the Universidad Nacional Autónoma de México (UNAM), Zaragoza Campus, in Mexico City (Sánchez-Rodríguez et al., 1998).

These tests were used as screening measurements for diagnosis of clinically healthy subjects.

**Alkaline unicellular electrophoresis**

The alkaline unicellular electrophoresis assay using blood samples was performed as described by Singh et al., 1988. The positive control was made with 200 µl of whole blood in 1 ml of Hanks Balanced Salt Solution (Ca²⁺, Mg²⁺-free) containing 200 µM H₂O₂ for 30 min, immersed in an ice-cold water; at the end of the treatment period, removed supernatant and the pellet the cells used for the assay.

The analysis of DNA damage was done in blinded fashion and the migration was calculated as the difference between length and diameter. The criterion for determining DNA damage was to follow the amounts of DNA in the tail: without DNA damage (DNA migration < 5%); with DNA damage (DNA migration > 5%). Another criterion for evaluation was degree DNA damage was low degree DNA damage when cells had 5–40% and high degree DNA damage when cells had > 40% (Fig. 1) (Anderson et al., 1994; Mendoza-Núñez et al., 2001); also we evaluated the magnitude of DNA damage by number of cells by individual. In this sense, it was used two categories: low magnitude when subjects had 1–5 cells with DNA damage and high magnitude when subjects had 6 more cells with DNA damage.

**Statistical analysis**

Data were processed by use of standard statistical software SPSS 10.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics are means ± standard deviation (SD) and percentages; median values with 25th and 75th percentiles. Mann–Whitney test, Kruskal–Wallis test, chi-square test or Fisher’s exact were used for statistical analyses as appropriate, with a significance level of P < 0.05, and odds ratio (OR) of logistic regression analysis with 95% confidence interval (CI) were performed.

**RESULTS**

**Biochemical characteristics and hepatic enzymes**

We found that serum GGT and AST were increased in healthy older adults and adults with chronic alcoholism, compared with healthy adults (P < 0.05); however, bilirubins and ALT serum levels in healthy adults and healthy older adults were similar (P > 0.05), although in alcoholics was increased (P < 0.05). Nevertheless, the values of hepatic enzymes and biochemical characteristics of the three study groups remained within reference values (Table 1).

**DNA damage**

We found a DNA damage frequency of 16% in healthy adults, in contrast with 55% in adults with chronic alcoholism and 62% in healthy older adults; these differences were statistically significant on comparison between healthy adults vs alcoholic adults (P < 0.05) and healthy adults vs elderly adults (P < 0.05). Likewise, we observed that DNA damage frequency in groups of healthy older adults and adult alcoholics was statistically similar (P = 0.82) (Fig. 2).

**DNA migration**

On comparing migration totals of the comet tail without taking into consideration nucleus and total migration, the group of
alcoholic adults demonstrated an average DNA migration value that was higher than in group of healthy elderly adults and the average was significantly higher than in group of healthy adults ($P < 0.05$) (Table 2 and Fig. 3).

**Cell damage magnitude**

The healthy adults not showed a magnitude of six or more cells with DNA damage in contrast with adult alcoholics and healthy older adults, in whom we found 19 and 35%, respectively, with six or more cells with DNA damage (Table 3).

**Degree of DNA damage**

With regard to degree of DNA damage, we observed in the healthy adult group a significantly lower percentage of DNA damage by $>40\%$ in comparison with adult alcoholics and healthy older adults ($P < 0.05$); nonetheless, the percentage of DNA damage $>40\%$ between adult alcoholics and healthy older adults was similar ($P > 0.05$) (Table 4).

**DNA damage-associated risk factors**

The multivariate logistic regression analysis showed that the alcohol ingestion and age ($>60$ years) were a risk factors for DNA damage (OR = 2.28, 95% CI = 1.1–4.73; $P < 0.05$; OR = 1.06, 95% CI = 1.02–1.11; $P < 0.05$, respectively). In relation of smoking the logistic regression analysis not showed significantly influence (Table 5).

**DISCUSSION**

Alcohol acts in a very varied manner within the organism and chronic alcohol consumption is associated with an increase in the incidence of a variety of diseases ranging from hepatitis to cancer (Brooks, 1997). It has been observed that high ethanol consumption results in an increase of OxS with the formation of FR and lipid peroxidation, the process of which produces cellular ageing. In this respect, it has been demonstrated that at the neurological level alcoholism provokes accelerated ageing due to biochemical changes provoked by ethanol in the nervous system; these changes are very similar to those occurring in normal ageing (Noonberg et al., 1985; Holden et al., 1988; Trujillo and Espinosa, 1992).

OxS has been linked to numerous chronic degenerative diseases, such as diabetes mellitus, cancer, rheumatoid arthritis, atherosclerosis, cataracts, and Alzheimer disease, because OxS provokes cell damage in different organs such as kidney, heart, brain, and liver in the same way in which the ageing of systems occurs and in which chronic ingestion of alcohol can favour these processes (Varga, 1991; Nohl, 1993; Poli, 1993).

On the other hand, it has been demonstrated that evaluation of DNA damage is a good biological marker for OxS; thus, this is considered a fragility indicator for older adults and one that can be useful for detecting cellular damage produced by ethanol (Duthie et al., 1996; Retana-Ugalde et al., 1997). In this regard, some in vitro studies show that chronic ethanol

**Table 1. Median values and 25th and 75th percentiles of total bilirubin and hepatic enzymes in healthy adults, adults with chronic alcoholism and healthy older adults**

<table>
<thead>
<tr>
<th></th>
<th>Healthy adults</th>
<th>Adults with chronic alcoholism</th>
<th>Healthy older adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.7 (0.56–0.9)</td>
<td>0.99 (0.78–1.35)$^1$</td>
<td>0.68 (0.47–0.9)</td>
</tr>
<tr>
<td>Conjugated bilirubin (mg/dl)</td>
<td>0.15 (0.1–0.2)</td>
<td>0.15 (0.14–0.29)$^1$</td>
<td>0.14 (0.12–0.16)</td>
</tr>
<tr>
<td>No-conjugated bilirubin (mg/dl)</td>
<td>0.56 (0.42–0.7)</td>
<td>0.77 (0.51–0.95)$^1$</td>
<td>0.54 (0.33–0.79)</td>
</tr>
<tr>
<td>AST $\times 10^{9}$ (U/l)</td>
<td>7.0 (4.0–10.0)</td>
<td>10.0 (6.0–12.0)$^*$</td>
<td>10.0 (6.0–14.0)$^*$</td>
</tr>
<tr>
<td>ALT $\times 10^{9}$ (U/l)</td>
<td>4.0 (3.0–6.0)</td>
<td>6.5 (4.0–10.0)$^1$</td>
<td>5.0 (3.0–8.0)</td>
</tr>
<tr>
<td>GGT $\times 10^{3}$ (U/l)</td>
<td>12.0 (9.0–15.0)</td>
<td>20.0 (11.0–26.0)$^*$</td>
<td>17.0 (11.0–23.0)$^*$</td>
</tr>
<tr>
<td>CHS $\times 10^{5}$ (U/l)</td>
<td>3.7 (2.5–5.1)</td>
<td>4.7 (4.1–5.9)</td>
<td>3.6 (2.3–6.3)</td>
</tr>
</tbody>
</table>

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; CHS, pseudocholinesterase.

$^*$Kruskal–Wallis test, $P < 0.05$, healthy adults vs healthy older adults and healthy adults vs adults with chronic alcoholism.

$^1$Kruskal–Wallis test, $P < 0.05$, healthy adults vs adults with chronic alcoholism and healthy older adults vs adults with chronic alcoholism.

**Fig. 2. Frequency of DNA damage in healthy adults, adults with chronic alcoholism and healthy older adults. Chi-square test. Percentage of DNA damage: adults with chronic alcoholism vs healthy adults $P < 0.001$; older adults vs healthy adults $P < 0.01$; adults with chronic alcoholism vs older adults $P > 0.05$.**
consumption increases DNA damage in lymphocytes, and that it is acetaldehyde, the product of ethanol metabolism, that causes this; similarly, the mechanism proposed is that of FR generation (Singh and Khan, 1995).

In terms of OxS, results reported in the scientific literature are inconsistent, above all in elderly population, because there are authors who note that ageing in itself is accompanied by OxS, and that therefore, there is greater damage to biomolecules in comparison with young adults (Martin and Rademaker, 1987; Pacifici and Davies, 1991; Markesbery, 1997). In this regard, in our study we observed greater DNA damage (Table 2).

### Table 2. Mean values ± SD of DNA migration in healthy adults, adults with chronic alcoholism and healthy older adults

<table>
<thead>
<tr>
<th>Migration (μm)</th>
<th>Healthy adults</th>
<th>Adults with chronic alcoholism</th>
<th>Healthy older adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comet tail without nucleus</td>
<td>21.8 ± 7.1</td>
<td>52.9 ± 33.4</td>
<td>33.6 ± 26.2</td>
</tr>
<tr>
<td>Total migration</td>
<td>41.3 ± 6.7</td>
<td>78.1 ± 33.2</td>
<td>58.6 ± 26.2</td>
</tr>
</tbody>
</table>

Mean values ± SD; Mann–Whitney test.

Total migration: healthy adults vs adults with chronic alcoholism, \( P = 0.03 \); healthy adults vs healthy older adults, \( P = 0.14 \); adults with chronic alcoholism vs healthy older adults, \( P = 0.09 \).

![Graphs](image-url)

Fig. 3. (a) The graphic shows DNA migration of cells of healthy adults. (b) The graphic shows DNA migration of cells of adults with chronic alcoholism. (c) The graphic shows DNA migration of cells of healthy older adults. The highest DNA migration was observed in adults with chronic alcoholism followed by the healthy older adults and healthy adults.
Percentage of subjects by number of cells with DNA damage for subject.

*R: Chi-square test, six and more cells: adults with chronic alcoholism vs healthy older adults, \( P = 0.16. \)

Table 3. DNA damage by number of cells in healthy adults, adults with chronic alcoholism and healthy older adults

<table>
<thead>
<tr>
<th>Cells with DNA damage</th>
<th>Healthy adults</th>
<th>Adults with chronic alcoholism</th>
<th>Healthy older adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>One cell</td>
<td>23 (84%)</td>
<td>23 (42%)</td>
<td>10 (38%)</td>
</tr>
<tr>
<td>Two cells</td>
<td>18 (69%)</td>
<td>19 (36%)</td>
<td>12 (46%)</td>
</tr>
<tr>
<td>Three cells</td>
<td>12 (46%)</td>
<td>15 (29%)</td>
<td>6 (23%)</td>
</tr>
<tr>
<td>Four cells</td>
<td>10 (38%)</td>
<td>11 (21%)</td>
<td>5 (19%)</td>
</tr>
<tr>
<td>Five cells</td>
<td>8 (31%)</td>
<td>10 (19%)</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Six or more cells</td>
<td>5 (19%)</td>
<td>8 (15%)</td>
<td>3 (11%)</td>
</tr>
</tbody>
</table>

Table 4. Degree of DNA damage in cells of healthy adults, adults with chronic alcoholism and healthy older adults

<table>
<thead>
<tr>
<th>Degree of DNA damage</th>
<th>5–40% Number of cells (%)</th>
<th>≥40% Number of cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy adults</td>
<td>5 (50%)</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>Adults with chronic alcoholism</td>
<td>8 (6%)</td>
<td>12 (94%)</td>
</tr>
<tr>
<td>Healthy older adults</td>
<td>12 (14%)</td>
<td>77 (86%)</td>
</tr>
</tbody>
</table>

The table shows the total number and percentage of cells with DNA damage by study group. Chi-square test, DNA damage degree ≥40% healthy adults vs adults with chronic alcoholism, \( P < 0.0001; \) healthy adults vs healthy older adults, \( P < 0.01; \) adults with chronic alcoholism vs healthy older adults, \( P > 0.05. \)

Table 5. Alcohol ingestion, age, tobacco and coffee as risk factors for DNA damage

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>OR(^1)</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol ingestion</td>
<td>2.28</td>
<td>1.10–4.73</td>
<td>0.026</td>
</tr>
<tr>
<td>Age</td>
<td>1.06</td>
<td>1.02–1.11</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoke (&lt;10 cigarettes)</td>
<td>1.45</td>
<td>0.5–4.17</td>
<td>0.492</td>
</tr>
<tr>
<td>Coffee ingestion</td>
<td>1.78</td>
<td>0.67–4.71</td>
<td>0.247</td>
</tr>
</tbody>
</table>

\(^1\)Logistic regression \( R^2 = 0.297; \) OR, odds ratio.

With respect to reported studies on alcoholic subjects, elevation of chromosomal changes, induction of sister chromatids, and breaking of DNA chains have been found. Similarly, studies have been conducted on ethanol and acetaldehyde-associated chromosomal-damage induction mechanisms; nonetheless, the results are unclear, because there are authors who mention that ethanol is an antioxidant, as well as the fact that ethanol is mutagenic, carcinogenic, and teratogenic in humans (Hayes, 1985; Singh and Khan, 1995; Phillips and Jenkinson, 2001; Tavares et al., 2001).

In such regard, the results obtained in the present study showed that the DNA damage frequency observed in adults with chronic alcoholism and in older adults is similar (elderly adults, 62% vs young alcoholics, 55%; \( P > 0.05 \)). In this sense, Varga (1991), points out that chronic exposure to alcohol favours premature ageing, because the cellular mass condensation observed during ageing coincides with lipid and protein accumulation observed in alcohol-related liver damage.

However, observing mean total of DNA damage migration in these groups, we note that for healthy adults \( 21.8 \mu m \pm 7.1 \) was found, while for older adults, we found \( 33.6 \mu m \pm 26.2 \), values that as previously indicated were lower than those for adults with chronic alcoholism, who demonstrated a value of \( 52.9 \mu m \pm 33.4 \), but not with a statistically significant difference (\( P = 0.09 \)). This allows us to suppose that despite the fact that some authors report that DNA damage increases with age (Singh et al., 1991a; Barnett and King, 1995) and with exposure to alcohol and acetaldehyde (Singh and Khan, 1995; Singh et al., 1995) the degree of damage is not the same, because in older adults this damage presents gradually as the subjects advance in age without significantly affecting the homeostasis of each individual; nevertheless, in alcoholics chronic exposure to ethanol provokes significant and lasting homeostatic alteration, propitiating a higher degree of DNA damage than that occurring with ageing.

Lastly, in risk analysis it is corroborated that although there are similarities in frequency, magnitude, and degree of DNA damage between alcoholics and healthy elderly adults the weight of alcohol ingestion as a risk factor for DNA damage is greater than age. Therefore our results suggest that there are not similarity between DNA damage in adults with chronic alcoholism and community-dwelling healthy older adults.

REFERENCES


