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Original Research

# Intercellular Adhesion Molecule-1 Lys469Glu Polymorphism, Systemic Redox Homeostasis and Gestational Diabetes Mellitus in Pregnant Women

Karolin Yanar PhD [a]; Seval Aydin PhD [a]; Bahadir Simsek MD [a]; İlhan Yaylim PhD [b]; Saime Turan MSc [b]; Mustafa Erinc Sitar MD [c]; Canan Cacina PhD [b]; Ozlem Kucukhuseyin PhD [b]; Abdullah Tuten MD [d]; Ufuk Cakatay PhD [a,*]; Ali Benian MD [d]

[a] *Department of Medical Biochemistry, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey*
[b] *Department of Molecular Medicine, Institute of Experimental Medicine, Istanbul University, Istanbul, Turkey*
[c] *Department of Clinical Biochemistry, Faculty of Medicine, Maltepe University, Istanbul, Turkey*
[d] *Department of Obstetrics and Gynecology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey*

**Key Messages**

- The gross oxidative stress observed in gestational diabetes may warrant a revision of the acceptable upper limit of glycated hemoglobin levels.
- The Lys469Glu ICAM-1 polymorphism does not seem to cause increased oxidative stress.
- Oxidative stress observed in gestational diabetes highlights the importance of early management.

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ABSTRACT

*Objectives:* Intercellular adhesion molecule-1 (ICAM-1) plays an important role in endothelial function. Hyperglycemia-induced impaired redox status is 1 of the well-known pathophysiologic characteristics of gestational diabetes mellitus (GDM), and it plays a crucial role in the causes of disease. Our aim was to clarify any possible relationship between the ICAM-1 Lys469Glu polymorphism and systemic redox status in women with and without GDM. Also, we investigated whether this polymorphism could be associated with a change for better or worse as evidenced by clinical and redox biomarkers.
*Methods:* The ICAM-1 polymorphism statuses of 89 pregnant women without GDM and 53 pregnant women with GDM were found. Stratifying patients based on GDM and polymorphism status, we investigated various redox homeostasis markers. The independent t test was used.
*Results:* Significantly higher systemic oxidative damage and diminished antioxidant defense were found in pregnant women with GDM. Also, results showed that whether pregnant women were carrying the Lys469Glu polymorphism or not did not seem to be associated with significant differences, as evidenced by comparable systemic oxidative damage.
*Conclusions:* Although no significant difference was observed between genotypes, the oxidative damage observed in patients with GDM warrants earlier screening and management in the light of new evidence.


*Mots clés :*
antioxydants
diabète sucré gestationnel
molécule d'adhérence intercellulaire 1
peroxydation des lipides
polymorphisme Lys469Glu
homéostasie redox

RÉSUMÉ

*Objectifs :* La molécule d'adhérence intercellulaire 1 (ICAM-1, de l'anglais *Intercellular adhesion molecule-1*) joue un rôle important dans le fonctionnement endothélial. L'altération de l'état redox induit par l'hyperglycémie est l'une (1) des caractéristiques physiopathologiques bien connues du diabète sucré gestationnel (DSG), qui joue un rôle crucial dans les causes de la maladie. Notre objectif était de clarifier toutes les relations possibles entre le polymorphisme Lys469Glu de l'ICAM-1 et l'état redox systémique chez les femmes atteintes ou non atteintes du DSG. De plus, nous avons examiné si ce

* Address for correspondence: Ufuk Cakatay, PhD, Department of Medical Biochemistry, Cerrahpasa Faculty of Medicine, Istanbul University, Kocamustafapasa, Istanbul 34098, Turkey.
*E-mail address:* ufuk.cakatay@istanbul.edu.tr

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polymorphisme pouvait être associé à une amélioration ou à une détérioration selon les biomarqueurs cliniques et redox.
*Méthodes :* Nous avons observé les polymorphismes de l'ICAM-1 chez 89 femmes enceintes non atteintes du DSG et 53 femmes enceintes atteintes du DSG. La stratification des patientes en fonction du DSG et du polymorphisme nous a permis d'examiner divers marqueurs d'homéostasie redox. Nous avons utilisé le test t pour échantillons indépendants.
*Résultats :* Nous avons observé un stress oxydatif systémique significativement élevé et une diminution des défenses antioxydantes chez les femmes enceintes atteintes de DSG. De plus, les résultats, qui montraient que peu importe si les femmes enceintes étaient porteuses ou non du polymorphisme Lys469Glu, ne semblaient pas être associés à des différences significatives, puisque le stress oxydatif systémique était comparable.
*Conclusions :* Bien que nous n'ayons observé aucune différence entre les génotypes, le stress oxydatif observé chez les patientes atteintes du DSG justifie à la lumière des nouvelles données probantes le dépistage et la prise charge précoces.



## Introduction

Diabetes Canada defines gestational diabetes mellitus (GDM) as the type of diabetes that is first recognized or begins during pregnancy (1). GDM affects nearly 1% to 14% of pregnant women and is characterized by both insulin resistance and decreased pancreatic beta-cell function. Risk factors that are linked to the development of GDM include unhealthy maternal lifestyle, higher body mass index during the prepregnancy period, family history of diabetes, polycystic ovarian syndrome, ethnicity, advancing age, waist circumference, smoking and drinking habits, antipsychotic drug usage and air pollution (2,3).

Intercellular adhesion molecule-1 (ICAM-1) is a transmembrane glycoprotein and is a member of the immunoglobulin superfamily. It is expressed on the surface of endothelial cells, fibroblasts and leukocytes. ICAM-1 has a total of 505 amino acids, and the Lys469Glu polymorphism includes lysine-to-glutamic acid alteration at the codon 469 of exon 6. The Lys469Glu polymorphism was previously studied in type 2 diabetes and diabetes-related disorders, such as diabetic retinopathy and diabetic nephropathy, but not in GDM (3–5).

It has been suggested that both diabetes and insulin resistance could lead to endothelial dysfunction and may also cause impaired antiatherogenic function of the vascular endothelium. Hyperglycemia is accepted as the main diagnostic hallmark of GDM and is related to impaired redox status by way of increased oxidative and glycoxidative modifications (6). Pancreatic beta cells are susceptible to reactive oxygen species (ROS)-mediated oxidative damage (7). Oxidatively modified lipids, proteins and DNA may lead to the occurrence of GDM-related pathologies (8). Cellular macromolecules, such as proteins, lipids and DNA, are the main targets of ROS and, therefore, are susceptible to oxidative modifications (9–11). Proteostasis (protein redox homeostasis) plays an essential role in normal cellular function. Impaired redox status leads not only to a high rate of global protein oxidation, with the formation of protein carbonyl groups (PCOs) and advanced oxidation protein products (AOPPs), but it also increases levels of specific protein oxidation biomarkers, such as dityrosine (DT), kynurenine (KYN) and N'-formylkynurenine (NFKYN). Moreover, it also causes the loss of protein thiol groups (P-SHs) (12). An additional outcome of this circumstance is the occurrence of lipid peroxidation, which commonly emerges by causing the formation of early-phase biomarkers, such as conjugated dienes and lipid hydroperoxides (LHPs), and progresses with the occurrence of late-phase highly reactive biomarkers, such as hydroxynonenal and malondialdehyde. Furthermore, it leads to decreased antioxidant capacity of biomarkers, such as thiol fractions, Cu,Zn-superoxide dismutase (Cu, Zn-SOD) and catalase activities.

Some of the important functions of ICAM-1 include leukocyte-endothelial interactions, platelet activity and vascular matrix reorganization. All of these are important in the wide spectrum of pathogenesis of cardiovascular diseases and diabetes. Therefore, polymorphisms in the genes encoding this protein could affect vital functions of this glycoprotein, and there could be an association with different polymorphisms and redox biomarkers. We were unable to find any research investigating the possible role of the ICAM-1 polymorphism in GDM occurrence and its relationship with systemic redox status. Also, the importance of the Lys469Glu polymorphism in GDM was not investigated until now. In the present study, we aimed to focus on investigating the relationship between the ICAM-1 polymorphism and various systemic redox homeostasis biomarkers in plasma constituents in pregnant women with and without GDM.

## Methods

### *Subjects*

We examined the ICAM-1 Lys469Glu gene polymorphism in 53 women with GDM and 89 pregnant women without GDM. They were followed up on at Cerrahpasa Faculty of Medicine, Department of Obstetrics and Gynecology, Istanbul University.

Between the 24th and 28th weeks of the gestational periods, venous blood samples were taken in the morning to avoid the effects of the circadian rhythm on redox status parameters (13).

### *Ethics protocol*

Informed consent was obtained from all patients enrolled in this study. The ethical protocol of the study was approved by the Medical Ethics Committee of Cerrahpasa Medical Faculty, Istanbul, Turkey (Ethics Committee issue #40230).

### *Chemicals and apparatuses*

All chemicals were supplied by Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, Missouri, United States). Plasma oxidative damage biomarker profiles of all pregnant women in this study were analyzed by spectrophotometric and spectrofluorimetric methods by BIOTEK Synergy $H_1$ optic reader (BioTek US, Winooski, Vermont, United States). Clinical chemistry parameters were assessed by a discrete otoanalyzer system (Roche, Porterville, California, United States). The homeostasis model assessment of insulin resistance was determined from glucose and insulin values by using the University of Oxford (Oxford, United Kingdom) online calculator (14).

### *Inclusion and exclusion criteria*

The inclusion criteria were singleton pregnancy with gestational age at enrollment between 24 and 28 weeks and without

relevant illness except for the possibility of GDM. The exclusion criteria were smoking, diabetes diagnosed prior to pregnancy, abnormal fasting glucose concentration (>7.5 mmol/L) prior to 24 weeks of gestation, GDM in a previous pregnancy, multiple pregnancies and pre-eclampsia.

*Creation of study groups*

Between the 24th and 28th weeks of pregnancy, following a 50 g glucose challenge test, venous blood samples were collected, and glucose tolerance tests with 100 g of glucose were performed in all pregnant women whose plasma glucose levels at 1 h were ≥7.8 mmol/L for the diagnosis of GDM, according to the Carpenter-Coustan criteria (15). Then, pregnant women were divided into the following 2 groups, according to the diagnostic test results, namely, with or without GDM.

*Genotyping*

Genomic DNA was extracted from whole blood samples by using a PureLink DNA extraction kit (Invitrogen, Carlsbad, California, United States). The purity of the DNA in the test samples was assessed by measuring the optic density at 260 nm. Genotyping was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism techniques. The primers for the ICAM-1 Lys469Glu polymorphism were selected as sense 5'-GGA ACC CAT TGC CCG AGC-3'; antisense 5'-GGT GAG GAT TGC ATT AGG TC-3'. The DNA template was amplified by a PCR technique using 3 mM $MgCl_2$, 0.2 mM of dNTP, 0.2 mM of primer and Taq polymerase (Fermentas, Vilnius, Lithuania) in a 25 µL final volume. The PCR conditions involved an initial denaturation for 5 min at 95°C, followed by 35 cycles of 94°C for 45 sec, annealing at 57°C for 45 sec and extension at 72°C for 45 sec. A final extension step at 72°C for 5 min was also studied. PCR products were digested with Bst UI (Fermantas) restriction enzyme at 37°C for 2 h followed by electrophoresis in 3% agarose gel. The genotypes were determined as AA (223 bp), AG (223,136,87 bp) or GG (136,87 bp) for the ICAM-1 Lys469Glu polymorphism.

*Assay of PCO groups*

An analysis of the PCO groups was performed according to the method previously described by Reznick and Packer (16). PCO groups react with 2,4-dinitrophenylhydrazine (DNPH) (1:4) reagent and form chromophoric dinitrophenylhydrazones. Plasma proteins were first precipitated with 20% (w/v) trichloroacetic acid and washed 3 times with 400 µL of an ethanol/ethyl acetate mixture (1:1). After centrifugation and washing procedures, the protein precipitates were dissolved in a 200 µL 6 M guanidine-HCl solution. Absorbance values of the samples were measured at 360 nm (DNPH, $\varepsilon=2.2\times10^4\ M^{-1}cm^{-1}$). PCO-bovine serum albumin (BSA) was used as a positive control. Untreated BSA and PCO-BSA were both prepared according to the method of Lenarczyk et al (17).

*Assay of advanced oxidation protein products*

AOPP concentrations in plasma samples were analyzed by modification of the Hanasand method (18). Test samples were prepared in the following way: 10 µL of plasma, 40 µL of phosphate-buffered saline and 200 µL of a citric acid solution (20 mmol/L) were mixed. After 1 min, 10 mL of 1.16 M potassium iodide was added, and the absorbance value of the reaction mixture was read at 340 nm. Then, 0 to 100 µmol/L chloramine-T standards were used to prepare a standard curve. AOPP concentrations of the samples were expressed as micromoles per litre of chloramine-T equivalents.

*Assay of advanced glycation end products*

Advanced glycation end product (AGE) levels were assayed spectrofluorometrically by the method of Munch et al (19). Plasma samples were diluted 1:20 in phosphate-buffered saline, pH 7.4, and then used for fluorescence spectroscopy. Fluorescence intensity was also recorded at the emission (440 nm upon excitation at 350 nm) and expressed in fluorescence units. Assay results are given as fluorescence units per milligram protein.

*Contents of dityrosine, kynurenine and N'-formylkynurenine*

Serum levels of DT, KYN and NFKYN were estimated based on their characteristic fluorescence. Fluorescence intensity was recorded at the emission maximum: 415, 480 and 434 nm upon excitation at 330, 365 and 325 nm, respectively.

*Assay of total, nonprotein and protein thiol fractions*

Plasma total (T-SH), nonprotein (NP-SH) and protein thiol (P-SH) were assayed in the following way, as described by Sedlak and Lindsay (20). We applied some optimizations of a previously described method in order to apply to small volumes of test samples. First, 20 mL of the plasma sample was mixed with 400 µL of 0.2 M Tris buffer, pH 8.2, and 20 µL of 0.01 M 5,5-dithiobis (2-nitrobenzoic acid) for the determination of T-SH groups. NP-SH samples were assayed as follows: 20 µL of plasma were precipitated with 400 µL of 50% trichloroacetic acid and then centrifuged for 15 min at 3000×g. Supernatant fractions were assayed as T-SH. The absorbance values of the resulting samples were read at 412 nm against a reagent blank. The value of the molar extinction coefficient of the thiol groups at 412 nm was $\varepsilon=13{,}100\ M^{-1}\ cm^{-1}$. The P-SH groups were calculated by subtracting the values of NP-SH from T-SH.

*Assay of lipid hydroperoxides*

Plasma LHP levels were assayed colorimetrically by the Wolff method, which uses oxidation of ferrous ions with xylenol orange FOX2 (ferrous oxidation with xylenol orange, v. 2) (21). LHPs oxidize ferrous ions into ferric ions in a diluted acid solution. Xylenol orange selectively binds ferric ions to form a blue-purple complex. Then, 5 µL aliquots of plasma were transferred into assay vials. The FOX2 reagent (950 µL) was then added, and the samples were mixed. After incubation with the FOX2 reagent at room temperature for 30 min, test samples were centrifuged at 3.000×g at 20°C for 10 min; supernatants were transferred into microplate wells, and absorbance was read at 560 nm against a reagent blank.

*Assay of Cu,Zn-superoxide dismutase activity*

Cu,Zn-SOD (extracellular 1.15.1.1) activity was assessed by using the method of Sun and Oberley, with some modifications (22). This method involves the inhibition of nitrobluetetrazolium reduction, with xanthine oxidase used as a superoxide generator. Enzyme activity was assessed by measuring the inhibition rate of substrate hydrolysis in the assay mixture containing 0.3 mmol/L xanthine, 0.6 mmol/L ethylenediaminetetraaceticacid disodium, 150 µmol/L nitrobluetetrazolium, 400 mmol/L sodium carbonate and 1 g/L BSA. The pH of the assay mixture was adjusted to 10.2. Then, 972 µL of assay mixture and 13 µL of xanthine oxidase (167 U/L) were added into a 25 µL test sample. Following 20 min of incubation, 250 µL of 0.8 mmol/L copper (II) chloride was added to the vial to stop the reaction. The final absorbance was read at 560 nm against a reagent blank. The percent of inhibition rate was calculated according to the following equation: $A_{blank}–A_{sample}/A_{blank}.100$. A single unit of Cu,Zn-SOD is defined as the amount of enzyme needed to exhibit a 50% dismutation of superoxide radical anion.

*Plasma ICAM-1 concentrations*

Plasma ICAM-1 concentrations were analyzed using a commercial sandwich enzyme-linked immunosorbent assay kit (Abcam, Cambridge, Massachusetts, United States).

*Statistical analyses*

Descriptive statistics are expressed as mean ± standard deviation (SD). Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS, v. 24.0, IBM, Armonk, New York, United States). Comparisons of the plasma redox status data of pregnant women were examined by using the Student t test for normally distributed data and the Mann-Whitney U test for nonparametric data. Observed genotype and allele frequencies of ICAM-1 Lys469Glu were compared by using the chi-square test; p<0.05 was considered statistically significant.

## Results

The demographic, anthropometric and clinical data of pregnant women with and without GDM were compared; relevant data are shown in Table 1. In pregnant women with GDM (n=53), glucose, total cholesterol, low-density lipoprotein-cholesterol, triglyceride, insulin resistance and glycated hemoglobin levels were higher than those in pregnant women without GDM (n=89).

*Redox status data of pregnant women with and without GDM*

In pregnant women with GDM (n=53), protein oxidation biomarkers AOPP, PCO, DT, KYN and NFKYN; early lipid peroxidation biomarker lipid hydroperoxide; plasma glycation biomarker; and AGE levels were found to be higher than in pregnant women without GDM (n=89). P-SH levels were found to be diminished in the group with GDM, which is a sign of more systemic oxidative damage. An antioxidative status biomarker, Cu,Zn-SOD, was significantly lower in the group with GDM than in the group without GDM. The genotypes and allele frequencies of ICAM-1 Lys469Glu in pregnant women with and without GDM are shown in Table 2.

A Hardy-Weinberg equilibrium analysis showed the genotype distribution for the ICAM-1 gene in GDM. Data are shown in Table 3.

*Demographic, anthropometric and clinical data of pregnant women according to ICAM-1*

*Lys469Glu genotypes.* Relevant data are given in Supplementary Table S1. In patients with GDM and the AA genotype, insulin resistance, glucose, glycated hemoglobin, total cholesterol, triglyceride and low-density lipoprotein cholesterol levels were found to be higher than those of controls. Also, in patients with GDM and the GA genotype, insulin resistance, glucose, glycated hemoglobin, total cholesterol, triglyceride, low-density lipoprotein cholesterol levels and birth weights were higher than those of the controls. On the other hand, in patients with GDM and the GG genotype, only glycated hemoglobin and triglyceride levels were found to be higher than those of the pregnant women without GDM.

**Table 1**
Comparison of the demographic, anthropometric and clinical data of pregnant women with and without gestational diabetes (GDM)

| Parameters | Without GDM n=89 | With GDM n=53 | p value |
|---|---|---|---|
| Age (y) | 31±4.4 | 32±5 | 0.447 |
| BMI (kg/m$^2$) | 28±2.6 | 29±3 | 0.330 |
| Waist circumference (cm) | 112±4 | 111±9 | 0.528 |
| Systolic BP (mmHg) | 108±8 | 109±9 | 0.576 |
| Diastolic BP (mmHg) | 66±8 | 68±8 | 0.061 |
| Insulin resistance (HOMA-IR) | 1.2±0.5 | 2.0±0.8 | 0.000† |
| Glucose (mmol/L) | 4.2±0.4 | 5.1±1.2 | 0.000† |
| Glycated hemoglobin (%) | 4.6±0.4 | 5.1±0.5 | 0.000† |
| Total cholesterol (mmol/L) | 5.1±0.4 | 5.4±0.6 | 0.005* |
| Triglyceride (mmol/L) | 2.0±0.3 | 2.3±0.3 | 0.000† |
| HDL cholesterol (mmol/L) | 1.4±0.1 | 1.4±0.2 | 0.511 |
| LDL cholesterol (mmol/L) | 2.6±0.3 | 2.9±0.6 | 0.004* |

*BMI,* body mass index; *BP,* blood pressure; *HOMA-IR,* homeostatic model assessment of insulin resistance; *HDL,* high-density lipoprotein; *LDL,* low-density lipoprotein.
*Note:* Data are presented as mean ± SD.
* p<0.01.
† p<0.001.

**Table 2**
Comparison of redox homeostasis biomarkers in pregnant women with and without gestational diabetes (GDM)

| Parameters | Without GDM n=89 | With GDM n=53 | p value |
|---|---|---|---|
| PCO (nmol/mg pr) | 0.97±0.2 | 1.2±0.2 | 0.000‡ |
| AOPP (μmol/L chloramine-T equivalent) | 80±43.0 | 99±39 | 0.015* |
| DT (FU/mg pr) | 297±40 | 332±31 | 0.000‡ |
| KYN (FU/mg pr) | 447±74 | 484±51 | 0.002† |
| NFKYN (FU/mg pr) | 350±45 | 399±28 | 0.000‡ |
| LHP (μmol/mg pr) | 0.2±0.1 | 0.3±0.2 | 0.002† |
| AGEs (FU/mg pr) | 338±52 | 424±38 | 0.000‡ |
| T-SH (nmol/mg pr) | 1.9±0.7 | 1.7±0.5 | 0.093 |
| NP-SH (nmol/mg pr) | 0.8±0.3 | 0.8±0.3 | 0.640 |
| P-SH (nmol/mg pr) | 1.1±0.7 | 0.8±0.5 | 0.043* |
| Cu,Zn-SOD (U/mg pr) | 1.2±0.3 | 0.9±0.2 | 0.000‡ |
| ICAM-1 (pg/mL) | 90±38 | 88±35 | 0.822 |

*AGEs,* advanced glycation end products; *AOPP,* advanced oxidation protein products; *Cu,Zn-SOD,* Cu,Zn-superoxide dismutase; *DT,* dityrosine; *FU,* fluorescence unit; *ICAM-1,* intracellular adhesion molecule-1; *KYN,* kynurenine; *LHP,* lipid hydroperoxide; *NFKYN,* N-formylkynurenine; *NP-SH,* nonprotein thiol; *PCO,* protein carbonyl groups; *pr,* protein; *pg,* picogram; *P-SH,* protein thiol; *T-SH,* total thiol; *U,* unit.
*Note:* Data are presented as mean ± SD.
* p<0.05.
† p<0.01.
‡ p<0.001.

**Table 3**
Genetic association of the ICAM-1 Lys469Glu polymorphism in pregnant women with and without gestational diabetes

| Genotype ICAM469 | With GDM n (%) | Without GDM n (%) |
|---|---|---|
| AA | 19 (35.9) | 26 (29.2) |
| GA | 21 (39.6) | 39 (43.8) |
| GG | 13 (24.5) | 24 (27) |
| p=0.71 | | |
| Alleles | | |
| A | 59 (55.6) | 91 (51.1) |
| G | 47 (44.4) | 87 (48.9) |
| p=0.46 | | |

*GDM,* gestational diabetes mellitus; *ICAM-1,* intracellular adhesion molecule-1.

*Redox status data of pregnant women according to ICAM-1 Lys469Glu genotypes*

Regardless of the genotype groups (AA or GG), protein oxidation biomarkers, such as PCO, DT, KYN and NFKYN, were significantly higher in women with GDM than in women without GDM. Data are shown in Table 4. AGEs were significantly higher in pregnant women with GDM, and the lipid peroxidation marker was also significantly higher in women with GDM. Cu,Zn-SOD activities were significantly diminished in both the AA and GG subgroups of women with GDM. Also, in an intergenotype comparison (AA and GG) of plasma oxidative product levels, we detected significantly more damage in pregnant women with GDM and the GG genotype than in pregnant women with GDM and the AA genotype for AOPP, DT, NFKYN and AGEs. To make sure that the intergroup results were not confounded by the severity of the GDM in the GG group, we

**Table 4**
Redox homeostasis biomarkers in pregnant women with and without gestational diabetes (GDM) with the ICAM-1 Lys469Glu polymorphism

| ICAM469 | AA | | | GG | | | AA vs. GG | |
|---|---|---|---|---|---|---|---|---|
| | Controls n=26 | GDM n=19 | p value | Controls n=24 | GDM n=13 | p value | p value GDM | p value non-GDM |
| PCO (nmol/mg pr) | 1.0±0.3 | 1.17±0.3 | 0.046* | 0.99±0.1 | 1.24±0.2 | 0.005* | 0.509 | 0.085 |
| AOPP (μmol/L Chloramine-T equivalent) | 79±47 | 86±35 | 0.625 | 94±39 | 114±26 | 0.140 | 0.025* | 0.301 |
| DT (FU/mg pr) | 271±22 | 318±39 | 0.000‡ | 313±41 | 345±16 | 0.008* | 0.033* | 0.001‡ |
| KYN (FU/mg pr) | 411±41 | 521±58 | 0.000‡ | 551±36 | 459±39 | 0.000‡ | 0.002† | 0.000‡ |
| NFKYN (FU/mg pr) | 347±43 | 396±37 | 0.001‡ | 321±25 | 421±17 | 0.000‡ | 0.036 | 0.032* |
| LHP (μmol/mg pr) | 0.19±0.1 | 0.35±0.26 | 0.029* | 0.22±0.2 | 0.27±0.1 | 0.463 | 0.347 | 0.429 |
| AGEs (FU/mg pr) | 319±32 | 412±39 | 0.000‡ | 323±52 | 453±34 | 0.000‡ | 0.005† | 0.770 |
| T-SH (nmol/mg pr) | 1.8±0.7 | 1.78±0.58 | 0.845 | 2.02±0.83 | 1.53±0.5 | 0.73 | 0.221 | 0.436 |
| NP-SH (nmol/mg pr) | 0.84±0.3 | 0.86±0.32 | 0.897 | 0.76±0.31 | 0.82±0.3 | 0.619 | 0.765 | 0.405 |
| P-SH (nmol/mg pr) | 0.98±0.6 | 0.92±0.58 | 0.757 | 1.26±0.92 | 0.69±0.4 | 0.047* | 0.241 | 0.267 |
| Cu,Zn-SOD (U/mg pr) | 1.09±0.2 | 0.82±0.16 | 0.000‡ | 1.27±0.22 | 0.9±0.2 | 0.003† | 0.266 | 0.231 |
| ICAM (pg/mL) | 94±39 | 92±41 | 0.902 | 80±25 | 85.10±38 | 0.710 | 0.654 | 0.263 |

*AGEs,* advanced glycation end products; *AOPP,* advanced oxidation protein products; *Cu,Zn-SOD,* Cu,Zn-superoxide dismutase; *DT,* dityrosine; *FU,* fluorescence unit; *ICAM,* intracellular adhesion molecule; *KYN,* kynurenine; *LHP,* lipid hydroperoxide; *NFKYN,* N-formylkynurenine; *NP-SH,* nonprotein thiol; *PCO,* protein carbonyl groups; *pr,* protein; *pg,* picogram; *P-SH,* protein thiol; *T-SH,* total thiol; *U,* unit.
*Note:* Data presented as mean ± SD.
* p<0.05.
† p<0.01.
‡ p<0.001.

adjusted the data for glycated hemoglobin levels and again detected a significant difference. Hence, these results imply that the GG polymorphism itself could be associated with elevated systemic oxidative stress. On the other hand, KYN levels were found to be significantly higher in patients with GDM and AA than in patients with GDM and GG, which supports the idea that there is less systemic oxidative stress in the GG group. Circulating ICAM-1 levels did not differ in any group.

## Discussion

GDM is a multifactorial disease that is diagnosed by impaired pancreatic beta-cell function first identified during the pregnancy period. Recently published reports indicate that people with GDM and diabetes may have signs of endothelial dysfunction (23). Systemic oxidative stress and hypercholesterolemia are known to be major mediators of endothelial dysfunction. Furthermore, proinflammatory cytokines and AGEs may lead to endothelial activation via the activation of the transcription factor NF-κB. Intercellular adhesion molecule-1 proteins play a role in T-cell activation, leukocyte-endothelial activation and inflammatory and anti-inflammatory pathways. After adjusting for possible confounding factors, it was found that elevated soluble ICAM-1 levels are associated with worse prognosis (24). It is still not clear how ICAM-1 expression increases in diabetes, but prognostic outcomes linking increased ICAM-1 and nephropathy seem convincing (24). It is suggested that complex intracellular signaling pathways involving mitogen-activated protein kinases and ROS may play a role in the upregulation of ICAM-1 protein (25,26). Recently, increased expression of ICAM-1 was reported to be tightly associated with proinflammatory cytokines, including interleukin-6, interleukin-1 beta and tumor necrosis factor alpha (27). This finding supports the hypothesis that the effects of antioxidative nuclear factor E2-related factor 2, ICAM-1 and ROS seem to be intertwined. Overproduction of ICAM-1 is shown to induce thickening in the renal tubular basement membrane and renal glomeruli, accelerating tubulointerstitial fibrosis (28). Therefore, it is suggested that the ICAM-1 gene could confer susceptibility to the development of diabetes. Patients with diabetes are found to have elevated soluble ICAM-1 levels (29). Whether increased ICAM-1 expression or the onset of diabetes triggers these alterations remains to be unraveled. Therefore, our hypothesis was, if the Lys469Glu polymorphism affects the inflammatory/anti-inflammatory functions of ICAM-1, this might render the carriers of this allele somehow susceptible to systemic redox imbalance.

Impaired regulation of systemic redox status leads to extensive oxidative alterations in various plasma constituents. Findings related to the systemic oxidative damage profile in GDM are limited. Studies investigating the importance of the ICAM-1 Lys469Glu polymorphism in patients with diabetes resulted in contradictory findings (8,30–32). Also, studies investigating the possible relationship between the ICAM-1 Lys469Glu polymorphism and diabetic retinopathy remained mostly inconclusive, and results varied significantly, depending on the populations studied (24). Previous studies had focused on finding an association between the ICAM polymorphism and diabetes/diabetes-related pathologies without investigating systemic oxidative damage in GDM, which is a relatively consistent method and could shed light on possible mechanisms. Because of the inconclusive and varying findings of the aforementioned studies concerning the importance of the Lys469Glu polymorphism and the reliability of a large spectrum of redox homeostasis markers used together, we aimed to evaluate the relationship between GDM and the ICAM-1 polymorphism via extensive systemic redox homeostasis research.

We would like to emphasize that our group with GDM consisted solely of patients who had no previous diagnosis of diabetes, and they were relatively healthy. Their impaired glucose-insulin-related morbidity was revealed only after glucose challenge tests. Even though they did not have any detected clinical problems before the glucose challenge test, their redox parameters say otherwise. Comparing the groups with and without GDM, we found that the group with GDM suffered from significant systemic oxidative stress as detected by PCO, AOPP, DT, KYN, NFKYN, lipid hydroperoxide, AGEs, PSH and Cu,Zn-SOD parameters. Shang et al reported significant decreases in Cu,Zn-SOD activities in patients with GDM, which was similar to our results (32). Therefore, the early debilitating alterations detected in this study emphasize the importance of early detection and prevention of GDM.

Our primary endpoint was investigating the plasma redox markers of pregnant women with and without GDM. In this study, it was clearly shown that patients with GDM suffer from significant oxidative stress compared to patients without GDM. As the secondary endpoint, our study was the first to evaluate the importance

of the ICAM-1 polymorphism as a risk factor for increased oxidative stress during pregnancy. The ICAM-1 Lys469Glu polymorphism and allele frequencies were compared in the groups with and without GDM, and no clear difference due to the polymorphism was detected. Our study groups consisted of a limited number of pregnant women with the Lys469Glu subtype, and the study groups consisted only of a limited number of pregnant women, so the resulting statistical frequencies may be influenced by this limitation. In some allele subgroups, we found considerable differences, but looking at the whole picture and taking into account all the markers we used, the Lys469Glu ICAM-1 polymorphism does not seem to be clearly associated with increased risk. Reports indicate that the glucose tolerance test is more sensitive in some conditions than the fasting glucose test (33). Also, it is clearly known that atherosclerotic alterations begin as early as fetal life. From a prevention and early-management point of view, even though pregnant women with GDM had relatively normal plasma glucose concentrations, the increased oxidative stress observed in the groups with GDM support these findings. To prevent morbidity and mortality and increasing healthcare costs, these results warrant further research into early detection and screening tests.

## Conclusions

The Lys469Glu ICAM-1 polymorphism does not affect oxidative stress markers significantly, and the plasma constituents of patients with GDM suffer extensive redox mismatch.

## Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the *Canadian Journal of Diabetes* at https://www.canadianjournalofdiabetes.com.

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## Author Disclosures

Conflicts of interest: None.

## Author Contributions

SA, UC, IY and KY are the principal investigators and take primary responsibility for the article; AB, AT, CC and MES recruited the patients; KY and ST also made contributions to work at the laboratory; KY, OK, BS and IY performed the statistical analyses; KY, UC, BS and IY wrote the article.

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## Supplementary Material

**Supplementary Table S1**
Comparison of the demographic, anthropometric and clinical data of pregnant women with and without gestational diabetes mellitus and the ICAM-1 Lys469Glu polymorphism

| ICAM469 | Without GDM (n=89) | | | With GDM (n=53) | | | p without GDM vs. with GDM | | |
|---|---|---|---|---|---|---|---|---|---|
| | AA (n=26) | GA (n=39) | GG (n=24) | AA (n=19) | GA (n=21) | GG (n=13) | AA | GA | GG |
| Age (y) | 31±4 | 32±4 | 31±4 | 30±6 | 33±3 | 32±5 | 0.512 | 0.295 | 0.649 |
| BMI (kg/m$^2$) | 29±2 | 28±2 | 28±3 | 28±3 | 29±2 | 29±3 | 0.943 | 0.526 | 0.330 |
| Waist circumference (cm) | 113±4 | 110±5 | 112±4 | 111±11 | 111±8 | 110±9 | 0.478 | 0.775 | 0.437 |
| Systolic BP (mmHg) | 108±10 | 109±7 | 107±9 | 109±10 | 110±7 | 108±10 | 0.770 | 0.744 | 0.737 |
| Diastolic BP (mmHg) | 68±8 | 64±8 | 65±7 | 67±8 | 69±8 | 69±8 | 0.940 | 0.058 | 0.202 |
| Insulin resistance (HOMA-IR) | 1.3±0.5 | 1.2±0.5 | 1.2±0.4 | 2.2±0.8 | 2.0±0.9 | 1.6±0.7 | 0.000‡ | 0.000‡ | 0.115 |
| Glucose (mmol/L) | 4.2±0.3 | 4.2±0.4 | 4.3±0.3 | 5.1±1.3 | 5.0±0.9 | 5.2±1.6 | 0.017* | 0.001† | 0.064 |
| Glycated hemoglobin (%) | 4.7±0.4 | 4.6±0.4 | 4.6±0.4 | 5.0±0.5 | 5.0±0.4 | 5.3±0.4 | 0.006† | 0.001† | 0.000‡ |
| Total cholesterol (mmol/L) | 4.9±0.4 | 5.1±0.4 | 5.1±0.4 | 5.6±0.7 | 5.5±0.7 | 5.0±0.6 | 0.014* | 0.037* | 0.893 |
| Triglyceride (mmol/L) | 1.8±0.3 | 2.1±0.3 | 1.9±0.3 | 2.3±0.4 | 2.2±0.4 | 2.3±0.3 | 0.000‡ | 0.337 | 0.031* |
| HDL cholesterol (mmol/L) | 1.4±0.1 | 1.5±0.1 | 1.5±0.1 | 1.4±0.1 | 1.5±0.2 | 1.4±0.1 | 0.696 | 0.899 | 0.240 |
| LDL cholesterol (mmol/L) | 2.5±0.2 | 2.5±0.3 | 2.6±0.3 | 3.1±0.5 | 2.9±0.6 | 2.6±0.6 | 0.008† | 0.030* | 0.673 |
| Birth weight (g) | 2870±231 | 2916±204 | 2967±261 | 3163±943 | 3383±630 | 3142±754 | 0.155 | 0.003† | 0.437 |
| Birth length (cm) | 49±0.8 | 50±0.9 | 50±0.9 | 48±4.8 | 50±3.1 | 49±4.0 | 0.360 | 0.817 | 0.532 |
| Gestational age at birth (weeks) | 38±0.7 | 38±0.9 | 38±0.6 | 37±3.1 | 39±1.7 | 37±2.0 | 0.122 | 0.980 | 0.443 |

*BMI*, body mass index; *BP*, blood pressure; *GDM*, gestational diabetes mellitus; *HDL*, high-density lipoprotein; *ICAM-1*, intracellular adhesion molecule-1; *LDL*, low-density lipoprotein.
*Note:* Data are presented as mean ± SD.
* p<0.05.
† p<0.01.
‡ p<0.001.