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RESEARCH

Serum AGEs in black South African patients with type 2 diabetes

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Objective: The objective of this study was to determine serum advanced glycation end products (AGEs) levels in black South African patients with and without type 2 diabetes (T2D) and to compare the results with those reported for other ethnic/race groups.
Design: Analytical cross-sectional study.
Setting and subjects: The study subjects consisted of 138 black South African patients with T2D and 81 non-diabetic patients at Dr George Mukhari Academic Hospital in Pretoria, South Africa.
Outcome measures: Serum total AGEs (TAGEs), Nε-carboxymethyl-lysine (CML), Nε-carboxyethyl-lysine (CEL) and fluorescent AGEs (FAGEs).
Results: Serum TAGEs, CML and CEL levels but not FAGEs were significantly higher in T2D patients than in non-diabetic patients. Serum TAGEs were lower than those reported for other ethnic/race groups whereas CML and FAGEs were within ranges reported for other ethnic/race groups. Only serum CEL levels were significantly higher in male than in female T2D patients and only serum FAGEs levels were negatively associated with age of the study subjects.
Conclusions: With the exception of FAGEs, serum AGEs levels are significantly higher in T2D than in non-diabetic black South Africans patients. Also, serum TAGE levels black South African appear to be lower than those reported for other ethnic/race groups.

Keywords: Black South Africans, serum AGEs, type 2 diabetes

Introduction

Advanced glycation end products (AGEs) are a heterogeneous group of compounds formed by the non-enzymatic reactions of proteins, lipids or nucleic acids with reducing sugars as part of the so-called 'Maillard reaction'.[1,2] AGEs accumulate gradually with age on long-lived protein such as collagen, but are formed at a more rapid rate in conditions associated with hyperglycaemia and oxidative stress such as diabetes mellitus.[2,3] AGEs are implicated in the development and progression of both microvascular complications (nephropathy, retinopathy and neuropathy) and macrovascular complications (myocardial infarction, stroke and peripheral artery disease) of diabetes.[3–6] It has been established that AGEs contribute to the development of vascular complications of diabetes mellitus through binding and cross-linking of both intracellular and extracellular matrix proteins as well as through the modulation of cellular function via binding to the receptor for advanced glycation end products (RAGE).[6,7]

Twin studies and comparison studies of different ethnic groups suggest that both circulating and tissue AGE levels may be genetically determined. In this regard, Leslie *et al.*[8] investigated whether genetic factors influence serum levels of Nε-carboxymethyl-lysine (CML), the most abundant circulating AGE in monozygotic and dizygotic twin pairs living under different environmental conditions and found that twin correlations for serum CML levels were significantly higher in monozygotic twin pairs (r = 0.71) than in dizygotic twin pairs. In support of the notion that tissue AGE levels may also be genetically determined, studies that have measured and compared tissue AGE levels among Caucasians, African Americans, Chinese, Arabs and South Asians have reported that skin-fluorescent AGE levels are higher among Caucasian groups compared with that in African Americans[9] and Chinese, as well as higher in Arabs compared with South Asians.[10] Although the genes responsible for the above-mentioned racial variations in both serum and tissue AGE levels are still unknown, it has been hypothesised that genetic polymorphism of the enzyme glyoxylase I that is involved in the degradation of some AGEs and their precursors in vivo may play a role in this regard.[11] Also, differences in diet and the way food is prepared among different ethnic groups may contribute to the observed racial differences in both circulating and tissue AGE levels.[12] To the best knowledge of the authors there are no published studies that have investigated and reported on circulating AGE levels among black African individuals and compared these with levels reported for other ethnic groups. Thus, the aim of the current study was to measure serum levels of the major types of circulating AGEs in black South African patients with and without diabetes, and to compare these with those measured in other ethnic groups using similar measurement techniques.

Study design and methods

Study design

The study was an analytical cross-sectional study.

Setting

The study was conducted at Dr George Mukhari Academic Hospital (DGMAH), a tertiary hospital near Pretoria, South Africa, that caters for mostly black African patients and serves as a training site for the Faculty of Health Sciences of the Sefako Makgatho Health Sciences University (formally the Medunsa campus of the University of Limpopo).

Subjects

In total, 138 subjects with type 2 diabetes (T2D) and 81 control subjects were randomly recruited into the study. Patients were excluded from the study if they had evidence of renal disease. All study subjects gave their informed consent after the aim of the study and their rights were fully explained to them. The control group was a convenience sample of patients with no history of diabetes that were admitted to orthopaedic wards at DGMAH. This group of patients was asked to fast overnight before blood samples were collected for the measurement of fasting plasma glucose, urea and creatinine. Only patients with FPG < 6.1 mmol/L, and estimated GFR > 60 ml/min were included in the final control sample. The study was conducted in accordance with the requirements of the research and ethics committee of the University of Limpopo (MREC/P/201/2013:PG).

Measurements

Body weight in light clothing was measured using a calibrated digital electronic scale, to the nearest 0.1 kg. Height was recorded to the nearest 0.5 cm using a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight divided by height in metres squared (kg/m^2). Blood pressure was measured with a blood pressure machine (EDAN Vital Signs Monitor®, model M3A (Dieckhoff & Ratschow Praxisdienst GmbH, Longuich, Germany) in a sitting position after the subject had rested for about 10 min. After an overnight fast, venous blood samples for the measurement of serum AGEs, lipid parameters, urea and creatinine were collected from all participants into blood collection tubes (BD Vacutainer®, Franklin Lakes, NJ, USA), and left to clot for 30 min and centrifuged at 4000 rpm for 15 min at 4°C. The resultant serum samples were then aliquotted and stored at -80 °C until analysed. For blood glucose and glycated haemoglobin (HbA1c) measurements blood samples were collected into citrate and EDTA blood tubes respectively. Serum total AGEs (TAGEs), CML and N$^{\varepsilon}$carboxyethyl-lysine (CEL) were measured respectively using STA-317, STA-316 and STA-300 Oxiselect™ ELISA kits (2BScientific, Upper Heyford, UK) according to the manufacturer's instructions. Fluorescent serum AGEs (FAGEs) were measured according to the method described by Münch *et al.*[13] In brief, 20 µl of serum was diluted to a volume of 10 ml with 20 mM phosphate buffered saline; pH 7.4. Fluorescence of the diluted sample was then measured spectrofluorometrically (excitation at 370 nm and emission at 440 nm) using a GloMax® Multi detection spectrofluorometer (Promega Corp, Madison, WI, USA). Fluorescent readings were expressed as arbitrary units (emission intensity/excitation intensity).

Fasting blood glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol, urea and creatinine were measured using commercially available kits adapted to the Beckman Coulter UniCel DXC 800 Synchron® Clinical System (Johannesburg, SA) available in the National Health Laboratory Services (NHLS) laboratory at the DGMAH. LDL-C was calculated from Friedwald's formula. HbA1c was measured using the immune chemiluminescent assay kit adapted to the Abbot Architect system Ci 8200® (Abbott Diagnostics Division, Johannesburg, SA) in the NHLS laboratory at DGMAH in accordance with the manufacturer's instructions.

Statistical analysis

Demographic and clinical data were expressed as mean ± SD and compared between T2D and non-diabetic patients using the Student t-test for independent samples. Distributions of the different types of serum AGEs were expressed in terms of whisker box plots as median (interquartile range) and compared between T2D and non-diabetic patients as well as between male and female T2D patients using the non-parametric Mann–Whitney U test. The Spearman rank correlation coefficient was used to determine correlations among the different study parameters. *p*-values of less than 0.05 were considered statistically significant. All statistical analysis and distributions were performed using SPSS version 23® software (IBM Corp, Armonk, NY, USA).

Results

Demographic and clinical characteristics of study subjects

Demographic and clinical characteristics of the T2D and non-diabetic patients are summarised in Table 1. Subjects with T2D had significantly higher HbA1c, fasting plasma glucose, BMI, and systolic blood pressure than the control group ($p < 0.05$). Mean HbA1c values were: 9.65 ± 1.18% (81 ± 0.99 mmol/mol) vs. 6.1 ± 2.6% (43 ± 5 mmol/l) ($p < 0.05$). Also, serum lipids did not differ between the T2D and control subjects. Some 86.2% of T2D study subjects had poor glycaemic control. The duration of T2D was more than 10 years in 42.0% of the T2D study subjects and was less than 10 years in 58.0% of the study subjects. Almost equal proportions of T2D subjects were treated with oral hypoglycaemic agents (OHAs) only or OHAs plus insulin (49.3% versus 50.7% respectively) (see Table 1).

Table 1: Demographic and clinical characteristics of T2D study subjects and control

Characteristic	T2D group (*n* = 138) Mean ± SD	Non-diabetic control group (*n* = 83) Mean ± SD	*p*-value
Age (years)	56.9 ± 9.4	51.1 ± 9.8	0.152
FPG (mmol/L)	11.6 ± 3.3	5.2 ± 6.3	0.012
HbA1c (%)	9.7 ± 1.2	6.1 ± 2.6	0.037
HbA1c (mmol/mol)	81 ± 0.99	43 ± 5	0.037
BMI (kg/m^2)	31.6 ± 4.7	26.8 ± 5.5	0.031
TC (mmol/L)	4.70 ± 1.80	4.23 ± 0.95	0.174
HDL (mmol/L)	1.1 ± 0.3	1.08 ± 5.5	0.416
LDL (mmol/L)	2.5 ± 0.15	2.1 ± 0.2	0.511
Triglyceride (mmol/L)	1.2 ± 0.5	1.32 ± 0.4	0.712
SBP (mmHg)	157 ± 16.9	138 ± 11.7	0.041
DBP (mmHg)	85 ± 10.8	84 ± 8.4	0.091
Urea (mmol/L)	6.0 ± 2.5	5.6 ± 1.3	0.421
Creatinine (µmol/L)	94 ± 55.9	86.4 ± 21.1	0.408
GFR (ml/min/1.73m^2)	101.6 ± 22.4	95.7 ± 23.1	0.116
Poor diabetic control (HbA1c > 7.0%), *n* (%)	119 (86.2)	–	–
Duration of diabetes (< 10 years), *n* (%)	80 (58.0)	–	–
Duration of diabetes (> 10 years), *n* (%)	58 (42.0)	–	–
Oral hypoglycaemic agents only, *n* (%)	68 (49.3)	–	–
Oral hypoglycaemic agents + insulin, *n* (%)	70 (50.7)	–	–

Notes: FBG = fasting blood glucose; LDL = low-density lipoprotein; HDL = high-density lipoprotein; HbA1c = haemoglobin A1c; SBP = systolic blood pressure; DBP = diastolic blood pressure; GFR = glomerular filtration rate; BMI = body mass index; TC = total cholesterol.

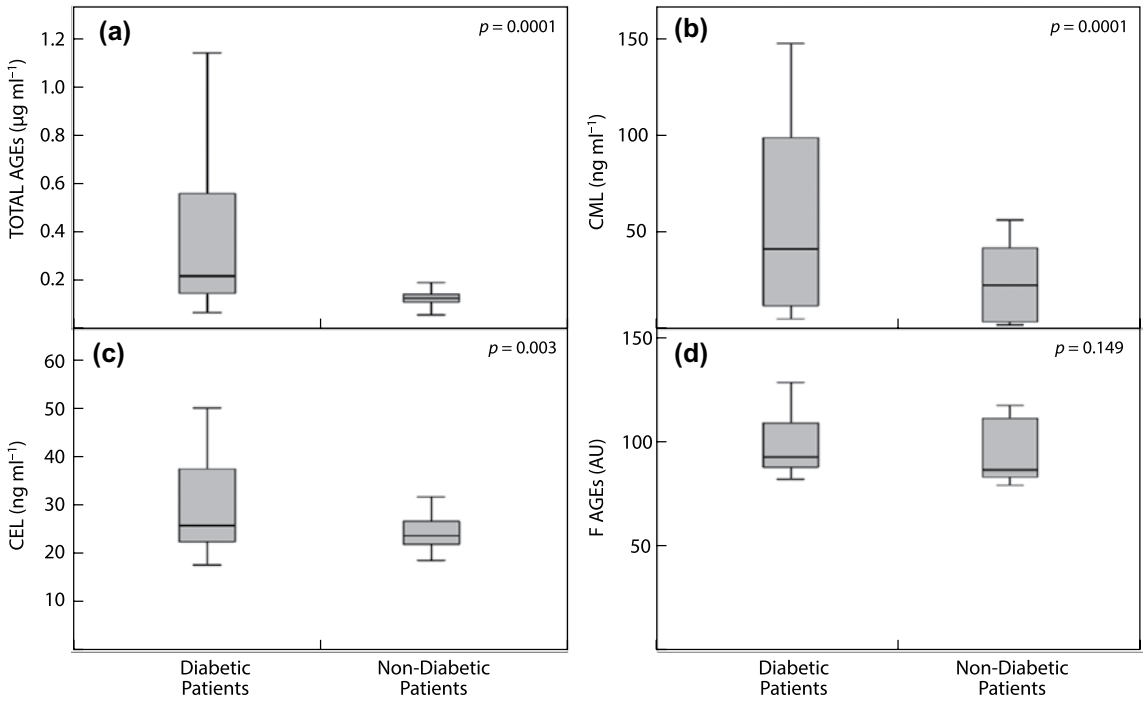

Figure 1: Comparison of serum levels of (A) total AGEs (TAGEs), (B) Nε-carboxymethyl-lysine (CML), (C) Nε-carboxyethyl-lysine (CEL) and (D) fluorescent AGEs (FAGEs) between type 2 diabetic and non-diabetic black South African patients. AU = arbitrary units.

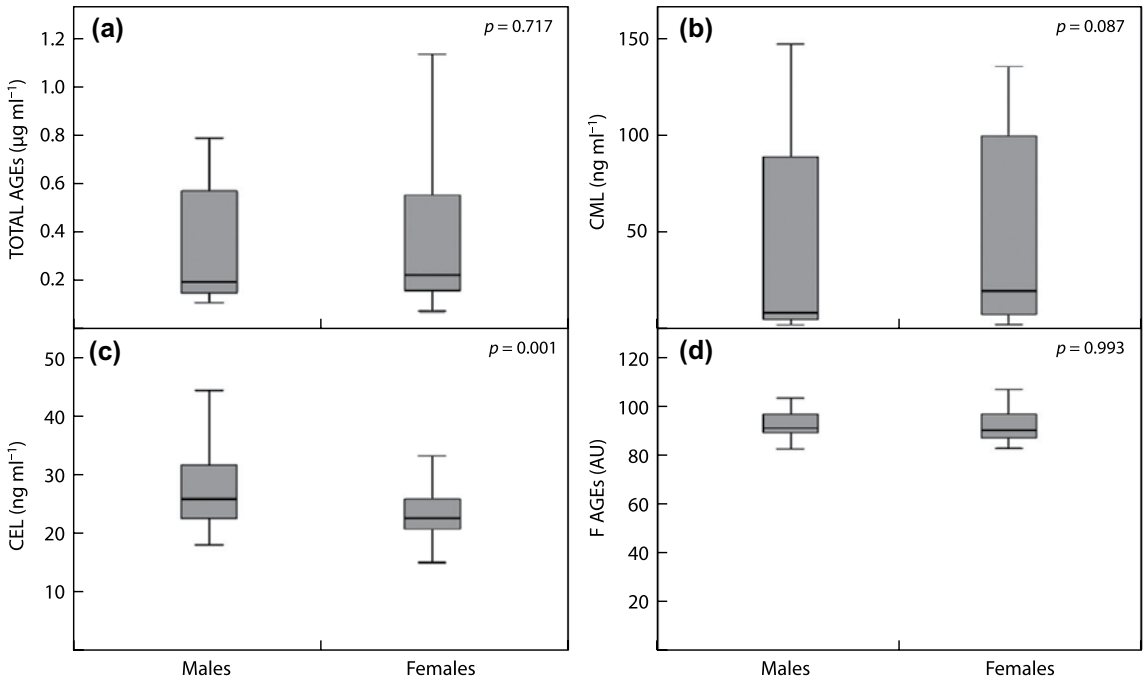

Figure 2: Comparison of serum levels of (A) total immunogenic AGEs (TIAGEs), (B) carboxymethyl-lysine (CML), (C) carboxyethyl-lysine (CEL) and (D) fluorescent AGEs (FAGEs) between male and female black South African type 2 diabetic patients.

Comparison of serum AGE levels of diabetic and non-diabetic study subjects

Box-plot distributions of serum levels of the different types of measured AGEs in T2D and non-diabetic patients after removal of outliers are shown in Figure 1. Serum TAGEs, CML and CEL levels of T2D patients were positively skewed whereas those of the non-diabetic patients were symmetrically distributed. Serum FAGEs levels of both T2D and non-diabetic patients groups were also positively skewed. With the exception of FAGEs the interquartile range (IQR) of all the measured serum AGEs were larger in the T2D group than in the non-diabetic patient group. Serum TAGEs, CML and CEL levels were significantly higher in the T2D patient group compared with the non-diabetic patient group; median (IQR) were 0.21 (0.18–0.58) vs. 0.16 (0.15–0.17) µg/mL, $p = 0.0001$; 47.5 (16.6–91.7) vs. 22.6 (2.2–47.4) ng/mL, $p = 0.0001$; and 27.2 (23.2–43.6) vs. 23.1 (20.2–26.8) ng/mL, $p = 0.001$, respectively. On the other hand there was no significant difference between serum FAGEs levels of the

Table 2a: Correlations between serum AGEs and BMI, age of the study subjects, lipids/lipoproteins as well as HbA1c among the non-diabetic control subjects

Factor		BMI	Age of subjects	LDL-C	HDL-C	TRIGs	HbA1c
TAGEs	r_s	–0.081	0.302	0.004	0.312*	0.120	–0.115
	p (1-tail)	0.331	0.671	0.131	0.031	0.071	0.062
CML	r_s	0.037	0.029	–0.117	0.063	–0.107	0.121
	p (1-tail)	0.251	0.132	0.151	0.350	0.111	0.051
CEL	r_s	0.171	–0.013	–0.169*	–0.011	0.062	0.081
	p (1-tail)	0.118	0.340	0.021	0.410	0.413	0.568
FAGEs	r_s	0.173	0.313*	–0.258*	0.028	0.225*	0.104
	p (1- tail)	0.210	0.044	0.025	0.244	0.031	0.094

Notes: TAGEs = total AGEs; CEL = varboxyethyl-lysine; LDL-C = low-density cholesterol; CML = carboxymethyl-lysine; FAGEs = fluorescent AGEs; HDL-C = high-density cholesterol; r_s = Spearman's correlation coefficient; BMI = body mass index; TRIGs = triglycerides; HbA1c = glycated haemoglobin.
*Significant at the 0.05 level (1-tailed).

Table 2b: Correlations between AGEs and BMI, duration of diabetes, age of the study subjects lipids/lipoproteins as well as HbA1c among the diabetic study subjects

Factor		BMI	Duration of diabetes	Age of subjects	LDL-C	HDL-C	TRIGs	HbA1c
TAGEs	r_s	–0.041	–0.232*	0.146	0.041	0.342*	0.210	–0.251
	p (1-tail)	0.374	0.034	0.358	0.400	0.015	0.094	0.057
	p (1-tail)#	0.413	–	0.018*	0.421	0.180	0.308	0.221
CML	r_s	.071	–0.005	0.179	–0.177	0.083	–0.198	0.171
	p (1-tail)	0.285	0.483	0.410	0.135	0.305	0.107	0.097
	p (1-tail)#	0.190	–	0.830	0.371	0.305	0.288	0.274
CEL	r_s	.160	–0.076	0.183	–0.289*	–0.008	0.005	0.100
	p (1-tail)	0.103	0.278	0.460	0.033	0.481	0.488	0.094
	p (1-tail)#	0.476	–	0.173	0.251	0.492	0.265	0.444
FAGEs	r_s	–.073	0.085	–0.243*	–0.285*	0.031	0.265*	0.174
	p (1- tail)	0.280	0.254	0.024	0.030	0.422	0.041	0.094
	p (1-tail)#	0.291	–	0.034*	0.436	0.492	0.087	0.492

Notes: TAGEs = total AGEs; CEL = carboxyethyl-lysine; LDL-C = low-density cholesterol; CML = carboxymethyl-lysine; FAGEs = fluorescent AGEs; LDL-C = low-density cholesterol; r = Spearman's correlation coefficient; BMI = body mass index; HDL-C = high-density cholesterol; HbA1c = glycated haemoglobin.
#*p*-value for comparison of r_s with that of the non-diabetic control group.
*Significant at the 0.05 level (1-tailed).

Table 3a: Correlation of the different types of serum AGEs measured in the non-diabetic control group with one another

Factor		TAGEs	CML	CEL	FAGEs
TAGEs	r_s	1.00	0.665**	–0.194	0.385
	p (1-tail)		0.000	0.080	0.091
CML	r_s	0.665**	1.00	0.350**	0.209
	p (1-tail)	0.00		0.000	0.101
CEL	r_s	–0.194	350**	1.00	0.102
	p (1-tail)	0.080	0.000		0.214
FAGEs	r_s	0.385	0.209	0.102	1.00
	p (1-tail)	0.091	0.101	0.214	

Note: r_s: Spearman correlation coefficient.
**Significant at the 0.01 level (1-tailed).

T2D patient group and that of the non-diabetic patient group; median (IQR), 94.2 (88.4–109) AU versus 88.9 (86.5–120) AU ($p = 0.149$).

Comparison of serum AGE levels of male and female diabetic subjects

Levels of the different types of serum AGEs measured in the T2D study subjects were stratified by gender and compared in order to determine whether serum AGE levels are influenced by gender. The results shown in Figure 2 indicate that with the exception of median CEL level, which was significantly higher in male than in female diabetic subjects (median [IQR], 27.2 [23.2–43.6] vs. 23.1 [20.2–26.8] ng/mL, $p = 0.001$); no significant differences between median TAGE, CML and FAGE levels of male and female diabetic study subjects ($p = 0.717$, 0.087 and 0.993 respectively) were observed.

Table 3b: Correlation of the different types of serum AGEs measured in diabetic subjects with one another

Factor		TAGEs	CML	CEL	FAGEs
TAGEs	r_s	1.00	0.160	0.191	0.002
	p (1-tail)		0.100	0.063	0.491
	p (1-tail)#		0.000**	0.017*	0.097
CML	r_s	0.160	1.00	0.597**	0.166
	p (1-tail)	0.100		0.000	0.101
	p (1-tail)#	0.000**		0.039*	0.405
CEL	r_s	0.191	0.597**	1.00	0.090
	p (1-tail)	0.063	0.000		0.248
	p (1-tail)#	0.017*	0.039*		0.472
FAGEs	r_s	0.002	0.166	0.090	1.00
	p (1-tail)	0.491	0.101	0.248	
	p (1-tail)#	0.097	0.405	0.472	

*Significant at the 0.05 level (1-tailed).
**Significant at the 0.01 level (1-tailed).
#*p*-value for comparison of r_s with that of the non-diabetic control group.

Correlations between BMI, duration of diabetes, age of the study subjects, lipids/lipoproteins and different types of serum AGEs in diabetic subjects

Factors known to be associated with poor glycaemic control such as BMI, duration of diabetes, age of the study subjects and lipid/lipoprotein levels were correlated with the different types of AGEs measured in the current study using the Spearman correlation coefficient (r_s). Results for the correlations of serum AGEs measured in non-diabetic control and T2D diabetic subjects are presented in Tables 2a and 2b respectively. As shown in Table 2a, for serum AGEs measured in non-diabetic control subjects, significant positive correlations were observed between TAGEs and HDL-C ($p < 0.05$) between FAGEs and TRIGs ($p < 0.05$) as well as between FAGEs and age of the study subjects ($p < 0.05$). Significant negative correlations were observed between CEL and LDL-C ($p < 0.05$) and between FAGEs and LDL-C ($p < 0.05$). For the serum AGEs measured in diabetic subjects, significant positive correlations were observed between TAGEs and HDL-C as well as between FAGEs and TRIGs ($p < 0.05$) (Table 2b). Significant negative correlations were observed between FAGEs and age of the study subjects, between TAGEs and duration of diabetes as well as between FAGEs and LDL-C ($p < 0.05$). In the current study, Spearman correlation coefficients obtained for the association between serum AGEs and BMI, age of the study subjects as well as lipid/lipoprotein parameters were compared with those obtained for the non-diabetic control group. In this regard, significant differences were observed between correlation coefficients of TAGEs and age of the study subjects as well as between FAGEs and age of the study subjects ($p < 0.05$) (Table 2b).

Correlation of the different types of serum AGEs with one another

The current study also investigated whether there were significant correlations among the different types of AGEs measured in the blood sera of the non-diabetic control group as well as those measured among the diabetic group. Results of these objectives are presented in Tables 3a and 3b respectively. As shown in Table 3a, among the serum AGEs measured in the non-diabetic control group, significant positive correlations were observed between CML and TAGEs ($p < 0.01$), as well as between CML and CEL ($p < 0.01$). Amongst the serum AGEs measured in diabetic subjects, a significant positive correlation was observed only between CML and CEL ($p < 0.01$) (Table 3b). When the correlation coefficients obtained amongst the measured serum AGEs in diabetic subjects were compared with those obtained amongst the measured serum AGEs in the non-diabetic control group, significant differences were observed between CML with TAGEs ($p < 0.01$), between CEL with TAGEs ($p < 0.05$) and between CML with CEL ($p < 0.05$).

Discussion

The current study has several main findings. First, as expected, significant differences were observed between some biochemical and clinical parameters of T2D patients and those of the non-diabetic patients. Second, there were significant differences in serum levels of TAGEs, CML and CEL but non-significant difference in serum levels of FAGEs between T2D and the non-diabetic patient groups. Third, only serum CEL levels were found to be significantly higher in male than in female T2D study subjects. Fourth, serum levels of FAGEs appeared to increase with increasing age in the non-diabetic control group and appeared to decrease with increasing age in T2D study subjects. Also, there were mixed associations between several serum AGEs and lipid/lipoprotein parameters as well as among some types of serum AGEs in both non-diabetic control and diabetic study subjects. Fifth, when correlations between serum AGEs measured in diabetic subjects and BMI, age of the study subjects, HbA1c as well as lipid/lipoprotein parameters were compared with those between serum AGEs measured in non-diabetic subjects and similar parameters, significant differences were observed for the correlations between FAGEs, TAGEs and age of the study subjects. Lastly, significant differences were observed between CML with TAGEs as well as between CML with CEL when correlation coefficients among the measured serum AGEs were compared between T2D subjects and non-diabetic controls.

As expected, biochemical indicators of glycaemic control (FBG and HbA1c) were found to be significantly higher in T2D study subjects compared with the non-diabetic control group. This observation can be attributed to a number of factors including: loss of glucose tolerance associated with the diabetic state, treatment non-compliance or failure as well as presence of determinants of poor glycaemic control such as obesity.[14,15] Serum levels of TAGEs, CML and CEL were found to be significantly higher in the T2D patient group compared with the non-diabetic patient group. These results, however, could not be compared with the results of other published studies, as subjects in these studies were in different environments and probably consumed different types of diets.

Unexpectedly, serum FAGE levels of T2D patients were not significantly different from those of non-diabetic patients. This observation might be attributed to the nature of the control group used in the current study. High serum AGE levels, in particular high serum level of pentosidine, the most abundant fluorescent AGE in plasma and tissues, have been associated with the development and progression of osteoporosis in diabetic and non-diabetic menopausal women.[16–18] Whether the high level of pentosidine observed in the cited studies is the cause or product of osteoporosis is currently not clear. It is possible that our patient control group, which was recruited from orthopaedic wards at DGMAH, may have included non-diabetic postmenopausal women with osteoporosis-related fractures. While this likelihood was not verified in the current study, it might explain the observed discrepancy between the results of the current study and those of similar previous studies with regard to the level of FAGEs.

The current study also investigated the effect of gender of T2D study subjects as well as of the age of both diabetic and non-diabetic subjects on levels of the different types of serum AGEs. Whereas there were no significant differences between serum TAGEs, CML and FAGEs for male compared with female T2D subjects, serum CEL levels were found to be significantly higher in males compared with female T2D subjects ($p < 0.01$). A possible explanation for this observation, which has never been reported before, may be the fact that, unlike the other types of AGEs, which are produced by multiple metabolic pathways and precursors, CEL is mainly produced by the glycolytic–methylglyoxal pathway.[2] It is thus possible that gender-specific hormones may influence the synthesis of CEL in a unique way.

Previous studies have shown that tissue levels of AGEs, in particular skin FAGEs, increase with age of the study subjects.[10,13] In the current study, there was no association between serum levels of AGEs, CML and CEL with age of both T2D and non-diabetic study subjects. However, a decrease in serum levels of FAGEs with increasing age of T2D patients and an increase in serum levels of FAGEs with increasing age of the control group was observed in the current study. The observation that serum FAGEs of the control group increased with increased age could also be attributed to the nature of the control group as explained above.

The observed significant negative associations between various serum AGEs and lipid/lipoprotein parameters cannot readily be explained, but the significant positive association observed between a number of serum AGEs and lipid/lipoprotein parameters could be attributed to the fact that some AGEs are known to be derived from lipid and lipoprotein oxidation.[2,3] Furthermore, the observed significant differences in the correlation coefficients between serum AGEs measured in diabetic patients and those measured in non-diabetic subjects could be attributed to the effect of hyperglycaemia on the formation of AGEs.[2,3]

Different types of circulating AGEs are described in the literature,[2,3] and whereas some AGEs such as CML, CEL, pentosidine and hydroimidazolones are immunogenic and can be measured using immunochemical methods, others such as pentosidine are both immunogenic and fluorescent and can also be measured using spectrofluorometric techniques.[13] In the current study we measured TAGEs by means of an ELISA with a polyclonal primary antibody directed against CML, pentosidine and other non-specified AGEs. Serum CML and CEL were measured by ELISA with monoclonal antibodies directed against their antigenic epitopes whereas FAGEs were measured spectrofluorometrically. When the results of these different measurement techniques were correlated with one another, significant positive correlations were observed between CML and TAGEs ($p < 0.01$) as well as between CML and CEL ($p < 0.05$). Whereas the former finding was expected due to the fact that CML is the major epitope for the polyclonal antibody used in the measurement of TAGEs, the latter finding was not expected due to the fact that CEL and CML are generally known to be produced by separate metabolic pathways.[2] To the best knowledge of the authors, the current study is the first to investigate the correlations among different serum AGEs in both T2D subjects and non-diabetic control subjects.

Previous studies that have investigated and reported on the association between serum AGE levels and HbA1c found weak or no correlation between serum AGE levels and HbA1c.[19,20] In agreement with these previous studies, the current study found weak non-significant correlations between the serum levels of measured AGEs and HbA1c in both T2D subjects and non-diabetic control subjects. An explanation that has been brought forward for this lack of association is the fact that AGEs can also be formed through other metabolic pathways beside glucose metabolic pathways; for example CML can be formed from oxidation of lipids.[2,20] Moreover, HbA1c and AGEs presumably reflect different pathways following hyperglycaemia and different duration of hyperglycaemia.

Currently serum AGEs are not routinely measured in most clinical laboratories, thus the result of this study may go a long way in raising awareness of the need to measure and monitor their levels in diabetic patients. Furthermore, as AGEs are involved in the development and progression of vascular complications of diabetes, their measurement and pharmacological intervention could help in the prevention of long-term complications of diabetes.

Limitations of the study

There are several limitations that should be taken into consideration when interpreting results of the current study. First, the sample size was small and study subjects were recruited from a single health institution, thus the findings could not be generalised beyond the study samples. Second, the current study is a cross-sectional study and therefore cause-and-effect relationships could not be inferred from the study results. Third, the possible confounding effect of exogenous dietary and smoking-related AGEs on serum AGE levels was not addressed in this study. Fourth, the control group selected for this study might have confounded the results, particularly those for the FAGEs. Lastly, the current study did not concurrently measure serum levels of AGEs for other South African race groups for comparison purposes. Despite these limitations, the authors believe that the results of this study are of great interest in that they are the first to describe the status of serum AGE levels among black South African T2D subjects.

Conclusions

The results of the current study suggest that serum AGE levels are significantly higher in T2D than in non-diabetic black South Africans, and with the exception of CEL are not influenced by gender. Also, serum FAGE levels appear to be positively associated with increasing age of study subjects in non-diabetic controls, but not in T2D subjects. In addition, the current study has revealed significant associations between various serum AGE levels and lipid/lipoprotein parameters as well as among the different types of serum AGEs and no significant association between serum AGEs and glycated haemoglobin among South African black patients with T2D.

References

1. Tessier FJ. The maillard reaction in the human body. The main discoveries and factors that affect glycation. Pathol Biol. 2010;58:214–9. http://dx.doi.org/10.1016/j.patbio.2009.09.014
2. Vistoli G, De Maddis D, Cipak A., et al. Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. Free Radic Res. 2013;47(sup1):3–27. http://dx.doi.org/10.3109/10715762.2013.815348
3. Singh VP, Bali A, Singh N, et al. Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol. 2013;18:1–14.
4. Nin JW, Jorsal A, Ferreira I, et al. Higher plasma levels of advanced glycation end products are associated with incident cardiovascular disease and all-cause mortality in type 1 diabetes: a 12-year follow-up study. Diabetes Care. 2011;34:442–7. http://dx.doi.org/10.2337/dc10-1087
5. Goh S-Y, Cooper ME. The role of advanced glycation end products in progression and complications of diabetes. J Clin Endocrinol Metab. 2008;93:1143–52. http://dx.doi.org/10.1210/jc.2007-1817

6. Jakus V, Rietbrock N. Advanced glycation end-products and the progress of diabetic vascular complications. Physiol Res. 2004;53:1–142.
7. Hegab Z, Gibbons S, Neyses L, et al. Role of advanced glycation end products in cardiovascular disease. World J Cardiol. 2012;4(4):90–102. http://dx.doi.org/10.4330/wjc.v4.i4.90
8. Leslie RDG, Beyan H, Sawtell P, et al. Level of an advanced glycated end product is genetically determined: a study of normal twins. Diabetes. 2003;52:2441–4. http://dx.doi.org/10.2337/diabetes.52.9.2441
9. Mensah GA, Mokdad AH, Ford ES, et al. State of disparities in cardiovascular health in the United States. Circulation. 2005;111:1233–41. http://dx.doi.org/10.1161/01.CIR.0000158136.76824.04
10. Mook-Kanamori MJ, Selim MM, Takiddin AH, et al. Ethnic and gender differences in advanced glycation end products measured by skin auto-fluorescence. Dematoendocrinol. 2013;5(2):325–30. http://dx.doi.org/10.4161/derm.26046
11. Kalousová M, Germanová A, Jáchymová M, et al. A419C (E111A) polymorphism of the glyoxalase i gene and vascular complications in chronic hemodialysis patients. Ann NY Acad Sci 2008;1126:268–71. http://dx.doi.org/10.1196/nyas.2008.1126.issue-1
12. Jara N, Leal MJ, Bunout D, et al. Dietary intake increases serum levels of carboxymethyllysine (CML) in diabetic patients. Nutr Hosp. 2012;27(4):1272–8.
13. Münch G, Keis R, Weßels A, et al. Determination of advanced glycation end products in serum by fluorescence spectroscopy and competitive ELISA. Eur J Clin Chem Clin Biochem. 1997;35(9):669–677.
14. Khattab M, Khader YS, Al-Khawaldeh A, et al. Factors associated with poor glycemic control among patients with type 2 diabetes. J Diabetes Complications. 2010;24:84–9. http://dx.doi.org/10.1016/j.jdiacomp.2008.12.008
15. Timothy GA. Factors influencing glycemic control in diabetes at three community health centres in Johannesburg [Internet];2011 [cited 6 July, 2014]. Available from http://wiredspace.wits.ac.za/jspui/bitstream.
16. Yang D-H, Chiang T-I, Chang IC, et al. Increased levels of circulating advanced glycation end-products in menopausal women with osteoporosis. Int J Med Sci. 2014;11(5):453–60. http://dx.doi.org/10.7150/ijms.8172
17. Yamamoto M, Yamaguchi T, Yamauchi M, et al. Serum pentosidine levels are positively associated with the presence of vertebral fractures in postmenopausal women with type 2 diabetes. J Clin Endocrinol Metab. 2008;93:1013–9. http://dx.doi.org/10.1210/jc.2007-1270
18. Schwartz AV, Garnero P, Hillier TA, et al. Pentosidine and increased fracture risk in older adults with type 2 diabetes. J Clin Endocrinol Metab. 2009;94:2380–6. http://dx.doi.org/10.1210/jc.2008-2498
19. Semba RD, Beck J, Sun K, et al. Relationship of a dominant advanced glycation end product, serum carboxymethyl-lysine, and abnormal glucose metabolism in adults: the baltimore longitudinal study of aging. J Nutr Health Aging 2010;14(7):23–31.
20. Ahmed KA, Muniandy S, Ismail IS. Role of Nε-(Carboxymethyl)Lysine in the development of ischemic heart disease in type 2 diabetes Mellitus. J Clin Biochem Nutr 2007;41(2):97–105. http://dx.doi.org/10.3164/jcbn.2007014

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