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Original Research

The effects of Advanced Glycation End Products (RAGE) -374T/A and Gly82Ser variants and soluble-RAGE levels to obesity in children

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Abstract: In recent years, studies related to advanced glycation end products (AGE) and their interaction with their receptors (RAGE) have advanced our knowledge of the roles of these molecules in different diseases. However, studies concerning AGE-RAGE interaction in obesity are limited and the results are conflicting. RAGE gene is located on 6p21.3, has several polymorphic sites including -374T/A, a functional polymorphism in the promoter region, and Gly82Ser, present within the ligand-binding domain. In the present study, the determination of possible risks in the development of obesity according to RAGE polymorhisms and plasma levels of RAGE (sRAGE) was aimed. 87 obese and 78 healthy children were included in this study. Genomic DNA was isolated with salting-out procedure. RAGE polymorphisms were analyzed by PCR based techniques. In contrast to Gly82Ser, -374T/A allelic and genotypic frequencies were not different between study groups. Ser(SerSer+GlySer genotype) allele frequency was higher in obese cases than controls (74.20%→25.80%,OR:2.573,95%CI:1.789-3.699;p<0.01). In obese cases, blood glycose (92.50±2.80→87.00±1.16; p=0.025) and HDL-C (46.14±2.75→39.84±1.82;p=0.057) levels were higher than TT genotype carriers. As for Gly82Ser polymorphism, HDL-C (p=0.004) and FT4 (p=0.020) levels were different in obese cases, the order was SerSer>GlySer>GlyGly for HDL-C, and opposite for FT4. Besides, Ser carriers had lower insulin (p=0.038) and homa-IR (p=0.081) levels than GG genotype. sRAGE levels were different between obese and control seperately or in combination with RAGE polymorphisms (p<0.05), the order of sRAGE was TT>TA>AA for -374T/A and SerSer>GlyGly>GlySer for Gly82Ser. According to our results SerSer genotype could have significant effects on sRAGE levels, and increased sRAGE levels and Gly82Ser polymorphism either combinatorially or seperately increased the propensity towards obesity.

Key words: Obesity, RAGE -374T/A, RAGE Gly82Ser, sRAGE, polymorphism.

Introduction

Obesity, known as fatness among people, is a complex and multifactorial disease which is characterized by the occurance of endocrine and metabolic alterations. The incidence of which is getting increased gradually in the world as well as in our country. In recent years it was well-known that this nutritional disease became a great worldwide health problem with increasing incidence as 25-30% in adolescents and children (1-3).

The development of obesity is rarely due to a primary disease. In most cases, there is any well defined reason for the development of obesity except traditional risk factors such as malnutritional habbits, variable physical activity, sedentary life style (1-4). Besides, obesity studies on twins revealed that the ratio of genetic interactions with BMI is about 60-84 % (5). Thus, it was accepted that endocrine, genetic and environmental factors, all play important roles in the etiopathogenesis of obesity (1-4). On the other hand, it was reported that obesity contributes to a number of health problems including diabetes, coronary heart disease, fatty liver disease, development of hypertension and certain cancers by affecting many metabolic pathways (1-4,6). It was both well known that storage of fatty acids due to obesity expands the mass of adipose tissue which has an important role in the regulation of fatty acid homeostasis of whole-body. Besides, recent studies reported that the synthesis and release of pro-inflammatory and anti-inflammatory cytokines from the adipose tissue mediate obesity related complications by affecting insulin sensivity, generation of reactive oxygen species (ROS) or inducing macrophage-derived factors which lead to a chronic low-grade inflammatory state in obesity (6). However, the molecular mechanism underlying the deregulation of adipokine release has not been fully explored. It was proposed that one reason for this mechanism can be the formation of advanced glycosylation end-products (AGE) or advanced lipoxidation end-products (ALE) (7-9).

The effects of AGEs are mediated by binding to specific receptors (*RAGE*s) found on the cell surface of organs and tissues, and recent studies investigating the role of AGEs and RAGEs in the complications due to obesity draw attention (10). In studies on nondiabetic mice with predisposition towards atherosclerosis, it was shown that deletion of *RAGE* increased serum adipokine levels but reduced adipocites and atherogenesis as well (11). Similarly, it was also reported that blockade of *RAGE* in both diabetic and nondiabetic mice reduced the risk of atherosclerosis to significant extent (12).

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RAGE gene locates on 6p21.3 chromosome in the major histocompatibility complex and posesses 11 exons (13). The *RAGE* gene product, 35 kDa polypeptide, is composed of three domains as signal transducing: cytoplasmic, membrane anchoring: transmembrane and signal capturing: extracellular domain. Among three RAGE isoforms, the free soluble *RAGE* (sRAGE) isoform, which is lacking of both cytoplasmic and transmembrane domain found in plasma and several tissues, and blocks intracellular signal transmission, which neutralizes the toxic and inflammatory effects of AGEs (14). In studies on diabetic and nondiabetic individuals, plasma *RAGE* levels was shown indirectly related to signs of metabolic syndrome involving parameters as insulin resistance and BMI (15). On the other hand, the visceral obesity individuals without known risk factors were shown to relate with the incremental lipid peroxidation and platelet activation (16). It was reported that these abnormalities are mediated by proinflammatory mediators and that this is related to the degree of abdominal obesity. Furthermore, it was also reported that AGEs increase platelet activation and thus *RAGE* expression on platelet cell surface (17).

Several polymorphic regions were described in the *RAGE* gene (13,18). A functional *RAGE* -374T/A polymorphism in the promoter of the *RAGE* gene has been shown to exert significant effects on transcriptional activity (18-20). On the other hand, *RAGE* Gly82Ser polymorphism of third exon plays a role in several diseases by activation of signalling pathways of inflammation and oxidative stress through changing the ligand binding affinity at relevant domain of *RAGE* receptor (13,18,21,22).

The concept that *RAGE* is one of the several factors responsible for the variability in the adipose tissue malfunctioning between individuals is getting gradually widespread. Thus, the aim of this study is to investigate *RAGE* levels and two functional polymorphisms of *RAGE* gene in obese patients to determine the association with adipose tissue malfunctioning as well as to explore the possible roles in the pathogenesis of obesity in order to contribute diagnosis, treatment and prognosis of the disease and to determine its distribution among the Turkish population.

Materials and Methods

Study participants and clinical investigation

This study was carried out 87 obese (30.30% girl, 42.50% boy) and 78 healthy (65.40% girl, 34.60% boy) children. Medical history, physical examination, assessment of glycemic control (hemoglobin A1c [HbA1c], fasting plasma glucose [FPG], insulin levels, and homeostasis model assessment of insulin resistance [HOMA-IR] index) was screened initially. The inclusion criteria for obesity were: BMI $\geq$ 30 kg/m^2 or BMI > 2 standard deviations above the WHO growth standard median or BMI > 95th percentile (1). BMI was calculated as weight in kilograms divided by the square of height in meters (23). Insulin resistance was calculated using the HOMA-IR index. Healthy children without any symptoms of obesity were selected for the control group. The study protocol was approved by both the Ethical Committee of the Istanbul Faculty of Medicine and the Research Fund of Istanbul University in accordance with the Declaration of Helsinki. Written informed parental consent and oral assent from children were obtained in accordance with ethics guidelines regarding the study.

Lipid measurement

Blood samples were collected into plain tubes from overnight fasted participants. The serum was immediately removed from these blood samples and frozen at −20◦C. Total cholesterol (TC) levels were measured by cholesterol oxidase-peroxidaseaminoantipyrine (CHOD-PAP) enzymatic calorimetric method. Serum high-density lipoprotein cholesterol (HDL-C) levels were measured by CHOD-PAP test, following precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic acid and magnesium ions. The glycerol phosphate oxidase-peroxidaseaminoantipyrine (GPO-PAP) enzymatic calorimetric test was used to measure serum triglyceride (TG) levels. Serum low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald formula.

sRAGE measurement

The plasma levels of sRAGE were determined using a commercially available enzyme linked immunosorbent assay (ELISA) kits (Biovendor Research and Diagnostic Products, Czech Republic) according to manufacturer's instructions. Measurements were performed in duplicate, and the results were averaged.

Polymerase chain reaction (PCR)-based detection of RAGE -374 T/A and RAGE Gly82Ser genotypes

Blood specimens were collected in tubes containing ethylenediaminetetraacetic acid (EDTA), and genomic DNA samples were extracted from leukocyte nuclei by salting-out procedure (24). The DNA samples were analysed for the *RAGE* -374 T/A and *RAGE* Gly82Ser polymorphism by polymerase chain reaction (PCR), restriction fragment length polymorphism, and agarose gel electrophoresis techniques as previously described (22-24). After amplification of the isolated DNA with PCR, the *RAGE* -374T/A and *RAGE* Gly82Ser polymorphisms were detected by cutting the PCR products with the restriction endonucleases *Tsp*509 I (New England Biolabs, Beverly, MA, USA) and Alu I (MBI Fermentas), respectively. The digested DNAs were then separated on 3% agarose gel electrophoresis, and the genotypes were typed by visualization under ultraviolet light (22-24).

Statistical methods

Statistical analysis was performed by using SPSS software package (revision 21, SPSS Inc., Chicago, IL, USA). The Hardy-Weinberg equilibrium was tested for all polymorphisms. Clinical laboratory data are expressed as mean±SD. Mean values were compared between patients and controls by unpaired Student's t-test. Differences in the distribution of genotypes and alleles between cases and controls were tested using the Chi-square-statistic and Fisher's-exact tests. Allele frequencies were estimated by gene counting methods. Values of $p<0.05$ were considered statistically significant.

Results

Clinical investigation

Demographic characteristics of the study groups were summarised in Table 1. All the study groups had similar distributions of gender and age. The levels of BMI, systolic and diastolic blood pressures, insulin, HOMA-IR, insulin resistance, TG, LDL-C, ALT and free T4 were found to be higher in obese cases than controls (*p<0.05*). In addition, HDL-C levels were higher in controls than obeses (*p<0.05*). The obese patient group had significantly higher sRAGE (519.55±37.20 → 854.53±58.70; *p<0.001*) and slighgtly higher glycose (89.10±0.84 → 86.16±1.26; *p=0.084*) levels when compared to the control group. The insulin resistance, dyslipidemia and hypertension frequencies in obese cases were 64.00%, 47.50% and 30.00%, respectively (*p<0.05*).

Distribution of RAGE -374 T/A and RAGE Gly82Ser variants

The distributions of *RAGE* -374 T/A and *RAGE* Gly82Ser genotypes and alleles among study groups were shown in Table 2. As seen in the Table 2 where *RAGE* -374 T/A genotype and allele distributions were consistent with Hardy–Weinberg equilibrium (*p>0.05*), the distribution of *RAGE* Gly82Ser genotypes were significantly different among study groups (*p<0.05*). The frequency of rare Ser allele (SerSer+GlySer genotypes) was significantly higher than GlyGly genotype in obese cases (75.90%→24.10%, Odds ratio, OR: 0.133, 95% Confidance interval, CI : 0.067-0.266; *p<0.05*). Besides, the frequency of Ser allele was significantly higher in obese cases than controls (74.20%→25.80%, OR: 2.573, 95% CI: 1.789-3.699; *p<0.01*). On the other hand it was found that the SerSer genotype was significantly increases the risk of obesity 4.5 times when compared with Gly allele (GlyGly+GlySer genotypes) (OR: 4.483, 95% CI: 1.013-19.833; *p=0.027*) (data not shown).

Association of the RAGE -374T/A and RAGE Gly82Ser variants with clinical parameters

In Table 3, the distributions of clinical parameters according to *RAGE* -374T/A and *RAGE* Gly82Ser variants were presented. There was no statistical association between genotype distributions of both polymorphisms and BMI, systolic and diastolic blood pressures (SBP, DBP), homa-IR, TC, TG, LDL-C, AST, ALT or TSH levels (*p>0.05*). However, it was found that in obese cases the blood glucose levels were different among the three *RAGE* -374 T/A genotypes (*p=0.068*). Besides, it was found that AA genotype carriers had higher blood glycose (92.50±2.80→87.00±1.16; *p=0.025*) and HDL-C (46.14±2.75→39.84±1.82; *p=0.057*) levels than TT genotype carriers in obese cases. As for *RAGE* Gly82Ser polymorphism, HDL-C (*p=0.004*) and FT4 (p=0.020) levels were different in obese cases and the order was SerSer>GlySer>GlyGly for HDL-C, and the opposite for FT4. In addition, SS genotype carriers had higher HDL-C levels than G allele (GG+GS genotypes) carriers (*p=0.007*) and G allele carriers had higher FT4 levels than SS genotype carriers (*p=0.025*). On the other hand, it was found that GlyGly genotype carriers had significantly higher blood insulin levels than SerSer genotype (27.89±3.07→22.23±1.38; *p=0.048*) carriers

Table 1. The baseline characteristics of the study population.

		CONTROLS	OBESE CASES	P value
Gender (female/male) (n)		51(65.40%) / 27 (34.60%)	50 (57. 50%) /37 (42.50%)	0.298
Age (years)		12.25±0.27	12.54±0.23	0.405
BMI (kg/m²)		18.27±0.54	31.04±0.32	**<0.001**
SBP (mmHg)		100.89±1.68	131.08±2.33	**<0.001**
DBP (mmHg)		64.60±1.26	80.12±1.24	**<0.001**
Glycose (mg/dl)		86.16±1.26	89.10±0.84	0.084
Insulin (µU/ml)		10.34±0.72	23.46±1.20	**<0.001**
Homa-IR		2.20±0.16	5.17±2.52	**<0.001**
IR	**absent**	70 (89.7%)	18 (21%)	**<0.001**
	present	6 (10.3%)	69 (79%)	
TC (mg/dl)		156.70±5.41	157.96±3.97	0.868
TG (mg/dl)		79.60±7.63	123.30±7.18	**0.002**
LDL-C (mg/dl)		91.24±4.68	110.65 ±4.00	**0.012**
HDL-C (mg/dl)		52.72±2.52	41.65±1.14	**<0.001**
sRAGE (pg/ml)		519.55±37.20	854.53±58.70	**<0.001**
AST (U/l)		25.29±3.90	29.91±1.01	0.202
ALT (U/l)		13.38±1.40	28.72±1.85	**<0.001**
TSH (mU/l)		2.65±0.30	3.31±0.17	0.058
Free-T4 (pmol/l)		13.46±0.49	14.85±0.19	**0.005**

The results are shown as meanSD. BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; HOMA: Homeostasis Assessment Model; IR: insulin resistance; IR: Insulin Resistance; TC: Total Cholesterol; TG: Triglyceride; LDL-C: low density lipopoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; sRAGE: soluble form of advanced glycation end products receptor; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TSH: Thyroid-stimulating hormone; T4: thyroxine; n: number of individuals, p<0.05 and bold values of p value indicates scientific significance.

Table 2. The distribution of *RAGE* -374T/A and *RAGE* Gly82Ser genotype and allele frequencies in the study groups.

***RAGE* -374 T/A**	**Genotypes**			**Alleles**	
Groups	**TT**	**TA**	**AA**	**T Allele**	**A Allele**
Controls	37 (47.4%)	26 (33.3%)	15 (19.3%)	100 (64.10%)	56 (35.90%)
Obese Cases	34 (39.1%)	39 (44.8%)	14 (16.1%)	107 (61.49%)	67 (38.51%)
***RAGE* Gly82Ser**	**Genotypes**			**Alleles**	
Groups	**GlyGly**	**GlySer**	**SerSer**	**Gly Allele**	**Ser Allele**
Controls	55 (70.50%)	21 (26.90%)	2 (2.60%)	131 (83.97%)	25 (16.03%)
Obese Cases	21 (24.10)	56 (64.40%)*	10 (11.50%)	98 (56.32%)	76 (48.68%)*

Chi-square test was used to compare genotypes in the study group. For determining allele frequencies gene count method was used. n, number of individuals; * indicates statistical significance (p<0.05).

Table 3. The distribution of clinical parameters according to *RAGE* -374T/A and *RAGE* Gly82Ser variants among the study groups.

		***RAGE* -374 T/A**				***RAGE* Gly82Ser**			
		TT Genotype	AT Genotype	AA Genotype	*p* value	GlyGly Genotype	GlySer Genotype	SerSer Genotype	*p* value
BMI (kg/m²)	Controls	18.21±0.50	18.69±0.57	17.75±0.62	0.594	18.03±0.34	18.86±0.75	17.79±2.56	N/A
	Obeses	32.12±0.84	30.47±0.81	30.091.35	0.284	31.53±0.88	31.24±0.73	28.95±1.22	0.357
SBP (mmHg)	Controls	100.67±2.35	104.47±3.35	96.15±3.11	0.218	102.80±1.88	96.32±3.35	105.00±15.00	N/A
	Obeses	133.00±3.42	131.54±3.76	125.38±5.65	0.534	131.32±3.15	132.10±3.17	122.86±6.80	0.541
DBP (mmHg)	Controls	65.17±1.77	66.84±2.07	60.00±2.99	0.143	64.51±1.61	63.68±2.05	75.00±5.00	N/A
	Obeses	81.50±1.96	79.26±1.82	79.23±3.48	0.684	81.05±2.41	80.17±1.51	77.14±5.22	0.726
Glycose (mg/dl)	Controls	84.93±1.90	88.00±2.30	87.50±2.19	0.560	85.77±1.29	94.00±2.00	79.00±0.00	N/A
	Obeses	87.00±1.16*	89.62±1.14	92.50±2.80*	0.068	87.40±1.26	89.45±1.19	90.60±1.70	0.483
Insulin (µU/ml)	Controls	10.16±0.99	10.46±1.71	10.51±1.57	0.977	10.24±0.82	10.26±1.83	11.48±0.00	N/A
	Obeses	25.10±2.09	22.15±1.66	23.02±2.95	0.533	27.89±3.07*	22.23±1.38*	21.19±2.18	0.113
Homa-IR	Controls	2.14±0.22	2.30±0.44	2.24±0.31	0.927	2.18±0.18	2.37±0.38	2.24±0.00	N/A
	Obeses	5.34±0.45	4.94±0.39	5.37±0.79	0.762	6.02±0.66	4.93±0.34	4.72±0.47	0.215
TC (mg/dl)	Controls	150.57±6.67	170.29±14.57	155.17±7.61	0.327	154.79±4.78	203.50±24.50	109.00±0.00	N/A
	Obeses	153.06±7.97	162.03±5.32	158.71±5.00	0.594	155.10±6.31	159.49±5.50	155.33±8.92	0.876
TG (mg/dl)	Controls	69.17±12.50	80.43±12.38	99.50±11.82	0.293	81.27±8.62	66.50±7.50	69.00±0.00	N/A
	Obeses	122.09±11.77	131.11±11.30	106.00±14.32	0.473	137.60±16.71	118.15±7.67	121.89±30.80	0.527
LDL-C (mg/dl)	Controls	86.82±5.64	95.75±11.72	94.60±6.33	0.680	88.32±4.27	134.95±9.05	68.00±0.00	N/A
	Obeses	107.14±8.40	114.55±4.95	108.97±5.16	0.696	106.26±5.75	113.94±5.59	101.31±9.36	0.517
HDL-Cl (mg/dl)	Controls	53.60±2.80	55.50±6.50	46.83±5.33	0.481	52.73±2.61	62.00±10.00	34.00±0.00	N/A
	Obeses	39.84±1.82*	41.47±1.70	46.14±2.75*	0.160	36.94±1.89*	41.99±1.39*	50.21±3.49*	**0.004**
AST (U/l)	Controls	26.75±6.98	25.50±1.50	19.00	0.855	26.40±5.55	22.00±0.00	23.00±0.00	N/A
	Obeses	30.78±1.94	29.94±1.44	27.69±0.98	0.588	28.25±1.68	30.63±1.40	29.56±1.94	0.609
ALT (U/l)	Controls	12.60±1.33	13.29±3.31	15.50±3.84	0.765	13.21±1.54	16.00±0.00	14.00±0.00	N/A
	Obeses	31.91±3.57	27.00±2.43	25.54±3.09	0.366	30.45±3.56	28.65±2.47	25.33±4.02	0.748
TSH (mU/l)	Controls	2.61±0.46	2.63±0.57	2.83±0.59	0.971	2.59±0.34	2.70±0.80	3.88±0.00	N/A
	Obeses	3.40±0.30	3.17±0.25	3.43±0.33	0.793	3.48±0.36	3.15±0.20	3.86±0.58	0.366
Free-T4 (pmol/l)	Controls	14.76±1.11	13.07±0.54	12.21±0.26	0.146	13.11±0.53	15.48±1.55	13.67±0.00	N/A
	Obeses	14.68±0.32	14.93±0.28	15.09±0.39	0.705	15.55±0.35*	14.81±0.22*	13.67±0.62*	**0.020**

The results are shown as meanSD. BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; Homeostasis Assessment Model; TC: Total Cholesterol; TG: Triglyceride; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; sRAGE: soluble form of advanced glycation end products receptor; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TSH: Thyroid-stimulating hormone; T4: thyroxine; n: number of individuals, statistical significance was defined as p<0.05 and bold values of p value indicates scientific significance of combined genotypes, * indicates scientific significance between two genotypes, N/A: Not available.

in obese cases. Besides, while Ser allele (SerSer+GlySer genotype) carriers had lower insulin (*p=0.038*), homa-IR (p=0.081), FT4 (p=0.041) levels than GG genotype carriers, it was found that GG genotype carriers had lower HDL-C levels than S allele carriers (*p=0.017*).

Association of sRAGE blood levels with RAGE -374T/A and RAGE Gly82Ser variants

The distribution of blood sRAGE levels according to *RAGE* -374T/A and *RAGE* Gly82Ser variants were shown in Table 4. It was seen that there were significant differences between obese and control cases according to sRAGE levels of each genotypes or alleles of declared polymorphisms separately (*p<0.05*). On the other hand among obese cases the distribution of sRAGE levels according to RAGE -374 T/A genotypes were not significant as well as RAGE Gly82Ser genotypes, however the order of sRAGE levels were as follows : TT>TA>AA and SerSer>GlyGly>GlySer, respectively. Besides, the blood levels of sRAGE in SerSer genotype carriers were higher than those possessing Gly allele (GlyGly+GlySer) (*p=0.071*). Therefore it may considered as SerSer genotype could have increasing effects on sRAGE levels.

Discussion

Enzymatic glycation is one of the important post-translational modification of proteins related to various biological processes involving differentiation, development-growth and defense (25). However, non-enzymatic glycolysation, which depends on the hyperglycemic state, leads to the generation of advanced glycation end products (AGEs) which has a plethora of deleterious effects (26) including cross-linking of proteins, modification of matrix components, platelet aggregation, defective vascular relaxation, abnormal lipoprotein metabolisms and, induction of cytotoxic, prothrombotic and proinflammatory pathways. All these effects were seen in extracellular matrix or vasculature by formation, or inside cardiac fibroblasts, monocytes or vascular smooth muscle cells by binding to specific AGE

Table 4. The serum levels of sRAGE according to RAGE -374 T/A and RAGE Gly82Ser variants.

Groups	Genotypes			Alleles	
RAGE -374 T/A	TT	TA	AA	T	A
Controls	477.59±50.57	595.59±76.02	491.22±66.29	526.29±43.45	557.41±54.00
Obese Cases	971.02±127.51	820.66±58.63	665.98±88.80	890.69±67.21	779.80±49.58
RAGE Gly82Ser	GlyGly	GlySer	SerSer	Gly	Ser
Controls	503.74±42.94	560.50±75.40	524.22±447.35	519.42±37.26	557.34±74.26
Obese Cases	887.36±171.01	789.84±56.30	1147.86±170.32	816.43±51.58	844.08±56.02

sRAGE: soluble form of advanced glycation end products receptor (pg/ml) ; The results are shown as mean±SD.

binding°rading membrane receptors (RAGEs). The first step for AGE formation is a non-enzymatic reaction between glucose and an amine group of macromolecules (proteins, lipids or DNA) to form a reversible Schiff base. Then with rearrangement reactions unstable Schiff bases converted into a more stabilized adduct, called Amadori products which is followed by the formation of irreversibly bounded AGEs by additional reactions including condensation and dehydrations. AGE formation depends on the concentration of glucose and turnover of macromolecules. In addition, all characteristic features of obesity such as hyperlipidemia, hyperglycemia and oxidative stress, play important roles in the formation of AGEs (13,27-32). Thus plasma AGE levels and the polymorphic sites of *RAGE* gene are gaining importance as being a candidate strategie for both diagnosis and theraphy of obesity, and we investigated associations of sRAGE levels and critical RAGE polymorphisms with the risk of obesity in a case control based study in a Turkish population.

In this study, in comply with literature, serum sRAGE levels were found to be higher in obese cases than healty controls. In addition, an association between *RAGE* Gly82 Ser polymorphism and obesity was observed in contrast to *RAGE* -374T/ A polymorphism. It was found that obese cases possessing SerSer genotype significantly have the risk of obesity as 4.5 fold than Gly allele carriers. Besides, it was found that SerSer genotype could have increasing effects on sRAGE levels, and the serum levels of HDL-C was in the order of SerSer>GlySer>GlyGly and the opposite for free-T4. Our finding of HDL-C was in comply with a recent study by Lorenzi *et al.* They investigated anti-sRAGE autoimmunity in obesity and found an inverse relationship between anti-sRAGE and HDL levels. They diagnosed decreased levels of sRAGE and anti-sRAGE and increased levels of HDL after gastric bypass in obese cases independently from glucose metabolism (33).

Studies regarding the association of this polymorphism with obesity were scarce. Kim *et al.* reported an association of Gly allele with proinflammatory responses under obese conditions than non-obese cases. They found higher serum sRAGE and lower high-sensitivity C-reactive protein (hs-CRP, as an inflammation marker) levels in Gly allele carrier obeses (34). On the other hand, Jang *et al.* investigated the association of Gly82Ser polymorphism with sRAGEs and inflammatory markers in non-diabetic non-obese Koreans, and similar to our report they reported the genotypic distribution as 70.41% GlyGly, 26.79% GlySer, 2.80% SerSer however, in contrast to our results they found a GlyGly>GlySer>SerSer order in sRAGE, insulin and HOMA-IR levels, and they reported the order of AGE levels and inflammatory markers as GlyGly<GlySer<SerSer in non-obese cases (35) as the results of Kim et al. (34) in obese cases. However there are conflicting results in the effects of RAGE on inflammation as Pullerits *et al.* reported decreased levels of blood sRAGE in rheumatoid arthritis patients (36). On the other hand, Han *et al.* reported a significant interaction between Ser allele and obesity in Chinese population with respect to osteoarthritis (37).

In this study as it was found that Ser allele carrier obeses have higher glycose, HDL-C and sRAGE levels and lower insulin, homa-IR and free-T4 levels, it was suggested that Ser allele increases the tendancy to insulin resistancy and formation of AGEs and was found more risky in the development of obesity. AGEs bind to RAGEs to activate intracellular signalling pathways; however if they cannot bind to these scavenger receptors they show some toxic effects in the blood stream. To compensate these toxic effects, in addition to evacuation from kidneys, sRAGEs found in the stream. As for our study, in obese cases, as expected, in the case of homozygous mutant SerSer genotype the higher blood sRAGE levels were detected to compensate the accumulated AGEs. In addition, higher serum HDL-C levels seen in obese cases carrying SerSer genotype could also be a compensation mechanism for impaired lipid metabolism according to high blood levels of glycose and latter formation of AGEs. On the other hand, it was known that formation of AGEs and binding with RAGEs induce prothrombic and proimflamamtory patways. Therefore, in contrast to Kim *et al.* (34), with the existance of this polymorphism, so do Ser allele, the accumulated AGEs might be more effective to induce inflammation. However, because of lacking acute inflammation marker levels and serum AGE levels in this report, our hypothesis needs to be clarified with future studies.

In conclusion, our results demonstrated that in addition to increasing effects on sRAGE levels of SerSer genotype, increased sRAGE levels and RAGE Gly82Ser polymorphism was together or seperately increase the propensity towards obesity.

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