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Evaluation of Nε-(carboxymethyl)lysine and lipid peroxidation in multiethnic Malaysian subjects with type 2 diabetes mellitus

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The glycoxidation product, Nε-(carboxymethyl)lysine (CML), is formed during reaction of glucose with protein under oxidative conditions. The aim of the study was to evaluate the levels of the most common glycoxidation product; Nε-(carboxymethyl)lysine (CML) and the most advanced lipoxidation product, malondialdehyde (MDA) in type 2 diabetic Malay, Indian, and Chinese ethnic groups in the Malaysian population. Studies were performed on age-matched Malay, Indian, and Chinese subjects with type 2 diabetes mellitus. Different biochemical parameters including serum levels of CML, MDA, lipid profile, glucose, and hemoglobin A1c (HbA1c) were measured by standard methods. The relationships between circulating CML and lipids, HbA1c, and MDA, were examined. Correlation studies between CML, lipids, HbA1c, and lipid peroxidation were performed. CML and MDA levels were significantly higher in Malay subjects than in Indian or Chinese patients (p <0.005). A significant difference was also observed in the levels of serum total cholesterol and LDL-cholesterol (p <0.05) between the groups in the study. A positive correlation was observed between serum levels of CML and MDA in Malay diabetic patients, but no such correlation was seen in either the Chinese or Indian populations. The results of this study demonstrate that ethnicity affects CML and MDA circulating levels in Malaysian subjects with type 2 diabetes mellitus.

Key words: Type 2 diabetes mellitus, Nε-(carboxymethyl) lysine, lipoxidation, multiethnic Malaysian subjects.

INTRODUCTION

Type 2 diabetes is the most common form of diabetes comprising 80 to 90% of all diabetic people. Type 2 diabetes mellitus (T2DM) is a global health problem affecting approximately 4.0% of adults in the world in 1995 (Harris et al., 1998) and this prevalence has been projected to increase to 5.4% by 2025 (King et al., 1998). Epidemiological data have considered hyperglycemia to be a major player in the development of macrovascular complications such as coronary artery disease (CAD) and stroke (Laakso, 1999). Macrovascular complications of diabetes mellitus (DM) are due to accelerated atherosclerosis and have an important role in the increased morbidity and mortality suffered by these individuals (Wingard and Barrett-Connor, 1995). For example, a recent meta-analysis study showed that the rate of fatal CAD is higher in diabetic patients than in non-diabetic individuals (5.4% vs. 1.6%) (Huxley et al., 2006). Diabetes causes oxidative stress, not only due to the increased production of mitochondrial reactive oxygen species (ROS) or an impaired endogenous capacity to scavenge free radicals (Brownlee, 2001), but also due to glucose auto-oxidation (Wolff et al., 1991), and to the non-enzymatic glycation of proteins (Brownlee, 2000). In vivo glycation and lipid peroxidation reactions may play a...
central role in diabetic complications. The glycoxidation product, N′(carboxymethyl)lysine (CML), is one of the major advanced glycation end products (AGEs) that forms under oxidative conditions (Bierhaus et al., 1998; Wells-Knecht et al., 1996). Ahmed et al. (2008) have shown the significant predictive power of high serum CML concentrations to the development of CAD in type 2 diabetes patients. They also reported that the association between CML and CAD is independent of other risk factors.

The International Diabetes Federation predicts that, by 2025, the South East Asia Region will have an estimated diabetes prevalence of 7.5% (81.6 million) and an impaired glucose tolerance prevalence of 13.5% (120.5 million) people (sicree et al., 2003). It has been found that ethnicity affects the morbidity and mortality associated with diabetes mellitus (Gu et al., 1998; Shaw et al., 1999; Saydah et al., 2001; Lowe et al., 1997). Of several studies that have assessed the risk of mortality associated with diabetes (Shaw et al., 1999; Saydah et al., 2001; Wei et al., 1998; Schernthaner, 1996), only one (Shaw et al., 1999) included significant numbers of Southeast Asians. The elucidation of these ethnic differences may be particularly relevant in Asia, since the region will experience the greatest increase in diabetes mellitus prevalence over the next 5 to 10 years (Zimmet et al., 2001).

According to the National Health and Morbidity Surveys of Malaysia, the prevalence of diabetes mellitus increased from 6.3% in 1986 (Public Health Institute, 1986) to 8.2% in 1996 (Public Health Institute, 1996), and to more than 10% in 2006 (Public Health Institute, 2006). Based on that prevalence rate, the estimated number of diabetic patients in Malaysia is 2.6 million, indicating that Malaysian individuals have a tendency to develop diabetes with prevalence variations among Malaysian ethnic groups.

Several studies have shown the higher prevalence of type 2 diabetes among Malays (Ali et al., 1993) and that obesity may be a more common feature of type 2 diabetes in Malays than in other Asian groups (Pan et al., 2004). In addition, a variety of studies in support of previous observations indicate that, Malays have a relatively high prevalence of the metabolic syndrome and type 2 diabetes compared with other Asian ethnic groups (Pan et al., 2004; Lim et al., 2000).

The population of Malaysia comprises three major ethnic groups: Malays, Indians, and Chinese. Therefore, Malaysia presents a good example to study the effect of ethnicity variations in the propensity to oxidative stress and CML formation, and subsequently the increased propensity to develop macrovascular complications in patients with T2DM. The aim of the present study is to determine whether or not ethnicity modifies advanced glycation, lipid peroxidation and the risk factors of macrovascular complications associated with type 2 diabetes mellitus among Malays, Chinese, and Indians living in Malaysia.

MATERIALS AND METHODS

Study population

This study was conducted on 171 patients with type 2 diabetes mellitus: 63 Malay, 58 Indian, and 50 Chinese participants. The patients were matched for age, gender, glucose levels, and diabetes duration. Smokers and former smokers as well as subjects with known hepatic diseases, diabetes complications, or under antioxidant medication were excluded from this study. Plasma and serum samples were obtained from patients who regularly attend the Diabetes Clinics at the University of Malaya Medical Centre. Type 2 DM was considered to be present if the fasting blood glucose was ≥7.0 mmol/L, the 2 h blood glucose in the oral glucose tolerance test (OGTT) was ≥11.1, and/or the patient is using glucose-lowering medication (WHO, 1999). A signed consent form was obtained from all patients prior to their inclusion in the study. The study was approved by the Faculty of Medicine, University of Malaya Medical Ethics Committee, and the local ethics committee for animal experimentation in the Faculty of Medicine, University of Malaya.

CML and malondialdehyde measurements

Polyclonal anti-CML antibodies were developed in female New Zealand white rabbits according to the method described by Ikeda et al. (1996) and these antibodies were used for the measurement of serum CML by competitive enzyme-linked immunosorbent assay (ELISA) in duplicates according to the method used by Makita et al. (1992) as described in our previous work (Ahmed et al., 2007). The absorbance at 405 nm was read using an ELISA reader (MRX Microplate Reader, Dynatech Laboratories Ltd, UK). The amount of plasma malondialdehyde (MDA), an index of lipid peroxidation, was spectrophotometrically determined by the thiobarbituric acid reactive substances (TBARS) method as previously described (Ohkawa et al., 1979). The measurements were performed in duplicates and the optical density of pink color developed in the test reaction was measured by spectrophotometer against distilled water as blank at 532 nm. MDA standard was prepared from acid hydrolysis of 1,1,3,3-tetraethoxypropane (TEP) according to the method of Tsaknis et al. (1998) with modifications. This stock-standard solution, freshly prepared every day, was used for the preparation of standards of malondialdehyde.

Other measurements

Glucose levels were measured using the hexokinase-glucose-6-phosphate dehydrogenase UV-method, in accordance with Kunst et al.’s (1983) method. HDL cholesterol, triglyceride and total cholesterol levels were measured by enzymatic methods using the Dimension Clinical Chemistry System (Dade Behring Inc., Newark, USA). LDL cholesterol was calculated according to Friedewald formula (Friedewald et al., 1972). Percent HbA1c was determined by COBAS INTEGRA systems (Roche diagnostics, F. Hoffmann-La Roche Ltd, Basel, Switzerland) according to the manufacturer’s instructions.

Statistical analysis

All data were checked for normality and expressed as the mean±standard deviation (SD). Chi-square (χ²) test was used for the analysis of categorical variables. Differences between groups in the mean values were assessed using one-way analysis of variance (ANOVA) followed by Bonferroni’s Post Hoc test to adjust the multiple comparisons. When data were not normally distributed,
Table 1. Comparison of demographic and biochemical parameters in the study of ethnic groups.

<table>
<thead>
<tr>
<th></th>
<th>Malays (n=63)</th>
<th>Chinese (n=50)</th>
<th>Indians (n=58)</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>29/34</td>
<td>23/27</td>
<td>30/28</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.1 ± 15.1</td>
<td>44.9 ± 18.8</td>
<td>47.3 ± 18.4</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>6.4 ± 3.1</td>
<td>6.8 ± 2.9</td>
<td>5.6 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78.7 ± 8.5</td>
<td>76.1 ± 7.3</td>
<td>75.7 ± 6.1</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>123.1 ± 18.7</td>
<td>118.2 ± 15.6</td>
<td>117.0 ± 14.8</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.95 ± 1.20</td>
<td>5.90 ± 0.58</td>
<td>6.45 ± 0.79</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.91 ± 0.86</td>
<td>3.03 ± 0.65</td>
<td>3.47 ± 0.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.0 ± 0.36</td>
<td>1.04 ± 0.29</td>
<td>1.05 ± 0.34</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.77 ± 0.91</td>
<td>5.33 ± 0.74</td>
<td>4.95 ± 0.85</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.43 ± 0.57</td>
<td>1.38 ± 0.65</td>
<td>1.22 ± 0.49</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>6.75 ± 2.81</td>
<td>5.98 ± 2.43</td>
<td>6.25 ± 2.60</td>
<td>NS</td>
</tr>
<tr>
<td>MDA (µmol/l)</td>
<td>513.9 ± 111.4</td>
<td>412.3 ± 58.8</td>
<td>431.1 ± 43.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CML (ng/ml)</td>
<td>513.9 ± 111.4</td>
<td>412.3 ± 58.8</td>
<td>431.1 ± 43.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: One-way ANOVA was used for statistical analysis, values are expressed as mean ± SD. *Differences are considered significant if P < 0.05. NS: the difference is not significant.

Table 2. Bivariate and multivariate correlation analysis between CML and clinical parameters in the ethnic groups.

<table>
<thead>
<tr>
<th></th>
<th>Malys</th>
<th>Chinese</th>
<th>Indians</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bivariate correlation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>P Value</td>
<td>β</td>
<td>P Value</td>
</tr>
<tr>
<td>MDA</td>
<td>0.40</td>
<td>0.001</td>
<td>0.325</td>
</tr>
<tr>
<td>LDL</td>
<td>0.43</td>
<td>&lt;0.001</td>
<td>0.356</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.32</td>
<td>0.009</td>
<td>0.255</td>
</tr>
<tr>
<td>MDA</td>
<td>0.07</td>
<td>0.62</td>
<td>0.006</td>
</tr>
<tr>
<td>LDL</td>
<td>0.15</td>
<td>0.28</td>
<td>0.026</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.29</td>
<td>0.04</td>
<td>0.277</td>
</tr>
<tr>
<td>MDA</td>
<td>0.33</td>
<td>0.013</td>
<td>0.30</td>
</tr>
<tr>
<td>LDL</td>
<td>0.32</td>
<td>0.016</td>
<td>0.33</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.03</td>
<td>0.80</td>
<td>0.14</td>
</tr>
</tbody>
</table>

analyses were carried out using Kruskall–Wallis test. Correlations between the parameters were analyzed by bivariate and multivariate correlation analysis. Statistical computations were calculated using SPSS 11.5 for Windows software (SPSS Inc, Chicago, IL, USA). A P value of <0.05 was considered statistically significant.

RESULTS

Demographic and biochemical characteristics of the study groups

Demographic and biochemical characteristics of the 3 ethnic groups are presented in Table 1. The mean ages of the Malay, Chinese, and Indian populations were: 45.1±15.1, 44.9±18.8, and 47.3±18.4 years, respectively. As Table 1 shows, Malays had significantly higher levels of CML (P<0.001), MDA (P<0.001), total cholesterol (P<0.001), and LDL (P<0.001) than the Indian and Chinese patients. Fasting blood glucose and HDL-cholesterol levels were not significantly different among the three groups. Although a statistical significance was not observed in the levels of HbA1c among the study groups, Post-hoc analysis revealed higher levels in Malays than in the Indian and Chinese subjects; P=0.009 vs. P= 0.006, respectively.

Correlations between CML and biochemical parameters in the study subgroups

Table 2 shows both bivariate and multivariate correlations between CML and different biochemical parameters in the three ethnic groups. Bivariate correlation analysis revealed that serum CML levels were significantly correlated with MDA in both Malay (r = 0.40, P = 0.001)
and Indian ($r = 0.33$, $P = 0.013$) diabetic subjects as shown in Figures 1A and B, respectively. In the meantime, a positive and significant correlation was observed between CML and LDL cholesterol among Malay and Indian individuals; $r = 0.43$, $P < 0.001$ vs. $r = 0.32$, $P < 0.016$, respectively. However, in the Chinese population, neither serum levels of MDA nor LDL were correlated with CML concentrations; $r = 0.07$, $P = 0.62$ vs. $r = 0.15$, $P = 0.28$, respectively. Additionally, only the Malay subjects had positive correlations between CML and HbA$_{1c}$ as confirmed by multivariate correlation analysis (Table 2).

**DISCUSSION**

N$^\epsilon$-(carboxymethyl)lysine is a glycoxidation product and its accumulation on tissue proteins has been contributed to the pathogenesis of many of the diabetic complications (Ahmed et al., 2007; Araki et al., 1992; Uesugi et al., 2000). In addition, it has been shown that CML is implicated in the impaired uptake of LDL leading to coronary artery diseases seen in type 2 diabetes (Ahmed et al., 2008). Malondialdehyde is a lipoxidation end product and is well characterized during the oxidation of polyunsaturated fatty acids on LDL (Requena et al., 1996). Although some researchers suggested that glucose may enhance free radical formation (Hicks et al., 1988; Mullarkey et al., 1990), the time course of the formation of lipoxidation end products indicates that oxidative modification occurs after more advanced glycation reactions have taken place. The present study revealed that CML levels and MDA were significantly elevated in Malay subjects when compared to those of the Indian or Chinese populations. This implies that over time modest increases in glucose and LDL concentrations can result in a significant increase of CML and lipid peroxidation products on long-lived proteins, and this might be affected by the lifestyle as well as the eating habits.

**Figure 1.** Relationship between CML and MDA in Malay (A) and Indian (B) diabetic patients.
Malays had the greatest incidence of high LDL and HbA\textsubscript{1c}, followed by the Indians and Chinese. Compared with the Chinese, the Malays and Indians were more likely to have a positive family history of diabetes mellitus. In addition, Indian patients were less likely to have associated hypertension than Malays. Correlation between CML and other clinical parameters showed that, the higher levels of CML were associated with the elevation of both MDA and LDL, which implies an enhanced oxidation process in the Malay and Indians ethnic groups. There have been no previous reports comparing Malays, Indians, and Chinese regarding the aforementioned health aspects. There are reports of enhancement of both oxidation and AGE-modification of LDL in diabetic patients (Bucala et al., 1993; 1994). Our results suggest that, in the Malaysian population, levels of HDL cholesterol correlated with MDA levels, indicating that HDL cholesterol was also subjected to oxidation due to a chronic increase in the glucose levels. This is in line with the previous reports which revealed that, HDL mole-cules are susceptible to structural modifications mediated by various mechanisms including oxidation and glycation. For example, Ferretti et al. (2001) have shown significant increase in TBARS and conjugated dienes in HDL incubated with glucose as compared to control HDL, indicating that that lipid peroxidation and HDL glycation are associated processes. Another study demonstrated that glycoxidized HDL (Gly-ox-HDL) affect the functions of endothelial cells (EC). Therefore, HDL exposure to hyperglycemic conditions could contribute to the accelerated atherosclerosis in diabetic patients (Matsunaga et al., 2001).

Ethnic differences in glycoxidation and lipid peroxidation products might be due to environmental, genetic, and/or dietary factors. It is also possible that the risk factors for diabetes-associated atherosclerosis could differ considerably between ethnic groups because of differences in diet, physical activity, body weight, and lifestyle variations. Information on ethnic differences in the risk factors of T2DM and its complications is, therefore, useful for describing the diabetes related health burden in a population as well as for the planning of practical preventive strategies (Bucala et al., 1993; 1994; Jarrett et al., 1982). In the NHMS III, diabetic patients showed different types of complications such as lower limb amputation (4.3%), strokes (3.4%), and kidney disorders (1.6%). As a result of the fact that, environmental changes can alter the tendency of patients to T2DM complications, knowledge of ethnic differences can be used to delay the onset of complications, and enable doctors to manage their patients better by using strategies for lifestyle changes such as dietary advice and cooking tips.

ACKNOWLEDGEMENTS

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REFERENCES


