Journal of Molecular Biomarkers & Diagnosis

Gaballah, et al., J Mol Biomark Diagn 2014, 5:6
DOI: 10.4172/2155-9929.1000203

Research Article Open Access

# Association between Angiotensin Converting Enzyme Insertion/Deletion Polymorphism and Soluble Receptor of Advanced Glycation End Products in Type 2 Diabetic Patients with and without Microalbuminuria

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**Rec date:** Oct 20, 2014, **Acc date:** Nov 19, 2014, **Pub date:** Nov 24, 2014



## Abstract

**Aims/Introduction:** Studies on the association between a deletion variant of the angiotensin-converting enzyme (ACE) gene and diabetic microalbuminuria and its impact on AGE/RAGE system remain inconclusive. Therefore, we aimed to study the association between ACE I/D polymorphism and the circulating levels of sRAGE (soluble receptor for advanced glycation end-products (sRAGE), pentosidine and advanced oxidation protein products (AOPPs) in type 2 diabetic patients with and without microalbuminuria.

**Subjects and methods:** ACE I/D polymorphism was analyzed using the amplified fragment length polymorphism Polymerase Chain Reaction method (AFLP). Circulating levels of sRAGE and pentosidine were assessed using enzyme linked immunosorbent assay, while AOPPs were assessed photometrically.

**Results:** within the diabetic normoalbuminuric group, DD and DI ACE genotypes were associated with significantly higher levels of sRAGE, Pentosidine and AOPPs (3280 ± 155 pg/mL, 289 ± 65 ng/ml and 192 ± 4.9 µmol/l ) respectively than those with the II genotype (2985 ± 310 pg/mL, 231 ± 66 ng/ml and 169 ± 7.5 µmol/l respectively, $p<0.05$). Also the microalbuminuric group exhibited a significant association between DD and DI ACE genotypes and higher levels of sRAGE, Pentosidine and AOPPs (10.2 ± 2.5%, 4975 ± 256 pg/mL, 305 ± 24 ng/ml and 207 ± 5.4 µmol/l ) respectively than those with the II genotype (4566 ± 219 pg/mL, 277 ± 15 ng/ml and 199 ± 7.6 µmol/l respectively, $p<0.05$).

**Conclusions:** Both ID and DD ACE genotypes might represent a risk factor for diabetic renal complications being associated with significantly higher levels of sRAGE, pentosidine and AOPPs.

**Keywords:** sRAGE; Microalbuminuria; ACE polymorphism

## Introduction

Diabetic nephropathy is a major cause of morbidity and early mortality in diabetes worldwide [1]. Although the exact pathogenesis of type 2 diabetic nephropathy is likely diverse in nature, there is accumulating evidence that several genetic and environmental factors contribute to its development and progression [2].

Genetic variations in the renin–angiotensin system (RAS) have been implicated in the pathogenesis of diabetic nephropathy [3]. Angiotensin-converting enzyme (ACE), a key component of RAS, plays a vital role in blood pressure regulation and electrolyte balance by converting angiotensin I to angiotensin II, which is a potent vasopressor and aldosterone-stimulating peptide [4].

Angiotensin converting enzyme (ACE) gene (encoding kininase II, EC 3.4.15.1) is located on chromosome 17q23 and consists of 26 exons and 25 introns. ACE polymorphism is based on the insertion [I] or deletion [D] within an intron 16 of a 287-base-pair (Alu Sequence) nonsense DNA domain, resulting in three genotypes (DD and II homozygotes, and ID heterozygotes) [5]. ACE polymorphism was found to be associated with plasma and renal expression levels of ACE as well as susceptibility to diabetic nephropathy [6].

In the setting of long-term hyperglycemia, free amino groups of proteins, lipids and nucleic acids, are nonenzymatically modified by glucose and its metabolites to form Schiff bases, which then rearrange to form Amadori products. Further modification by the Maillard reaction, nonenzymaticgly cation, leads to formation of advanced glycation end-products (AGEs) which are then accumulated in the vascular wall [7]. Pentosidine is one of the well characterized AGEs, which acts as a marker for the formation and accumulation of AGEs in diabetes [8].

Binding of AGE to the receptor for AGEs (RAGE) generates free radicals, evokes vascular inflammation, and activates cell signaling mechanisms coupled to increased transforming growth factor -β and vascular endothelial growth factorexpression that contribute to the pathogenesis of diabetic microvascular complications [9].

The receptor for AGEs is a member of the immunoglobulin superfamily of transmembrane cell surface molecules [10]. The soluble form of RAGE (sRAGE) can be found in the circulation and consists of only the extracellular ligand-binding domains of RAGE and lacks the transmembrane and intracytoplasmic domains. These soluble

receptors can be formed by cleavage of the membrane-bound full-length receptor by metalloproteinases or by alternative splicing of the RAGE gene [11].

Recently, it has been suggested that inhibition of angiotensin-converting enzyme (ACE) reduces the accumulation of AGEs in diabetes partly by increasing the expression and secretion of sRAGE into the plasma [12]. However, the studies on the association between ACE I/D polymorphism and sRAGE in type 2 diabetic nephropathy remain inconclusive.

Therefore, we aimed to investigate the effect of ACE I/D polymorphism on the serum levels of sRAGE and other oxidative-glycation biomarkers such as pentosidine and advanced oxidation protein products (AOPPs) in type 2 diabetic patients with and without microalbuminuria, in order to identify the potential genetic and biochemical risk factors for diabetic nephropathy which may benefit in early diagnosis and in developing effective therapeutic interventions.

## Subjects and Methods

This study was conducted on 150 subjects, including 100 patients with Type 2 diabetes mellitus diagnosed according to the American Diabetes Association criteria 2012 [13], who were presented to the outpatient clinics ofInternal Medicine Department, Tanta University Hospital. These patients were further subdivided into two groups: Group I consisted of 50 patients (36 females and 14 males) with normoalbuminuria (urinary albumin to creatinine ratio was 30 mg/g), and Group II consisted of 50 patients (38 females and 12 males) with microalbuminuria (urinary albumin to creatinine ratio was 30-299 mg/g). The selected type 2 diabetic patients were under an antidiabetic regimen of oral hypoglycemic drugs and/or insulin, and patients receiving antihypertensive agents were excluded from the study to avoid fallacies due to effects of these drugs on the studied parameters. Fifty subjects (40 females and 10 males), who visited the other departments of the hospital, with no evidence of Type 2 DM (according to fasting blood sugar), hypertension, and/or renal disease were selected to represent the control group (Group III).

All patients gave their written informed consent before participation. The study protocol was approved by the local ethics committee, and was in accordance with the principles of the Declaration of Helsinki II. Patients with serum creatinine higher than 2 mg/dl, history of proteinuria before the onset of diabetes, liver dysfunction, malignancy, known adrenal disease, inflammatory renal disease, urinary tract infection or benign prostatic hyperplasia were excluded from the study.

Urine sampling: First voided morning urine samples were used for estimation of urinary albumin and creatinine levels using commercial kits obtained from BioSystems Co. (Spain) and Diamond Diagnostics Co. (Egypt) respectively.

Blood sampling: After 12 hours of overnight fasting, 7ml of venous blood samples were taken from each investigated subject. 3.5 ml whole blood was separated in EDTA 5% coated tubes and stored at –20°C for genotyping for the ACE I/D polymorphism. The remaining sample was transferred slowly into a dry sterile centrifuge tube, allowed to clot at room temperature, centrifuged at 2000 rpm for 10 minutes and serum was separated and stored at -70°C until the time of analysis.

### Laboratory investigations

Fasting and 2 hours postprandial blood Sugar was measured by the oxidase method (Biodiagnostic., Egypt). Serum urea and creatinine were measured by commercial kits from BioSystems Co. (Spain) and Diamond Diagnostics Co. (Egypt) respectively. The estimated glomeular filtration rate (eGFR) was calculated using the four-variable Modification of Diet in Renal Disease (MDRD) Study equation of Levery et al., [14]. Total lipid profile including total cholesterol (TC), triglycerides (TAG) and high density lipoprotein cholesterol (HDL-C) were measured by enzymatic-colorimetric methods (Biodiagnostic., Egypt). Low density lipoprotein cholesterol (LDL-C) concentration was calculated friedwald formula [15]; LDL-C=TC-[(TG/5) +HDL- C] (mg/dl).Fasting serum insulin levels were estimated using a commercial ELISA kit (Cat# 1606-15, Diagnostic Automation/Cortez Diagnostics, Inc., CA 91302, USA). Insulin resistance was assessed by the homeostatic model assessment (HOMA-IR), calculated as: Fasting glucose (mg/dl) *fasting insulin (μIU/mL) /405 [16]. Serum soluble receptor of advanced glycation end products (s RAGE) was estimated using solid-phase sandwich enzyme immunoassay (ELISA) kit (Cat# SK00112-02, AVISCERA BIOSCIENCE INC., CA 95051, USA) according to the manufacturer's protocol. Serum pentosidine was measured with sandwich ELISA kit (Cat# CEA264Ge, USCNK Life Science Inc., Wu Han, China), according to the manufacturer's protocol. Advanced oxidation protein products (AOPPs) were quantified according to the method by Witko-Sarsat et al., [17]. 200 μl of serum diluted 1:5 in phosphate-buffered saline were placed into each well of a 96-well microtitre plate and added 20 μl of acetic acid to each well. For the standards, we added 10 μl of 1.16 M potassium iodide to 200 μl of chloramine-T solution (0 to 100 μmol/L) in a well and then added 20 μl of acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm against a blank consisting of 200 μl of phosphate-buffered saline, 10 μl of 1.16 M potassium iodide, and 20 μl of acetic acid. AOPPs concentrations are expressed as μmol/L of chloramine-T equivalents.

### DNA extraction and genotyping for the ACE insertion/ Deletion polymorphisms

DNA was extracted from the whole blood with EDTA using the GF-1 total DNA Extraction Kit supplied by (Vivantis Inc., CA, USA). Polymerase Chain Reaction (PCR) was performed to determine the different genotypes of ACE I/D. The specific primers were: Fwd: 5'-CTG GAG ACC ACT CCC ATC CTT TCT -3'; and Rev:5'-GAT GTG GCC ATC ACA TTC GTC AGA T -3'[18]. Briefly, PCR was carried out using 20 pmol of each primer, 200 μMdNTPs, 1.5 mM $MgCl_2$, 1U Taq DNA polymerase enzyme in a 10X PCR buffer and 300–500 ng genomic DNA in total volume of 25 μl. The PCR protocol consisted of 5 min at 94°C, 40 cycles of 30 s at 94°C, 30 s at 60°C and 1 min at 72°C and then, 10 min at 72°C for final extension. A 12 μl of PCR products was electrophoresed on a 1.5% agarose gel. The presence of ACE II genotype produces a 490-bp fragment, DD genotype produces a 190-bp fragment, and ID genotype produces two fragments of 490 and 190-bp that were confirmed in the staining by ethidium bromide as fluorescent dye.

## Statistical Analysis

The data were analyzed using statistical package for the social science (SPSS) version 20.0 software (SPSS Inc., Chicago, IL, USA). Frequencies of genotypes/alleles were determined by the gene counting method. Quantitative data were expressed as mean

±Standard deviation. Multiple comparisons were performed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Correlations were analyzed using the Pearson test.

## Results

The clinical and demographic characteristics of the studied groups are demonstrated in Table 1. No statistically significant difference was detected between the studied groups regarding age, sex and body mass index, systolic and diastolic blood pressure. However, there was a statistically significant difference as regards disease duration ($p<0.05$). Table 2 demonstrated a statistical comparison between the studied groups with respect to the laboratory biochemical findings using ANOVA test followed by Tukey's test. Fasting, 2-h post prandial blood glucose, TAG, TC, LDL, urinary Albumin/Creatinine ratio, serum creatinine, blood urea, insulin resistance (HOMA/IR) and fasting insulin levels were significantly higher in the micro-albuminuric type 2 diabetic group compared to the other two groups ($p<0.05$). Estimated GFR and HDL levels were significantly lower in the micro-albuminuric type 2 diabetic group compared to the other two groups ($p<0.05$). Of note, serum sRAGE, pentosidine and AOPPs levels in the type 2 diabetic microalbuminuric group were significantly higher than the normoalbuminuric group (4859 ± 317 vs. 3275 ± 270 pg/mL, 298 ± 26 vs. 273 ± 85 pg/mL and 203.1 ± 7.3vs. 189 ± 5.1 µmol/l) respectively, ($p<0.001$).

| | | Normoalbuminuric type 2 diabetes(n=50) | Microalbuminuric type 2 diabetes(n=50) | Control (n=50) | Statistical test | |
|---|---|---|---|---|---|---|
| | | | | | F/t/$X^2$ | P value |
| Age (years) | | 54.1 ± 12.4 | 53.3 ± 8.6 | 48.2 ± 8.1 | f =2.05 | 0.09 |
| sex | male | 36 (72%) | 28 (76 %) | 40 (80 %) | $X^2$=0.43 | 0.8 |
| | female | 14 (28%) | 12 (24 %) | 10 (20%) | | |
| Body mass index (kg/$m^2$) | | 27.85 ± 3.24 | 28.74 ± 4.39 | 26 ± 2.9 | f =1.11 | 0.4 |
| Disease duration( years) | | 5.7 ± 2.6 | 8.4 ± 4.9 | - | t=2.43 | 0.01* |
| SBP (mmHg) | | 125.8 ± 8.9 | 123.4 ± 9.1 | 111.4 ± 6.5 | f =2.65 | 0.054 |
| DBP (mmHg) | | 84 ± 7.8 | 80.6 ± 8.5 | 78.2 ± 6.7 | f =1.08 | 0.6 |
| SBP: systolic blood pressure, DBP:diastolic blood pressure , P was considered significant at <0.05 | | | | | | |

**Table 1:** Clinical and demographic characteristics of all of the studied groups.

| | Normoalbuminuric type 2 diabetes (n=50) | Micro-albuminuric type 2 diabetes (n=50) | Control (n=50) | ANOVA | | Tukey's test | | |
|---|---|---|---|---|---|---|---|---|
| | | | | f | p | P1 | P2 | P3 |
| Fasting blood glucse (mg/dl) | 214.5 ± 54.7 | 246.5 ± 65.8 | 78.1 ± 7.8 | 121.1 | <0.001* | 0.003* | <0.001* | <0.001* |
| 2-h PP blood glucose (mg/dl) | 280.1 ± 63.7 | 361.3 ± 54.2 | 95.1 ± 15.9 | 228.4 | <0.001* | <0.001* | <0.001* | <0.001* |
| HDL (mg/dl) | 48.1 ± 8.9 | 39.6 ± 6.2 | 48.9 ± 5.1 | 23.5 | <0.001* | <0.001* | 0.6 | <0.001* |
| TAG (mg/dl) | 116.5 ± 42.7 | 139.9 ± 36.8 | 96.7 ± 15.1 | 5.2 | 0.007 | 0.02* | 0.9 | 0.01* |
| TC (mg/dl) | 230.4 ± 61.3 | 271.5 ± 51.6 | 171.8 ± 32.7 | 18.8 | <0.001* | 0.009* | 0.01* | <0.001* |
| LDL (mg/dl) | 161.1 ± 63.7 | 204.5 ± 49.3 | 103.9 ± 28.7 | 21.1 | <0.001* | <0.001* | 0.26 | <0.001* |
| Fasting insulin (µIU/ml) | 8.6 ± 5.6 | 13.3 ± 4.9 | 4.1 ± 1.5 | 21.6 | <0.001* | <0.001* | <0.001* | <0.001* |
| HOMA-IR | 4.7 ± 3.8 | 7.9 ± 4.5 | 1.1 ± 0.3 | 23.8 | <0.001* | 0.001* | <0.001* | <0.001* |
| Urinary Albumin/ Creatinine ratio (UACR, mg/g) | 16.9 ± 9.5 | 74.6 ± 33.1 | 18.1 ± 6.3 | 109.3 | <0.001* | <0.001* | 0.001* | <0.001* |
| Urea (mg %) | 33.5 ± 9.1 | 73.4 ± 18.7 | 25.7 ± 6.8 | 135.3 | <0.001* | <0.001* | 0.22 | <0.001* |
| Serum creatinine (mg/dl) | 0.79 ± 0.3 | 0.94 ± 0.7 | 0.7 ± 0.1 | 5.5 | 0.001* | <0.001* | 0.52 | <0.001* |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| estimated GFR (eGFR ,ml/min per 1.73 $m^2$ ) | 95.6 ± 29.3 | 71.5 ± 25.3 | 94.4 ± 17.3 | 4.8 | 0.011* | 0.02* | 0.98 | 0.02* |
| sRAGE, pg/mL | 3275 ± 270 | 4859 ± 317 | 1562 ± 386 | 748.7 | <0.001* | <0.001* | <0.001* | <0.001* |
| Pentosidine pg/mL | 273 ± 85 | 298 ± 26 | 257 ± 43 | 7.08 | 0.002* | 0.943 | 0.003* | 0.008* |
| AOPPs (µmol/l) | 189 ± 5.1 | 203.1 ± 7.3 | 125.83 ± 4.05 | 1409.7 | <0.001* | <0.001* | <0.001* | <0.001* |
| Data presented as means ± SD. P was calculated by one way ANOVA test followed by Tukey's post-hoc test. P was considered significant at <0.05.; *Significant; P1 comparison between group I and II; P2 comparison between group I and III; P3 comparison between group II and III. | | | | | | | | |

**Table 2:** Statistical comparisons between the studied parameters.

Three genotypes were recognized II with one band at 490 bp, DD with one band at 190 bp and ID a heterozygote type with 2 bands at 490 and 190 bp as illustrated in Figure 1. Data for the distribution of angiotensin converting enzyme insertion/deletion (ACE I/D) genotypes and alleles in the studied groups is illustrated are shown in Table 3. The frequencies of the DD and ID genotypes of ACE were insignificantly higher in diabetic patients (22 and 46%respectively) than controls (18 and 42% respectively, p>0.05), while II genotype frequency was insignificantly lowerin diabetic patients than controls (32 vs. 40%, p>0.05). Furthermore, in diabetic patients with microalbumiuria the frequencies of ACE II, ID, and DD genotypes were 28, 48 and 24% respectively that was insignificantly different from diabetic patients with normoalbumiuria (36, 44 and 20% respectively). The odds ratio for the variant genotypes (II, ID, and DD) were 0.70, 1.17 and 1.28 and the 95% CI were (0.34-1.42, 0.59-2.33 and 0.54-3.04) respectively. Moreover, the frequency of ACE D allele was insignificantly higher (p>0.05) in the diabetic patients (45%) than controls (39%), with being insignificantly higher (p>0.05) in microalbuminuric (48%) compared to normoalbuminuric (42%) diabetic patients also, I allele frequency was insignificantly lower (p>0.05) in the diabetic patients (55%) than controls (61%).

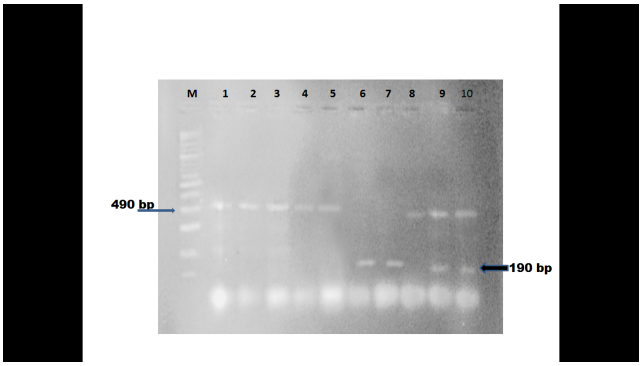


Figure 1: Gel Electrophoresis demonstrating the different genotype of ACE (I/D) polymorphism after PCR analysis. Lane M shows DNA marker (100bp), Lanes (1-5,8)show I/I homozygous (490bp), Lanes 9,10 show I/D heterozygous (490bp and190bp), Lans 6,7 show D/D homozygous (190bp)

| ACE Genotype | Normo-albuminuric type 2 diabetes | | Micro-albuminuric type 2 diabetes | | Total type 2 diabetes | | Controls | | Odds ratio | | P-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | N | % | OR | 95% CI | |
| Overall | 50 | 100% | 50 | 100% | 100 | 100% | 50 | 100% | | | |
| II | 18 | 36% | 14 | 28% | 32 | 32% | 20 | 40% | 0.70 | 0.34 to 1.42 | 0.36 |
| ID | 22 | 44% | 24 | 48% | 46 | 46% | 21 | 42% | 1.17 | 0.59 to 2.33 | 0.72 |
| DD | 10 | 20% | 12 | 24% | 22 | 22% | 9 | 18% | 1.28 | 0.54 to 3.04 | 0.67 |
| ID+DD | 32 | 64% | 36 | 72% | 68 | 68% | 30 | 60% | 1.41 | 0.70 to 2.86 | 0.36 |
| I Allele | 58 | 58% | 52 | 52% | 110 | 55% | 61 | 61% | 0.78 | 0.47 to 1.27 | 0.38 |
| D Allele | 42 | 42% | 48 | 48% | 90 | 45% | 39 | 39% | 1.27 | 0.78 to 2.08 | 0.38 |
| ACE: angiotensin converting enzyme, OR: odds ratio, CI: confidence interval, NS: Non significant at p value >0.05 | | | | | | | | | | | |

**Table 3:** The distribution of angiotensin converting enzyme insertion/deletion (ACE I/D) genotypes and alleles in the studied groups

Comparing the studied parameters in diabetic patients with the risky genotypes DD and DI to those with the II genotype revealed that these risky genotypes were significantly associated with higher serum levels of TC, LDL, fasting insulin, sRAGE, Pentosidine, AOPPs (p <0.05), these data are summarized in Table 4.

Within the diabetic normoalbuminuric group, the risky genotypes DD and DI were associated with significantly higher levels of sRAGE, Pentosidine and AOPPs (3280 ± 155 pg/mL, 289 ± 65 ng/ml and 192 ± 4.9 µmol/l) respectively than those with II genotype (2985 ± 310 pg/mL, 231 ± 66 ng/ml and 169 ± 7.5 µmol/l respectively, p<0.05). Similarly, the diabetic patients with microalbuminuria exhibited a significant association between the risky genotypes DD and DI and higher levels of sRAGE, Pentosidine and AOPPs (4975 ± 256 pg/mL, 305±24 ng/ml and 207 ± 5.4 µmol/l) respectively than those with the II genotype (4566 ± 219 pg/mL, 277 ± 15ng/ml and 199 ± 7.6 µmol/l respectively, p<0.05), (Table 5).

The correlation between serum levels of sRAGE, Pentosidine and AOPPs with other studied parameters in diabetic patient groups (I and II) was tested using pearson's correlation, where there were significant positive correlations between each of these 3 parameters and fasting, 2 hours postprandial blood glucose, TC, LDL, fasting insulin, insulin resistance (HOMA/IR), blood urea and serum creatinine levels (p<0.05). Likewise, sRAGE levels were significantly correlated with pentosidine (r=0.63, p<0.01) and AOPPs (r=0.52, p<0.01) levels in the studied diabetic groups. Levels of pentosidine showed significant positive correlation with AOPPs serum levels (r=0.59, p<0.01) (Tables 6 and 7) showed forward stepwise multiple logistic regression analysis of factors that might be independently associated with microalbuminuria. It was performed on a number of predictors including serum levels of sRAGE, pentosidine, AOPPs, fasting Insulin and insulin resistance (HOMA/IR) as independent variables, and the development of microalbuminuria as the dependent variable. It was found that serum levels of sRAGE followed by pentosidine then AOPPs were the most important predictors for the development of microalbuminuria in type 2 diabetic patients.

| **Parameters** | **ACE genotypes** | | **T-test** | |
|---|---|---|---|---|
| | II | ID/DD | t | P-value |
| Age (years) | 52.9 ± 1.2 | 53.3 ± 2.5 | 0.72 | 0.47 |
| Duration (years) | 5.9 ± 3.9 | 6.4 ± 4.8 | 0.36 | 0.71 |
| Fasting blood glucose (mg/dl) | 225.8 ± 17.8 | 240.9 ± 33.2 | 1.70 | 0.09 |
| 2-h PP blood glucose (mg/dl) | 301 ± 84.4 | 315 ± 47.3 | 0.75 | 0.45 |
| HDL (mg/dl) | 47.5 ± 6.5 | 43.3 ± 8.8 | 1.70 | 0.09 |
| TAG (mg/dl) | 171 ± 43.1 | 195.5 ± 55.4 | 1.55 | 0.12 |
| TC (mg/dl) | 234.5 ± 42.8 | 281 ± 45.7 | 3.42 | 0.001* |
| LDL (mg/dl) | 195.5 ± 36.2 | 228.7 ± 49.1 | 2.40 | 0.01* |
| Fasting insulin (µIU/ml) | 9.1 ± 4.5 | 13.1 ± 5.6 | 2.49 | 0.01* |
| HOMA-IR | 6.1 ± 3.3 | 6.5 ± 4.2 | 0.33 | 0.73 |
| Urinary Albumin/Creatinine ratio (UACR , mg/g) | 37.9 ± 4.6 | 40.5 ± 5.8 | 1.57 | 0.12 |
| Urea (mg %) | 47.4 ± 14.8 | 49.9 ± 17.2 | 0.50 | 0.61 |
| Serum creatinine (mg/dl) | 0.93 ± 0.45 | 0.98 ± 0.33 | 0.44 | 0.65 |
| estimated GFR (eGFR ,ml/min per 1.73m$^2$ ) | 88.26 ± 49.1 | 81.33 ± 27.9 | 0.63 | 0.52 |
| sRAGE, pg/mL | 4450 ± 311 | 4667 ± 367 | 2.04 | 0.04* |
| Pentosidine ng/ml | 249 ± 37 | 285 ± 46 | 2.73 | 0.008* |
| AOPP(µmol/l) | 178 ± 8.7 | 189 ± 3.4 | 6.45 | <0.001* |
| Data presented as means± SD. P was calculated by T-test. P was considered significant at <0.05.;*Significant HOMA-IR: Homeostatic Model Assessment of Insulin Resistance, sRAGE: soluble receptor for advanced glycation end-products, AOPP: advanced oxidation protein products | | | | |

**Table 4:** association between distribution of angiotensin converting enzyme insertion/deletion (ACE I/ D) genotypes and the studied parameters in T2 diabetic patients with and without microalbuminuria

| | **Normo-albuminuric type 2 diabetes** | | **Micro-albuminuric type 2 diabetes** | |
|---|---|---|---|---|
| | ACE genotypes | T-test | ACE genotypes | T-test |

| | II (n=18) | ID/DD (n=32) | t | P-value | II (n=14) | ID/DD (n=36) | t | P-value |
|---|---|---|---|---|---|---|---|---|
| sRAGE, pg/mL | 2985 ± 310 | 3280 ± 155 | 3.19 | 0.004 | 4566 ± 219 | 4975 ± 256 | 3.71 | 0.001 |
| Pentosidine ng/ml | 231 ± 66 | 289 ± 65 | 2.13 | 0.044 | 277 ± 15 | 305 ± 24 | 2.85 | 0.008 |
| AOPPs (µmol/l) | 169 ± 7.5 | 192 ± 4.9 | 9.30 | <0.001* | 199 ± 7.6 | 207 ± 5.4 | 2.96 | 0.006 |
| Data presented as means ± SD. P was calculated by T-test. P was considered significant at <0.05.;*SignificantsRAGE: soluble receptor for advanced glycation end-products, AOPP: advanced oxidation protein products | | | | | | | | |

**Table 5:** Serum levels of the studied parameters in various genotypes of ACE.

## Discussion

Despite being intronic, the insertion/deletion (I/D) polymorphism of the ACE gene is of a functional significance as it accounts for a large proportion of serum ACE activity variability. Besides, it has been implicated as a risk factor for a number of pathologies, such as myocardial infarction, stroke, and hypertension [19]. However, the contribution of ACE gene variants to diabetic nephropathy development and progression is controversial and varies among different ethnic/racial groups [20].

The current study shows that the frequencies of both ID and DD ACE genotypes as well as the minor D allele frequency were insignificantly higher in the diabetic patients compared to controls. Likewise, these risky genotypes (ID and DD) were insignificantly higher in microalbuminuric than normoalbuminuric diabetic patients.

Noteworthy, the positive association between ACE minor D allele and type 2 diabetic nephropathy was previously reported in Indian [5], Iranian [21] and Tunisian [22] populations. Moreover, a recent meta-analysis of 63 studies comprising 26,580 subjects found an evidence for a significant association between the I/D polymorphism of the ACE gene and the risk of type 2 diabetic nephropathy [3].

The highest systemic and renal ACE levels were found to be strongly associated with DD and DI genotypes, lending support to the hypothesis that ACE DD genotype may confer risk for diabetic nephropathy through producing greater amounts of angiotensin II [23]. Conceivably, angiotensin II is a vasoactive peptide causing an increase in the intraglomerular pressure; it also induces renal interstitial fibrosis by triggering the release of cytokines such as platelet-derived growth factor, fibronectin, and transforming growth factor-β thus accelerating the progression to chronic renal failure [24]. Moreover, it has been reported that ACE (I/D) polymorphism influences the beneficial effect of ACE inhibition on the progression of diabetic nephropathy which becomes most evident among II and DI carriers [25].

On the other hand, continuous hyperglycemia and oxidative stress causes increased generation of advanced glycation end-products (AGEs) [26]. Binding of the receptor for AGEs (RAGE) with AGEs elicits inflammatory and/or thrombogenic responses, thus participating in the development and progression of diabetic angiopathies [27].

Soluble RAGE (sRAGE) is a naturally occurring inhibitor of AGE-RAGE which functions as a decoy for RAGE ligands [28]. Renin-angiotensin system has been recently identified as a key modulator for AGEs in diabetes, where blockade of ACE reduces the accumulation of AGEs and attenuates AGEs -mediated signaling pathways [29]. However, the impact of ACE I/D gene polymorphism on circulating sRAGE levels in diabetic nephropathy still remains obscure. The present study revealed a significant elevation of serum soluble RAGE levels in type 2 diabetic patients compared to control subjects. This finding concurs with previous studies done by Ng et al., [30] and Aubert et al., [31] who reported elevated serum sRAGE level in response to circulating AGEs in type 2 diabetic patients. Well in line, Fujisawa et al., [32] documented that sRAGE levels were associated with the increased risk of cardiovascular disease in type 2 diabetic subjects and suggested that sRAGE may be a potential predictive biomarker of RAGE-mediated vascular injury.

Accumulating evidence corroborates the notion that circulating sRAGE levels are elevated in type 2 diabetes. Initially, AGEs are positive regulators of cell expression of RAGE which is found to be enhanced in diabetic atherosclerotic plaques, nephropathy, and retinopathy [33]. In addition, endogenous sRAGE could be generated from the cleavage of cell surface RAGE [9]. Following this line of argument, it is conceivable that sRAGE is positively associated with circulating AGEs levels in diabetes. Further bolstering this viewpoint, the finding that soluble RAGE belongs to the same immunoglobulin superfamily that includes soluble adhesion molecules which were found to be upregulated in type 2 diabetes [34], thus elevation of circulating sRAGE in a related pattern seems to be reasonable.

In addition, Nakamura et al., [35] recently found that serum levels of sRAGE are positively correlated with inflammatory biomarkers such as tumor necrosis factor-alpha (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) which were found to be elevatedin type 2 diabetic patients. Another plausible mechanism is that hyperinsulinemia, observed in type 2 diabetes, can stimulate proteolytic cleavage of RAGE and shedding of sRAGE from the membrane bound receptor as first reported by Lam et al., [36] thus participates in elevating circulating levels of s RAGE in type 2 diabetic patients

On the other hand, the finding of elevated sRAGE levels contrasts the study by Basta et al., [37] who concluded that the circulating sRAGE is inversely associated with glycemic control. Moreover, Tam et al., [38] showed that serum level of sRAGE is down-regulated in Type 2 diabetes, although the AGE expression is enhanced. These conflicting results may be attributed to a number of factors such as ethnicity or use of certain medications.

Concomitantly, the present study reported a significant elevation of sRAGE levels in microalbuminuric than normoalbuminuric type 2 diabetic patients. This finding is in keeping with the study by Daroux et al. [39] who reported that serum sRAGE levels and circulating

AGES were associated with the severity of nephropathy in type 2 diabetes. Well in line, Nakashima et al., [40] concluded that serum sRAGE levels increased in patients with renal dysfunction, mainly hemodialysis patients. Taken together, these observations suggest that sRAGE level might be a predictive biomarker of renal vascular injury in patients with type 2 diabetes, where its upregulation might serve as a counter-regulatory mechanism to counteract the vasculotoxic effect of AGEs accumulation [40].

To further assess the association of different ACE genotypes with serum sRAGE levels, both normoalbuminuric and microalbuminuric diabetic patients were subdivided according to ACE genotype, where serum sRAGE levels were significantly associated with the minor D allele carriers (DD and DI genotypes) compared to II genotype in the two diabetic groups. This observed significant positive association can be supported by the findings thatthe D allele carrier isbe associated with higher ACE serum levels andactivity, subsequently resulting in producing higher levels of Angiotensin II [23]. Moreover, angiotensin II was found to increase RAGE levels in endothelial cells, and stimulated s RAGE formation in vitro [41].

Accordingly, it seems plausible that the D allele carriers will have higher circulatingsRAGE than the I allele carriers, which is consistent with our data.The notion of the pathophysiological crosstalk between ACE and sRAGE was further underscored by the study by Ono et al., [42] who revealed that angiotensin II type 1 receptor blockers, not only inhibit the angiotensin II-elicited sRAGE generation by endothelial cells, but also decrease serum levels of sRAGE in diabetic patients with essential hypertension. Moreover, Kamioka et al., [12] concluded that AGE-triggered NADPH- oxidase signaling pathways, including matrix metalloproteinase-9 and apoptosis, were attenuated by rennin angiotensin system blockade, which may be beneficial for treating diabetic vascular complications.

Moreover, our data revealed that both pentosidine and AOPPs levels were significantly elevated in microalbuminuric group compared to normoalbuminuric type 2 diabetic and control groups, with being significantly associated with the risky ACE genotypes ID and DD in both diabetic groups.

In harmony with these findings, Sato et al., [43] and Kerkeniet al., [8] reported that pentosidine levels were positively associated with microvascular complications in type 2 diabetic patients. Moreover, previous studies showed that pentosidine levels were increased markedly with the severity of microangiopathy because the kidney is the main elimination site for pentosidine [44].

Pentosidine might be involved in nephrotoxicity via modifying the protein's tertiary structure through cross-linking, altering enzymatic activity and impairing receptor recognition [45]. Moreover, the sustained AGE-RAGE interaction could generate cellular reactive oxygen species and activate pro-inflammatory pathways by increasing vascular endothelial growth factor, MCP-1 and ICAM-1 expression in microvascular endothelial cells [46]; it also activates nuclear factor-kappa B, NADPH oxidase, apoptosis, angiogenesis and tissue remodelling [47]. This scenariofits well with the study done by Yamagishi et al. [9] who postulated that renal AGE accumulation can itself become a perpetuating factor in inducing mesangial cell loss and dysfunction, glomerulosclerosis and tubulointerstitial fibrosis thus ending in renal damage.

| | **sRAGE, pg/mL** | | **Pentosidine pg/mL** | | **AOPPs (µmol/l)** | |
|---|---|---|---|---|---|---|
| | r | P-value | r | P-value | r | P-value |
| Age (years) | 0.16 | 0.25 | 0.09 | 0.53 | 0.14 | 0.31 |
| Duration | 0.14 | 0.32 | 0.23 | 0.10 | 0.22 | 0.11 |
| Fasting blood glucse (mg/dl) | 0.33 | 0.01* | 0.39 | 0.005* | 0.28 | 0.04* |
| 2-h PP blood glucose (mg/dl) | 0.39 | 0.005* | 0.28 | 0.04* | 0.28 | 0.04 |
| HDL (mg/dl) | -0.02 | 0.87 | -0.25 | 0.07 | -0.01 | 0.90 |
| TAG (mg/dl) | 0.06 | 0.66 | 0.25 | 0.07 | 0.26 | 0.06 |
| TC (mg/dl) | 0.40 | 0.004* | 0.29 | 0.04* | 0.33 | 0.01* |
| LDL (mg/dl) | 0.64 | 0.001* | 0.28 | 0.04* | 0.60 | 0.001* |
| Fasting insulin (µIU/ml) | 0.36 | 0.009* | 0.62 | 0.000* | 0.56 | 0.001* |
| HOMA-IR | 0.46 | 0.001* | 0.56 | 0.000* | 0.62 | 0.001* |
| UACR | 0.15 | 0.28 | 0.04 | 0.78 | 0.05 | 0.71 |
| Urea (mg %) | 0.51 | 0.000* | 0.30 | 0.03* | 0.11 | 0.42 |
| Serum creatinine (mg/dl) | 0.34 | 0.015* | 0.32 | 0.02* | 0.06 | 0.64 |
| estimated GFR (eGFR ,ml/min per 1.73m$^2$ ) | -0.12 | 0.39 | -0.17 | 0.21 | -0.08 | 0.58 |
| sRAGE, pg/mL | | | 0.63 | 0.001* | 0.52 | 0.001* |
| Pentosidine pg/mL | 0.63 | 0.001* | | | 0.59 | 0.001* |

| | | | | | | |
|---|---|---|---|---|---|---|
| AOPPs | 0.52 | 0.001* | 0.59 | 0.001* | | |

r= Pearson's correlation coefficient, P was considered significant at <0.05.; *Significant

HOMA-IR: Homeostatic Model Assessment of Insulin Resistance, sRAGE: soluble receptor for advanced glycation end-products, AOPP: advanced oxidation protein products

**Table 6:** showed Pearson's correlation between serum levels of sRAGE, Pentosidine and AOPP with other studied parameters in diabetic patient groups.

| Variables | B | S.E. | P-value | OR | 95.0% C.I. for odd | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| sRAGE | 0.155 | 0.103 | 0.000* | 5.454 | 3.954 | 15.429 |
| pentosidine | 0.253 | 0.091 | 0.005* | 1.288 | 1.078 | 1.539 |
| AOPPs | -0.091 | 0.036 | 0.012* | 0.913 | 0.851 | 0.980 |
| Fasting insulin | 0.987 | 0.435 | 0.023* | 2.683 | 1.143 | 6.295 |
| HOMA/IR | -0.677 | 0.447 | 0.130 | 0.508 | 0.212 | 1.221 |

B: regression coefficient , S.E: Standard error, OR: Odds ratio CI, confidence interval

**Table 7:** Multiple logistic regression analysis with background elimination method using 5 biologically important variables as independent variables, and the development of microalbuminuria as the dependent variable.

Furthermore, Piwowar et al., [48] reported increased plasma AOPPs concentrations in type 2 diabetic nephropathy lending support to our data. The role of AOPPs in the pathogenesis of diabetic nephropathy was underscored by experimental studies which demonstrated that AOPPs promoted renal oxidative stress, inflammation, glomerular hypertrophy, and enhanced expression of fibronectin leading to albuminuria [49]. Moreover, AOPPs accumulation resulted in podocyte apoptosis by a cascade of signaling events coupled with intracellular oxidative stress [50]. Taken together, it is tempting to speculate that serum pentosidine and AOPPs might be useful biomarkers of diabetic microvascular complications.

The reported significant association between AOPPs and ID and DD risky genotypeswas corroborated by the intriguing finding that rennin angiotensin blockade led to increased antioxidant defences in mouse renal tissues and decreased plasma AOPPs levels [51].

Likewise, our data demonstrated a similar pattern of association between pentosidine and the risky ACE genotypes in type 2 diabetic patients.Relevant to this notion, Matsui et al., [52] unambiguously underscored the concept of an existing crosstalk between RAS and AGE in glomerular endothelial cells where AGE significantly stimulated angiotensin II production; also angiotensin II type 1 receptor blockade suppressed AGE-induced reactive oxygen species and inflammatory reactions, thus may play a protective role against diabetic nephropathy.

The limitation of our study is the relatively small sample size; a larger number of subjects should be analyzed for the verification of our results. Also, addition of another group representing diabetic patients with macroalbuminuria and/or a group with end stage renal disease could further confirm the findings of this paper.

In conclusion, the present study provided evidence that AGE/RAGE axis plays a pivotal role in the pathogenesis of diabetic renal microvascular complications, thus it might serve as biomarker of renal vascular injury in type 2 diabetic patients. It also revealed that both ID and DD ACE genotypes might represent a potential risk factor for developing diabetic renal complications being associated with significantly higher levels of sRAGE, pentosidine and AOPPs.

## References

1. Gohda T, Mima A, Moon JY, Kanasaki K (2014) Combat diabetic nephropathy: from pathogenesis to treatment. J Diabetes Res 2014: 207140.
2. Tang ZH, Fang Z, Zhou L (2013) Human genetics of diabetic vascular complications. J Genet 92: 677-694.
3. Wang F, Fang Q, Yu N, Zhao D, Zhang Y, et al. (2012) Association between genetic polymorphism of the angiotensin-converting enzyme and diabetic nephropathy: a meta-analysis comprising 26,580 subjects. J Renin Angiotensin Aldosterone Syst 13: 161-74.
4. Bernstein KE, Ong FS, Blackwell WL, Shah KH, Giani JF, et al. (2012) A modern understanding of the traditional and nontraditional biological functions of angiotensin-converting enzyme. Pharmacol Rev 65: 1-46.
5. Ahluwalia TS, Ahuja M, Rai TS, Kohli HS, Bhansali A, et al. (2009) ACE variants interact with the RAS pathway to confer risk and protection against type 2 diabetic nephropathy.DNA Cell Biol 28: 141-150.
6. Rahimi Z (2012) ACE insertion/deletion (I/D) polymorphism and diabetic nephropathy. J Nephropathol 1: 143-151.
7. Del Turco S, Basta G (2012) An update on advanced glycationendproducts and atherosclerosis. Biofactors 38: 266-274.
8. Kerkeni M, Saïdi A, Bouzidi H, Letaief A, Ben Yahia S, et al. (2013) Pentosidine as a biomarker for microvascular complications in type 2 diabetic patients. Diab Vasc Dis Res 10: 239-245.
9. Yamagishi S, Maeda S, Matsui T, Ueda S, Fukami K, et al. (2012) Role of advanced glycation end products (AGEs) and oxidative stress in vascular complications in diabetes. Biochim Biophys Acta 1820: 663-671.
10. Ramasamy R, Yan SF, Schmidt AM (2012) Advanced glycation end products: from precursors to RAGE: round and round we go. Amino Acids 42: 1151-1161.
11. Manigrasso MB,Juranek J, Ramasamy R, Schmidt AM (2014) Unlocking the biology of RAGE in diabetic microvascular complications. Trends Endocrinol Metab 25: 15-22.
12. Kamioka M, Ishibashi T, Sugimoto K, Uekita H, Nagai R, et al. (2010) Blockade of renin-angiotensin system attenuates advanced glycation end products-mediated signaling pathways. J Atheroscler Thromb 17: 590-600.
13. American Diabetes Association. (2012) Standards of medical care in diabetes--2012. Diabetes Care.; 35 Suppl 1:S11-63. Review.
14. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, et al. (2003) National Kidney Foundation practice guidelines47.
15. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.ClinChem 18: 499-502.

16. Duseja A, Thumburu KK, Das A, Dhiman RK, Chawla YK, et al. (2007) Insulin tolerance test is comparable to homeostasis model assessment for insulin resistance in patients with nonalcoholic fatty liver disease. Indian J Gastroenterol 26: 170-173.
17. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, et al. (1996) Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 49: 1304-1313.
18. Rahimi Z, Felehgari V, Rahimi M, Mozafari H, Yari K, et al. (2011) The frequency of factor V Leiden mutation, ACE gene polymorphism, serum ACE activity and response to ACE inhibitor and angiotensin II receptor antagonist drugs in Iranians type II diabetic patients with microalbuminuria. Mol Biol Rep. 38: 2117-2123.
19. Yang J, Zhao Y, Hao P, Meng X, Dong M, et al. (2013) Impact of angiotensin I converting enzyme insertion/deletion polymorphisms on dilated cardiomyopathy and hypertrophic cardiomyopathy risk. PLoS One 8: e63309.
20. Mtiraoui N, Ezzidi I, Turki A, Chaieb M, Mahjoub T, et al. (2011) Renin-angiotensin-aldosterone system genotypes and haplotypes affect the susceptibility to nephropathy in type 2 diabetes patients. J Renin Angiotensin Aldosterone Syst 12: 572-580.
21. Nikzamir A, Esteghamati A, Feghhi M, Nakhjavani M, Rashidi A, et al. (2009) The insertion/deletion polymorphism of the angiotensin-converting enzyme gene is associated with progression, but not development, of albuminuria in Iranian patients with type 2 diabetes. J Renin Angiotensin Aldosterone Syst 10: 109-114.
22. Ezzidi I, Mtiraoui N, Kacem M, Chaieb M, Mahjoub T, et al. (2009) Identification of specific angiotensin-converting enzyme variants and haplotypes that confer risk and protection against type 2 diabetic nephropathy. Diabetes Metab Res Rev. 25: 717-724.
23. Yu ZY, Chen LS, Zhang LC, Zhou TB (2012) Meta-analysis of the relationship between ACE I/D gene polymorphism and end-stage renal disease in patients with diabetic nephropathy.Nephrology (Carlton) 17: 480-487.
24. Montezano AC, Nguyen Dinh Cat A, Rios FJ, Touyz RM (2014) Angiotensin II and vascular injury.CurrHypertens Rep 16: 431.
25. Volkan-Salanci B, Dagdelen S, Alikasifoglu M, Erbas T, Hayran M, et al. (2009) Impact of rennin angiotensin system polymorphism on renal haemo-dynamic responsiveness to acute angiotensin-converting enzyme inhibition in type 2 diabetes mellitus. J Renin Angiotensin Aldosterone Syst 10: 41-50.
26. Arora MK, Singh UK (2014) Oxidative stress: meeting multiple targets in pathogenesis of diabetic nephropathy. Curr Drug Targets 15: 531-538.
27. Singh VP, Bali A, Singh N, Jaggi AS (2014) Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol 18: 1-14.
28. Chawla D, Bansal S, Banerjee BD, Madhu SV, Kalra OP, et al. (2014) Role of advanced glycation end product (AGE)-induced receptor (RAGE) expression in diabetic vascular complications. Microvasc Res 95: 1-6.
29. Jeganathan VS (2011) The therapeutic implications of renin-angiotensin system blockade in diabetic retinopathy. Curr Pharm Biotechnol 12: 392-395.
30. Ng ZX, Chua KH, Iqbal T, Kuppusamy UR (2013) Soluble Receptor for Advanced Glycation End-Product (sRAGE)/Pentosidine Ratio: A Potential Risk Factor Determinant for Type 2 Diabetic Retinopathy. Int J MolSci 14: 7480-7491.
31. Aubert CE, Michel PL, Gillery P, Jaisson S, Fonfrede M, et al. (2014) Association of peripheral neuropathy with circulating advanced glycation end products, soluble receptor for advanced glycation end products and other risk factors in patients with type 2diabetes. Diabetes Metab Res Rev30: 679-685.
32. Fujisawa K, Katakami N, Kaneto H, Naka T, Takahara M, et al. (2013) Circulating soluble RAGE as a predictive biomarker of cardiovascular event risk in patients with type 2 diabetes. Atherosclerosis 227: 425-428.
33. Mahajan N, Dhawan V (2013) Receptor for advanced glycation end products (RAGE) in vascular and inflammatory diseases. Int J Cardiol 168: 1788-1794.
34. Pham MN, Hawa MI, Roden M, Schernthaner G, Pozzilli P, et al. (2012) Increased serum concentrations of adhesion molecules but not of chemokines in patients with Type 2 diabetes compared with patients with Type 1 diabetes and latent autoimmune diabetes in adult age: action LADA 5. Diabet Med 29: 470-478.
35. Nakamura K, Yamagishi S, Adachi H, Matsui T, Kurita-Nakamura Y, et al. (2008) Circulating advanced glycation end products (AGEs) and soluble form of receptor for AGEs (sRAGE) are independent determinants of serum monocyte chemoattractant protein-1 (MCP-1) levels in patients with type 2 diabetes. Diabetes Metab Res Rev 24:109-114.
36. Lam JK, Wang Y, Shiu SW, Wong Y, Betteridge DJ, et al. (2013) Effect of insulin on the soluble receptor for advanced glycation end products (RAGE). Diabet Med 30: 702-709.
37. Basta G, Sironi AM, Lazzerini G, Del Turco S, Buzzigoli E, et al. (2006) Circulating soluble receptor for advanced glycation end products is inversely associated with glycemic control and S100A12 protein. J Clin Endocrinol Metab 91: 4628-4634.
38. Tam XH, Shiu SW, Leng L, Bucala R, Betteridge DJ, et al. (2011) Enhanced expression of receptor for advanced glycation end-products is associated with low circulating soluble isoforms of the receptor in Type 2 diabetes. Clin Sci (Lond) 120: 81-89.
39. Daroux M, Prévost G, Maillard-Lefebvre H, Gaxatte C, D'Agati VD, et al. (2010) Advanced glycation end-products: implications for diabetic and non-diabetic nephropathies. Diabetes Metab 36: 1-10.
40. Nakashima A, Carrero JJ, Qureshi AR, Miyamoto T, Anderstam B, et al. (2010) Effect of circulating soluble receptor for advanced glycation end products (sRAGE) and the proinflammatory RAGE ligand (EN-RAGE, S100A12) on mortality in hemodialysis patients. Clin J Am Soc Nephrol 5: 2213-2219.
41. Rüster C, Bondeva T, Franke S, Tanaka N, Yamamoto H, et al. (2009) Angiotensin II upregulates RAGE expression on podocytes: role of AT2 receptors. Am J Nephrol 29: 538-550.
42. Ono Y, Mizuno K, Takahashi M, Miura Y, Watanabe T (2013) Suppression of advanced glycation and lipoxidation end products by angiotensin II type-1 receptor blocker candesartan in type 2 diabetic patients with essential hypertension. Fukushima J Med Sci 59: 69-75.
43. Sato E, Nagaoka T, Yokota H, Takahashi A, Yoshida A (2012) Correlation between plasma pentosidine concentrations and retinal hemodynamics in patients with type 2 diabetes. Am J Ophthalmol 153: 903-909.
44. Nishizawa Y, Koyama H, Inaba M (2012) AGEs and cardiovascular diseases in patients with end-stage renal diseases. J Ren Nutr 22: 128-133.
45. Gradinaru D, Borsa C, Ionescu C, Margina D (2013) Advanced oxidative and glycoxidative protein damage markers in the elderly with type 2 diabetes. J Proteomics 92: 313-322.
46. Ishibashi Y, Matsui T, Ueda S, Fukami K, Yamagishi S (2014) Advanced glycation end products potentiate citrated plasma-evoked oxidative and inflammatory reactions in endothelial cells by up-regulating protease-activated receptor-1 expression. Cardiovasc Diabetol 13:60.
47. Tobon-Velasco JC, Cuevas E, Torres-Ramos MA, Santamaria A (2014) Receptor for AGEs (RAGE) as Mediator of NF-kB Pathway Activation in Neuroinflammation and Oxidative Stress.CNS NeurolDisord Drug Targets.
48. Piwowar A, Knapik-Kordecka M, Warwas M (2010) Comparison of the usefulness of plasma levels of oxidatively modified forms of albumin in estimating kidney dysfunction in diabetic patients. Clin Invest Med 33: E109.
49. Colombo G, Clerici M, Giustarini D, Rossi R, Milzani A, et al. (2012) Redox albuminomics: oxidized albumin in human diseases. Antioxid Redox Signal 17: 1515-1527.
50. Zhou LL, Cao W, Xie C, Tian J, Zhou Z, et al. (2012) The receptor of advanced glycation end products plays a central role in advanced oxidation protein products-induced podocyte apoptosis. Kidney Int 82: 759-770.

51. Portero-Otín M, Pamplona R, Boada J, Jové M, Gonzalo H, et al. (2008) Inhibition of renin angiotensin system decreases renal protein oxidative damage in diabetic rats. Biochem Biophys Res Commun 368: 528-535.

52. Matsui T, Nishino Y, Maeda S, Takeuchi M, Yamagishi S (2011) Irbesartan inhibits advanced glycation end product (AGE)-induced up-regulation of vascular cell adhesion molecule-1 (VCAM-1) mRNA levels in glomerular endothelial cells. Microvasc Res 81: 269-273.