

Gly82Ser Polymorphism of the Receptor for Advanced Glycation End Products Gene (RAGE) and the Endogenous Secretory RAGE (esRAGE) and its Association with Diabetic Retinopathy in Type 2 Diabetic Patients

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ABSTRACT

The binding of advanced glycation end-products (AGEs) to their receptor (RAGE) may play an important role in the development of diabetic retinopathy (DR). Recently, endogenous secretory RAGE (esRAGE) has been identified as an alternative splicing form of RAGE able to capture AGEs, and exerts protection against AGEs-induced endothelial cell injury. A Gly82Ser polymorphism in exon 3 of RAGE gene was identified and thought to have an effect on the functions of its protein. This study was planned to investigate the frequency of the Gly82Ser polymorphism in RAGE gene and the role of esRAGE as a biological marker for DR in type2 diabetes and its association with the severity of DR. Thirty-five patients with type2 diabetes were recruited into the study. They were subclassified into 15 patients with no clinically apparent retinopathy (No DR), 12 patients with nonproliferative DR (NPDR), and 8 patients with proliferative DR (PDR). Twenty, age matched, healthy subjects were included as controls. Serum esRAGE level was measured by enzyme-linked immunosorbent assay. Genotype frequencies of Gly82Ser polymorphism were studied by polymerase chain reaction amplification and restriction fragment length polymorphism analysis using AluI enzyme. The results showed no significant difference between serum esRAGE levels in both controls and diabetic patients with No DR ($P = 0.15$). Among the diabetic subjects, there was a significant decrease of serum esRAGE levels between patients with No DR and patients with NPDR ($P = 0.008$) and a more significant decrease between diabetic patients with No DR and patients with PDR ($P = 0.001$). The low serum esRAGE diabetic patients had higher risk to develop DR than those with high serum esRAGE level (odds ratio = 4.7, 95% confidence interval = 1.07-20.65, $P = 0.02$). There were no significant differences in genotyping frequencies or allele frequencies between controls and diabetic patients with No DR, or patient with DR ($P < 0.05$). In conclusion, serum esRAGE level showed a significant association with the severity of DR and, hence, it could be used as a prognostic tool to predict the development and progression of DR. esRAGE could be a novel and potential protective factor for DR. Gly82Ser polymorphisms in RAGE gene are not associated with the susceptibility of type 2 diabetes, or with the development of DR in type 2 diabetic subjects.

INTRODUCTION

One of the most common complications of diabetes is diabetic retinopathy (DR). Several pathways of glucose metabolism have been postulated as possible mechanisms for the development of diabetic vascular complications. There is substantial evidence to support the involvement of advanced glycation endproducts (AGEs) binding to its receptor (RAGE) in the development of diabetic microvascular complications.¹

AGEs is formed from the nonenzymatic reaction of glucose and/or α -oxoaldehydes with amino groups of proteins, lipids, and DNA. These heterogeneous adducts can modify the structure and function of proteins and lead to intramolecular and intermolecular cross-link formation.² In the vascular endothelium, the engagement of RAGE by AGEs causes changes in endothelial cells and pericytes that are characteristic of diabetic microangiopathy. AGEs stimulate the growth of microvascular endothelial cells with an induction of vascular endothelial growth factor, leading to angiogenesis,³ and inhibit prostacyclin production and stimulate plasminogen activator inhibitor-1 synthesis by endothelial cells, leading to thrombogenesis.⁴ AGEs also exhibit a growth inhibitory action on pericytes, which would lead to ‘‘pericyte loss’’, the earliest histological hallmark of DR.⁵

RAGE is a member of the immunoglobulin superfamily, and was originally described as a

transmembrane multiligand receptor.⁶ Yonekura et al. found that human vascular cells express a novel splice variant coding for the sRAGE protein, termed endogenous secretory RAGE (esRAGE) that neutralizes AGEs actions on endothelial cells and pericytes.^{7,8} According to their findings, that variant may exert protective effects by preventing ligand triggered RAGE-dependent cellular activation. This may be particularly crucial in the diabetic state in which AGEs accumulate at an extremely high rate and shown to be a major factor in the vascular cell derangement.⁹

Genetic polymorphism in RAGE gene may also alter AGEs processing in tissues or reactions after the binding of AGEs to RAGE. The gene encoding RAGE is located on chromosome 6 in the major histocompatibility complex.¹⁰ The RAGE gene contains 11 exons and a 3' untranslated region. Within the exons, a common variant (Gly82Ser) and 3 rare changes (Thr18Pro, Gly329Ala, Ala389Gln) have been identified.¹¹ Some reports^{12,13} suggested that Gly82Ser polymorphism in the RAGE gene were associated with DR in type 2 diabetes, whereas others^{14,15} did not support such significant linkage.

The purpose of the current study is to clarify the frequency of RAGE Gly82Ser polymorphism in DR patients and the role of esRAGE in the development of DR and their correlation with the severity of DR in type 2 diabetic patients.

SUBJECTS & METHODS

Study subjects:

The study was carried out in Faculty of Medicine, Zagazig University. The patients were collected from Ophthalmic Outpatient Clinic of Zagazig- University-Hospital, and the biochemical assessment was done at Medical Biochemistry Department. Thirty-five patients with type 2 diabetes were recruited for the study; of these 15 patients with no clinically apparent retinopathy (No DR), 12 patients with nonproliferative diabetic retinopathy (NPDR), and 8 patients with proliferative diabetic retinopathy (PDR). The patients with No DR were chosen to have diabetic duration comparable with patients with retinal microangiopathy. Twenty nondiabetic age-matched healthy subjects were enrolled as controls. Exclusion criteria for diabetic patients included the presence of clinical nephropathy (urinary albumin excretion (UAE) >300 µg albumin/ mg creatinine) and patients treated with angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers. A written informed consent was obtained from all subjects included in the study.

Eye examination:

Comprehensive eye examinations included direct and indirect ophthalmoscopy (with pupils dilated), as well as retinal color photographs using Zeiss FF 450 plus digital camera and, whenever indicated, fundus fluorescein angiography. The fundus photography was obtained on four fields which included macula, disc, superior temporal, and inferior

temporal quadrants. The photographs were graded using the Early Treatment Diabetic Retinopathy Study grading system.¹⁶

Biochemical analysis:

All blood and urine specimens were collected between 9:00 and 11:00 a.m. after the subjects had fasted overnight. Venous blood specimens were collected in ethylene diamine tetra acetic acid (EDTA)-treated and plain tubes. The tubes were placed on ice until they were delivered to the laboratory (within 1-3 hours). After plasma and serum were separated, the samples were stored at -20°C. The whole EDTA-blood was used for estimation of glycated hemoglobin (HbA_{1c}).

Fasting blood glucose levels were measured by the glucose oxidase method according to **Trinder** (Genzyme Diagnostics PEI Inc PE, Canada C1E 2B9).¹⁷ Affinity chromatographic quantitation of HbA_{1c} was performed according to Abraham et al., (Kit was purchased from STANBIO).¹⁸ Urine creatinine were estimated with the colorimetric method (kit was purchased from Randox) according to Batels.¹⁹ UAE was measured in spot urine sample from albumin/creatinine ratio (ACR); a solid phase enzyme-linked immunosorbent assay (ELISA) was used to measure urinary albumin on spot urine sample (Orgentec diagnostika GmbH).²⁰

Spot urine ACR ratio was calculated for diabetic patients.

Serum levels of esRAGE were measured by ELISA according to **katkami et al.**²¹ It was done using the B-Bridge esRAGE ELISA Kit (B-Bridge International, Sunnyvale,

USA) following manufacturers' instructions.

Genotyping of RAGE Gly82Ser:

DNA extraction was performed according to Bubbon using a Pure gene DNA purification kit (Gentra, Minneapolis, USA) following the manufacturer's protocol.²² A polymerase chain reaction (PCR)-RFLP assay was used to determine the RAGE Gly82Ser polymorphism as described by Hudson et al.¹⁴ PCR was done on 25 μ L reaction volume containing 25 pmol of forward 5'-CACTGTTTAGGCCCTGCTTC-3' and reverse primer 5'-GGAATTCT-TACGGTAGACACGG-3' (Biosource Europe SA, Belgium, Netherlands, Germany), 200 mM of each deoxynucleotide phosphates (Sigma Chemical Co. St Louis, MO USA), 2 mM MgCl₂, 1 unit of Taq polymerase (Life Tech Inc; Gaithersburg a USA), 2 μ L of 10x PCR buffer (75mM Tris-HCl, 20mM (NH₄)₂SO₄ and 0.1% Tween 20 (ABgene), and 200 ng genomic DNA. The amplification was carried out using thermal cycler PTC-100 machine (MJ Reasearch Inc; Watertomn, Mass USA) after initial denaturation at 95°C for 5 minutes, the DNA was amplified by 35 cycles of 94°C for 30 seconds, 62°C for 40 seconds, 72°C for 45 seconds, followed by a final extension for 5 minutes at 72°C.

Restriction endonuclease digestion:

The polymorphism causing a Gly to Ser change at codon number 82 (GGC/AGC) resulted in the formation of an *AluI* restriction site. The digestion was performed with *AluI* (*Arthrobacter luteus*) (Life Technologies) for 16 hours at 37°C in total volume 25 μ L that contained: 3

μ L of 10x buffer (10mM Tris-HCl (pH 7.4), 100mM sodium citrate, 0.1mM EDTA, 1mM dinitrothreitol), 0.5 μ L of bovine serum albumin, 1.5 μ L of *AluI* (10U/ μ L), 15 μ L of PCR product, and 5 μ L of deionized water. Then the digestion products were electrophoresed at 100 mA and 70 volts on a 2% agarose gel for 1.5 hours using the EC 360 Submarine Gel electrophoresis system (Maxicell, EC 360 M-E-C apparatus Cooperation. St Petersburg Florida USA). The results were visualized by ethidium bromide staining under transillumination with 100 bp ladder (Pharmacia Biotech, USA) and then photographed. 189, 49 bp bands represent a wild type for Gly82 allele (82Gly/Gly). 122, 67, 49 bp bands represent homozygote for the Ser82 allele (82Ser/Ser). 189, 122, 67, 49 bp bands represent a heterozygote for the Gly82 and Ser82 alleles (82Gly/Ser), (Fig 1).

Statistical analysis: The data was processed by the SPSSX (SPSS Inc.,

Chicago, IL, version 11, USA) statistical package. All results are expressed as mean \pm SD. Comparison among groups was made by Student's *t*-test (unpaired). One-way analysis of variance (ANOVA) was used to compare the groups on continuous variables. Exact Fisher test was used to compare independent samples. Odds ratio was used to calculate the risk. For all statistical tests, $P < 0.05$ was considered as the level of significance.

RESULTS

There was no statistically significant difference between controls and diabetic patients as

regard age ($P = 0.15$). There were no statistically significant differences between the diabetic subjects with different grades of DR as regard: age of the patients ($P = 0.76$), duration of diabetes ($P = 0.13$), fasting blood

glucose level ($P = 0.11$), HbA_{1c} ($P = 0.84$); but, there was a significant difference as regard UAE ($P = 0.02$). The patients' clinical and biochemical characteristics are shown in Table 1.

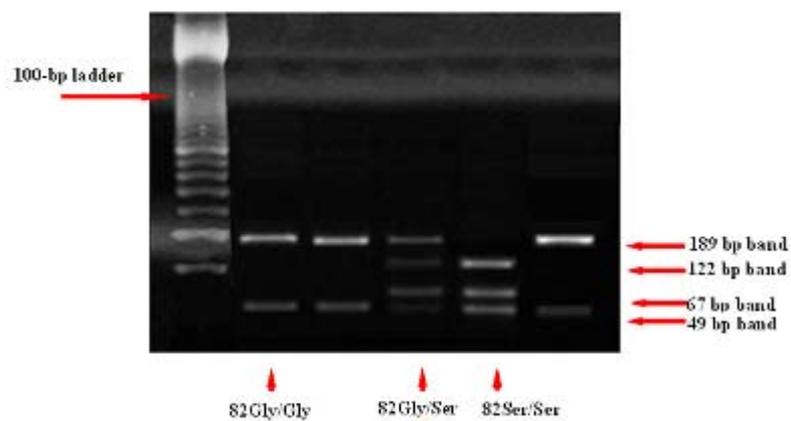


Figure 1. PCR-based RFLP assay for Gly82 and Ser82 RAGE alleles

Table 1. Clinical and Biochemical Characteristics of Studied Subjects

	Controls (n = 20)	Diabetic patients (n,35)			F value	P value
		No DR (n = 15)	NPDR (n = 12)	PDR (n = 8)		
Gender (%);					--	--
male:	12 (60)	9 (60)	5 (42)	4 (50)		
female:	8 (40)	6 (40)	7 (58)	4 (50)		
Age (yrs); range:	45-65	49-66	50-62	50-65		0.15 [†]
mean ± SD:	54.9±6.2	57.0±5.2	57.9±3.9	56.4±4.8	0.27 [‡]	0.76 [‡]
Blood glucose (mg/dL); range:	70-92	135-185	135-220	140-230	2.42 [‡]	0.10 [‡]
mean ± SD:	79.6±7.4	158.6±14.8	170.3±24.5	179.8± 30.7		
HbA _{1c} %; range:	4.1-5.9	4.9-10.1	4.0-11.1	4.0-11.3	0.17 [‡]	0.84 [‡]
mean ± SD:	4.7±0.9	7.5±1.4	7.9±1.9	7.7±2.3		
UAE*; range:	3.8-16.4	4.2-18.0	8.9-21.7	10.7-28.5	4.32 [‡]	0.02 [‡]
mean ± SD:	9.1±4.1	9.7±3.8	12.4±4.1	15.5±6.1		

*UAE = Urinary albumin excretion, (μg albumin/mg creatinine).

[†]t test between controls and diabetic patients.

[‡]ANOVA (single factor) between diabetic patients.

In the control subjects, the mean level of serum esRAGE was 0.10 ± 0.03 ng/ml. In diabetic patients, the mean level of serum esRAGE was 0.12 ± 0.04 ng/ml., 0.09 ± 0.01 ng/ml., and 0.07 ± 0.02 ng/ml. in patients with No DR, NPDR, and PDR respectively. There was no significant difference between levels of serum esRAGE between healthy controls and diabetic patients with No DR ($P = 0.15$). Among the diabetic patients; the serum level of esRAGE was significantly lower in patients with NPDR when compared to diabetic patients with No DR ($P = 0.008$), and

a more significant decrease in patients with PDR compared to diabetic patients with No DR ($P = 0.001$) (Table 2). When DR was graded arbitrary into level 1 = No DR, level 2 = NPDR, and level 3 = PDR, a significant negative correlation was found between serum esRAGE and the severity of DR ($R^2 = 0.30$, $P < 0.001$) (Fig 2). No significant correlation was found between HbA_{1c} and serum esRAGE level ($R^2 = 0.02$, $P = 0.47$) (Fig 3). Also, there was no significant correlation between UAE and serum esRAGE levels in the DR patients ($R^2 = 0.03$, $P = 0.29$) (Fig 4).

Table 2. Serum esRAGE (ng/ml.) in Controls and Diabetic Patients.

	Controls	Diabetic patients (n,35)		
	(n, 20)	No DR (n = 15)	NPDR (n = 12)	PDR (n = 8)
Range	0.05-0.18	0.07-0.19	0.07-0.11	0.05-0.10
Mean \pm SD	0.10 ± 0.03	0.12 ± 0.04	0.09 ± 0.01	0.08 ± 0.02
P value	--	0.149*	0.008 [†]	0.001 [†]

No DR = no diabetic retinopathy (DR). NPDR = nonproliferative DR. PDR = proliferative DR

*t test versus controls.

[†]t test versus No DR patients

Odds ratio was used for calculation of the risk of the development of DR; the median value of serum levels of esRAGE among all 35 diabetic patients was used as a cut-off value, it was equal to 0.09 ng/ml. The diabetic patients were divided as low serum esRAGE subjects (equal to or less than the median value), and high serum esRAGE subjects (higher than the median value). Odds ratio was 4.7 (95% confidence interval = 1.07-20.65, $P = 0.02$).

In control subjects, 19 persons had homozygous 82Gly/Gly (95%), one person (5%) had heterozygous 82Gly/Ser, and none had homozygous 82Ser/Ser; and the allele frequency for the Gly82 was 97.5% (39/40), and the allele frequency for Ser82 allele was 2.5% (1/40). In the No DR patients, 12 patients had homozygous 82Gly/Gly (80%), 2 patients (13.3%) had heterozygous 82Gly/Ser, and one patient (6.7%) had homozygous 82Ser/Ser; and the allele frequency for the Gly82 allele was 86.7% (26/30)

and the allele frequency for the Ser82 allele was 13.3% (4/30). In the DR patients, 18 patients had homozygous 82Gly/Gly (90%), one patient (5%) had heterozygous 82Gly/Ser, and one patient (5%) had homozygous 82Ser/Ser; and the allele frequency for the Gly82 allele was 92.5% (37/40) and the allele frequency for the Ser82 allele was 7.5% (3/40). There were no statistically significant differences between controls and diabetic patients with No DR as regard Gly82Ser genotyping frequencies (Gly/Gly, $P = 0.23$; Gly/Ser, $P = 0.50$; or Ser/Ser, not determined). There were also no statistically significant differences between controls and diabetic patients with DR as regard Gly82Ser genotyping frequencies (Gly/Gly, $P =$

0.62; Gly/Ser, $P = 1.00$; or Ser/Ser, not determined). There were no statistically significant differences between diabetic patients with No DR and diabetic patients with DR as regard Gly82Ser genotyping frequencies (Gly/Gly, $P = 0.45$; Gly/Ser, $P = 0.46$; or Ser/Ser, $P = 0.86$). There were no statistically significant differences as regard allele frequencies between controls and diabetic patients with No DR ($P = 0.11$ for both Gly82 and Ser82 alleles), between controls and diabetic patients with DR ($P = 0.37$ for both Gly82 and Ser82 alleles), or between diabetic patients with No DR and diabetic patients with DR ($P = 0.45$ for both Gly82 and Ser82 alleles) (Table 3).

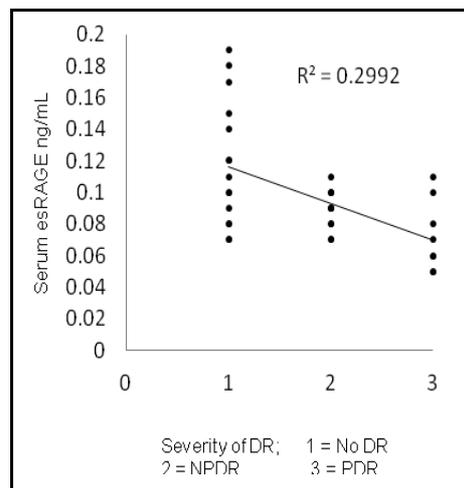


Figure 2. Regression and correlation between serum RAGE and severity of diabetic retinopathy (DR). NPDR = nonproliferative DR; PDR = proliferative DR. $P < 0.001$.

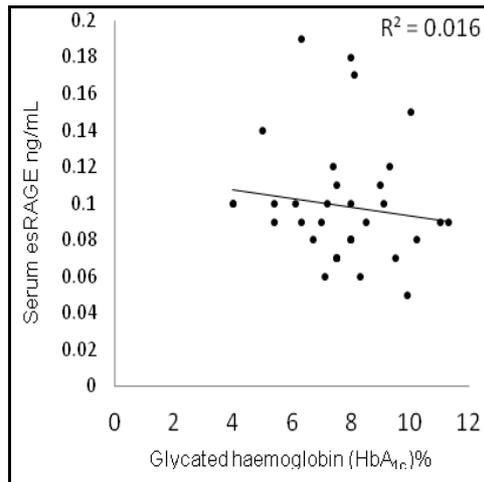


Figure 3. Regression and correlation between serum RAGE and glycated Hemoglobin HbA_{1c}%. *P* = 0.47

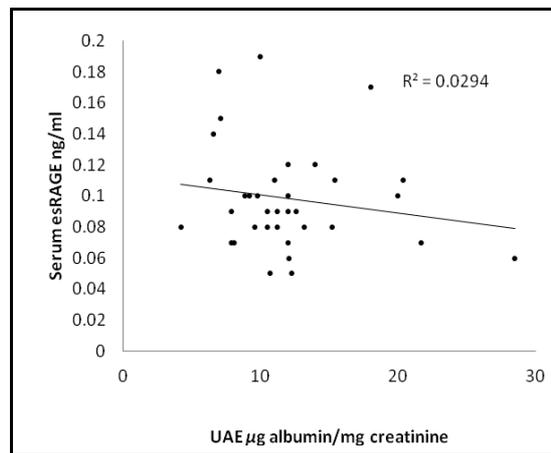


Figure 4. Regression and correlation between serum esRAGE and urinary albumin excretion (UAE). *P* = 0.29

Table 3. Frequency of Gly82 and Ser82 RAGE Alleles in the Studied Groups.

Population	82Gly/Gly	82Gly/Ser	82Ser/Ser	Gly82 allele	Ser82 allele
Controls (n = 20) Number (%)	19 (95)	1 (5)	0 (0)	39/40 (97.5)	1/40 (2.5)
No DR (n = 15) Number (%)	12 (80)	2 (13.3)	1 (6.7)	26/30 (86.7)	4/30 (13.3)
DR (n = 20) Number (%)	18 (90)	1 (5)	1 (5)	37/40 (92.5)	3/40 (7.5)
<i>P</i> value (Exact Fisher test)	0.23*	0.50*	--*	0.11*	0.11*
	0.62 [†]	1.00 [†]	-- [†]	0.36 [†]	0.36 [†]
	0.45 [‡]	0.50 [‡]	0.86 [‡]	0.45 [‡]	0.45 [‡]

*Between controls and diabetic patients with no diabetic retinopathy (DR).

[†]Between controls and patients with DR.

[‡]Between diabetic patients with No DR and patients with DR.

DISCUSSION

In the present study, serum level of esRAGE was shown to decrease in DR patients compared to control subjects, and that decrease strongly associated with the development and progression of DR. Also, there is no association between HbA_{1c} and severity of DR, which means that the decreased serum esRAGE levels with the severity of DR was independent of blood glucose control. Further, decreased serum esRAGE level was inferred in patients with DR without any diabetic renal affection. Lastly, the results showed that Gly82Ser polymorphism in RAGE gene were not associated with the susceptibility of type 2 diabetes, or with the development of DR in type 2 diabetic subjects.

As serum levels of esRAGE could be influenced by agents that block the renin-angiotensin system.²³ Patients treated with angiotensin-converting enzyme inhibitors or

angiotensin-receptor blockers were excluded from the study. Patients with clinical nephropathy were also excluded, so the association between serum esRAGE level and severity of DR could be assessed without the influence of underlying kidney affection. The current study revealed that UAE levels in NPDR patients and PDR patients were slightly higher but statistically significant than that in patients with No DR, and there was no significant correlation between UAE and serum esRAGE levels in the DR patients. These results suggest that the kidney state as reflected by UAE does not decrease serum esRAGE and the association between the DR and serum esRAGE was not confounded by possible diabetic kidney affection or drugs.

To reduce the influence of circadian variation, all blood and urine samples were collected between 9:00 and 11:00 a.m. after the subjects had fasted overnight. We calculated the UAE from the calculation of ACR in a

spot urine sample as this method has certain advantages. It is a more convenient test for the patients and may be less prone to errors due to improper collection methods. The calculation of ACR also exhibits a higher sensitivity and specificity in the detection of microalbuminuria.²⁴

We are interested particularly in Gly82Ser polymorphism because of relatively high prevalence and the polymorphism results in the creation of an *AluI* restriction site (AG^{CT}). The nucleotide change can be rapidly screened by PCR-RFLP method. Gly82Ser polymorphism is said to have significance because it occurs at a predicted N-linked glycosylation site and in the same immunoglobulin variable domain as the AGEs binding site.¹⁴

For prediction and prevention of DR, identification and quantification of risk factors are essential. **Rema et al.** stated that the main risk factors for DR were uncontrolled glycemia, as reflected by HbA_{1c}, and duration of diabetes.²⁵ In the present study the patients with No DR were chosen to have diabetic duration comparable with patients with DR, and our results showed no significant difference as regard diabetic duration between diabetic patients. Also, we found no significant correlation between HbA_{1c} levels and stages of DR. Therefore, it could be speculated that other factors besides HbA_{1c} or diabetic duration may influence the development of DR. Accumulating evidence indicates that the AGE-RAGE system plays a pivotal role in the development of diabetic vascular complications and its blockage by esRAGE should be a candidate molecular target for

overcoming diabetic vascular complications.⁸ Our speculation is supported by a major epidemiologic study done by Porta & Bandello who stated that although HbA_{1c} is closely associated with diabetic complications but there can be discernible and sometimes perplexing heterogeneity in the development of DR, they also found no threshold levels for HbA_{1c}. Moreover, in our study, no significant correlation between HbA_{1c} and serum esRAGE levels was found. This suggests that the serum esRAGE levels are not affected by the fluctuation of blood glucose level, at least in a short term. That finding means that the association of increased serum esRAGE level with the severity of DR was independent of HbA_{1c}.

We found no significant difference in the serum levels of esRAGE between nondiabetic controls and diabetic patients with No DR. But, among the diabetic patients, serum esRAGE levels were significantly decreased in NPDR, and PDR when compared with patients with No DR. We could explain these results by assuming that, the serum esRAGE level is not related, at least directly, to the events that trigger the onset of type 2 diabetes but after the onset of DR, serum esRAGE level affects the course of the development of retinopathy. Because among diabetic patients; low serum esRAGE patients get retinopathy earlier than high serum esRAGE patients, so we could speculate that serum esRAGE might protect against the development of DR.

We assume that individual variations in levels of serum esRAGE

could be a determinant for individual differences in susceptibility or resistance to the development of DR, as our study revealed that serum esRAGE was significantly decreased in diabetic patients with different severities of DR. Diabetic subjects with low serum esRAGE (equal to or lower than 0.09 ng/ml.) have 4.7 fold higher risk to develop DR than those with high esRAGE serum levels (higher than 0.09 ng/ml.). It could be hypothesized that the role of serum esRAGE in the development of DR might be that esRAGE may act as a decoy of AGEs to maintain vascular homeostasis and could protect vessels against AGEs toxicity in type 2 diabetic patients. The interaction of AGEs with its receptor RAGE alters intracellular signaling, gene expression, release of proinflammatory molecules, and production of reactive oxygen species that might contribute towards the pathology of DR.²⁷

Our hypothesis that serum esRAGE can act as decoy is consistent with the results of Grossin et al who found that, in type 2 diabetes serum esRAGE levels were significantly lower in patients with complications versus those without complications.²⁸ Also; in type 1 diabetes, Katakami et al., found that a low serum esRAGE level has been related to the severity of DR.²¹ In context with diabetic vascular complications, Koyama et al.,²⁹ reported that in type 2 diabetes, a decrease of serum esRAGE level has been associated to a high incidence of cardiovascular mortality. Of note that recombinant esRAGE can successfully prevent diabetic microvascular and macrovascular

complications both in vitro and in animal models in which RAGE ligands mediate vascular and inflammatory stress.³⁰ These observations support our findings that serum esRAGE might play a protective factor for the development DR.

Nowadays, genome-wide association study is encouraging and widely conducted to help in developing more accurate diagnostic and therapeutic strategies of various kinds of human diseases.³¹ Our candidate single nucleotide polymorphism (Gly82Ser) investigation in type 2 diabetes was to find out whether the polymorphism was associated with DR or not. First, there were no significant differences in genotyping frequencies or allele frequencies between controls and diabetic patients with No DR, or patient with DR. So, we can state that the Gly82Ser polymorphism was not associated with the susceptibility of type 2 diabetes. Previous studies have supported that suggestion.^{32,33} Second, there were no significant differences in genotyping frequencies or allele frequencies between diabetic patients with No DR, or patient with DR. So, we could suggest that the Gly82Ser polymorphism was not associated with the development of DR. These results could be explained as the effect of the Gly82Ser polymorphism on receptor function occurs at a predicted N-linked glycosylation site and in the same immunoglobulin variable domain as the AGEs binding site. But because the tertiary structure of RAGE is unknown, it is impossible to predict any structural effects of that polymorphism. Our results are in

parallel with the results of Liu and Xiang³⁴ who had shown that Gly82Ser is not associated with DR in type 2 diabetes in the Chinese population. There are a few reports on association between Gly82Ser polymorphism of RAGE gene and diabetic complications such as skin complications¹² and DR.¹³

We believe that our results have potential clinical implications for the prevention of retinal microangiopathy in diabetic patients and the present findings suggest the opportunity of exploring preventive strategies aiming at raising serum esRAGE levels. But this study has some limitations: (a) A cross-sectional approach was used to provide evidence of an association between serum esRAGE and the severity of DR. (b) The study was carried out in clinic populations; hence, the values of serum esRAGE derived from this study cannot be generalized unless similar studies are replicated and validated in different populations. Moreover, to address the predictive potential of serum esRAGE as a biomarker for DR, prospective studies have to be carried out. Further prospective genome wide association study on the relation between RAGE gene and related genes and DR is needed.

In conclusion, we suggest that serum esRAGE measurement could be used as a simple biomarker in primary screening programs to predict the development and progression of DR. Once validated in replicative studies, it would serve as a specific biomarker not only for an early diagnosis of DR but also to prevent them; if we could invent a means to selectively induce this splice variant, it would help

increase resistance to the development of the disease. Our results showed that Gly82Ser polymorphism in RAGE gene are not associated with the susceptibility of type 2 diabetes, or with the development of DR in type 2 diabetic subjects.

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التعدد الشكلي جليسين ٨٢ سيرين لجين مستقبلات منتجات الارتباط السكري و المستقبلات المفرزة الداخليه للراچ و علاقتها باعتلال الشبكيه السكري في مرضى البوال السكري النوع الثاني

تهدف هذه الدراسة إستقصاء مدي فاعلية قياس المستقبلات المفرزة الداخليه للراچ في مصل الدم للتنبؤ بحدوث وتطور إعتلال الشبكية السكري في مرضى البوال السكري النوع الثاني و مدي إنتشار التعدد الشكلي جليسين ٨٢ سيرين لمستقبلات منتجات الارتباط السكري في هؤلاء المرضى .

تم إجراء هذه الدراسة في قسمي طب وجراحة العيون والكيمياء الحيوية الطبيه بكلية الطب جامعة الزقازيق و قد اشتملت على ٣٥ مريضاً بالبوال السكري من النوع الثاني و تم تقسيمهم الى ١٥ مريض لا يعانون من إعتلال الشبكية السكري و ١٢ مريض يعانون من إعتلال الشبكية السكري الغير متشعب و ٨ مرضى يعانون من إعتلال الشبكية السكري المتشعب ، ومجموعة ضابطه اشتملت على ٢٠ أصحاء لا يعانون من أمراض أو يتعاطون أدويه تتعارض مع الدراسة و تم قياس مستوى المستقبلات المفرزة الداخليه للراچ في مصل الدم وإستخدم التفاعل التسلسلي عديد البلمرة للكشف عن التعدد الشكلي للمستقبلات لجين المستقبلات.

تشير نتائج الدراسة إلى أنه ليس هناك إختلاف ذو دلالة إحصائية في مستوى المستقبلات المفرزة الداخليه للراچ في مصل الدم بين المجموعة الضابطه والمرضى الذين لا يعانون من إعتلال الشبكية السكري ، لكن وجد إنخفاض ذو دلالة إحصائية في مستوى المستقبلات المفرزة الداخليه للراچ في المرضى الذين يعانون من إعتلال الشبكية السكري الغير متشعب عنه في المجموعة الضابطه ، كما وجد إنخفاض ذو دلالة إحصائية في مستوى المستقبلات المفرزة الداخليه للراچ في المرضى الذين يعانون من إعتلال الشبكية السكري المتشعب عنه في المجموعة الضابطه. وليس هناك إختلاف ذو دلالة إحصائية في معدل وجود التعدد الشكلي جليسين ٨٢ سيرين لمستقبلات منتجات الارتباط السكري بين مرضى البوال السكري الغير مصابين باعتلال الشبكية السكري عنه في الأصحاء ومرضى البوال السكري المصابين باعتلال الشبكية السكري.

الخلاصه: نستخلص مما سبق أنه يمكن أن تستخدم المستقبلات المفرزة الداخليه للراچ للتنبؤ بحدوث وتطور إعتلال الشبكية السكري في مرضى البوال السكري النوع الثاني ويمكن إستخدامه للعلاج إذا وجدت إستراتيجية لزيادة مستواه في الدم. . أن التعدد الشكلي جليسين ٨٢ سيرين لجين مستقبلات منتجات الارتباط السكري ليس له علاقه بالقابليه لحدوث مرض البوال السكري من النوع الثاني أو حدوث إعتلال الشبكية السكري في مرضى البوال السكري من النوع الثاني.