POSSIBLE IMPLICATION OF LECAM-1 GENE P213S POLYMORPHISM IN THE RISK FOR ADVANCED STAGES OF DIABETIC NEPHROPATHY IN PATIENTS WITH TYPE 1 DIABETES

Nicolae Mircea PANDURU[*], Danut CIMPONERIU[**], Pompilia APOSTOL[**], Maria MOTA[***], Eugen MOTA[***], Monica STAVARACHI[**], Mihai TOMA[**], Irina RADU[**], Anne-Marie CRACIUN[*], Cristian SERAFINCEANU[*], Dan Mircea CHETA[*]

[*] *"N. C. Paulescu" National Institute for Diabetes Nutrition and Metabolic Diseases, Bucharest, Romania*
[**] *Human Genetics and Molecular Diagnostic Laboratory, Institute of Genetics, University of Bucharest, Romania*
[***] *Diabetes Nutrition and Metabolic Diseases Department, Craiova University of Medicine and Pharmacy, Romania*
Corresponding author: Nicolae Mircea Panduru, "N. C. Paulescu" National Institute for Diabetes Nutrition and Metabolic Diseases, 2nd Diabetes Clinic, 5-7 Ion Movila, 020475 Bucharest, Romania, tel. 0040314199693, fax. 0040314199918, e-mail: nicoly_pmn@yahoo.com

Abstract. Diabetic nephropathy has an unclarified pathogenesis, with multifactorial aetiology which includes metabolic and haemodynamic abnormalities, aberrant signaling of numerous cytokines or growth factors and genetic susceptibility. Inflammation and low birth weight seems to be predisposing factors for diabetic nephropathy. In both processes levels of L-selectin (CD62L) may play an important role. The genetic heritability of diabetic nephropathy and CD62L levels sustain the investigation of relationship between LECAM polymorphisms and the disease risk. The aim of the study was to investigate a possible relationship between Pro213Ser polymorphism in LECAM-1 gene and advanced stages of diabetic nephropathy in type 1 diabetes. We enrolled unrelated Caucasian patients with type 1 diabetes mellitus, fall into control group – diabetic patients with duration of disease over 20 years without microalbuminuria (n = 83) and nephropathy group – patients with overt nephropathy or end stage renal disease – ESRD (n = 121). Pro213Ser polymorphism genotyping was achieved using PCR-RFLP technique. Genotype spread analysis indicates that ProSer and SerSer are more frequent in the nephropathy group (ProSer = 41.95% and SerSer = 6.02 %), compared with the control group (ProSer = 20.53% and SerSer = 1.73%). The corrected OR's due to the small number of patients pointed the possibility that the 213Ser is the risk allele (OR_{Ser} = 1.634; p=0.04), and 213Pro is the protective one (OR_{Pro} = 0.602; p=0.04) regarding diabetic nephropathy. Thus our results suggest a possible association between the P213S polymorphism and advanced stages of diabetic nephropathy in type 1 diabetic patients. Additional researches are required in order to acknowledge the mentioned results and to clarify the mechanism by which this polymorphism intervenes in the disease pathogenesis.

Keywords: *Pro213Ser polymorphism*, diabetic nephropathy, type 1 diabetes, LECAM-1 gene.

INTRODUCTION

Diabetic nephropathy (DN) is a condition characterised by the decrease of the glomerular filtration rate and persistent albuminuria, accompanied by high blood pressure as well as elevated mortality caused by cardiovascular diseases. Currently, the pathogenesis of this disorder is not fully understood. The multifactorial aetiology of ND includes metabolic and haemodynamic abnormalities, aberrant signalling of numerous cytokines or growth factors and genetic susceptibility.

Acute hyperglycaemia (postprandial) determines endothelial dysfunction and doubles the production of reactive oxygen species by the ***polymorphonuclear*** and mononuclear leucocytes [32]. The free radicals stimulate the proinflammatory cytokines release which induces the adhesion molecules expression [25], probably by nuclear factor – Kb [2]. Chronic hyperglycaemia and mechanic stress caused by the intraglomerular high blood pressure can independently lead to the increased expression and to release of monocitary ***chemoatracting*** protein gene (MCP-1) [8, 9], followed by glomerular infiltration [27]. Therefore, acute or chronic hyperglycaemia and mechanic stress determines inflammation triggering [17], activation of the immune cells with cytokines release, followed by increased prosclerotic response to TGF β1 [23]. Prevention of the glomerular monocitary infiltration and immune cells activation has antiproteinuric and renoprotective effect [14, 31].

Glomerular infiltration supposes recruitment of monocyctes, ***neutrophils*** and then lymphocytes to the vascular wall, followed by its crossing through diapedesis and cells movement through the interstitial spaces due to chemoattraction. Adherence and cells rolling on the vascular wall require an increased circulating level of L-selectin (CD62L) and the interaction between CD62L from ***neutrophils surface*** and E-selectin expressed on the endothelial cells [10, 24, 33]. This molecule is also similarly involved in the interaction of other cells important for glomerular infiltration [11]. In addition it has been found that the circulating levels of CD62L are high both in patients with type 1 DM [29] and in apparently healthy, first degree relatives [15], but the expression of this molecule is lower at the lymphocytes and ***neutrophils*** surface in type 2 diabetic patients with DN, compared with those who have not microvascular complications [19]. Thus, the role of CD62L in pathogenesis of diabetes mellitus (DM) and its complications remains to be clarified.

Since only 30-40% of the overall patients with type 1 DM develop clinically DN, it is supposed that there is a genetic predisposition for this complication, fact acknowledged by the increased incidence of the disease in studies with normo- and macroalbuminuric pairs of twins [3, 28]. Genetic heritability of the DN and CD62L levels, corroborated with a possible involvement of L-selectin in the pathogenesis of this disease, sustain the investigation of functional mutations at the level of this gene in patients with type 1 DM and different degree of vascular complications.

The L-selectin gene (SELL, LECAM-1) has ten exons and is located 1(q23-q25) [22]. Polymorphism rs4987310 from the sixth exon (coding for short repetitive consensus domain 1) consists in a C/T transition which entails the amino acid switch at the

level of the 213 codon from Proline to Serine. This polymorphism seems to influence the interaction between leucocytes and endothelium [18], the risk of Graves' disease in Chinese population [4] and of DN [12] but not retinopathy in Japanese population with type 2 DM [13].

The purpose of this research is to investigate if Pro213Ser polymorphism in LECAM-1 gene is associated with advanced chronic renal disease in type 1 DM patients.

MATERIALS AND METHODS

A number of 204 unrelated Caucasian patients with type 1 DM have been included in the study. The study enrolment was performed after an informed consent was signed, in accordance with the declaration of Helsinki. They have been considered as control group (83 patients with diabetes duration over 20 years without microalbuminuria) or nephropathy group (121 patients with overt nephropathy or end stage renal disease – ESRD). The diagnostic of type 1 DM was based on inaugural ketoacidotic coma, insulin treatment during the first 12 months from this episode and the level of peptide C < 0.3 nmol/l. Patients included in the nephropathy group have a glomerular filtration rate (GFR) ≤ 59 ml/min/1,73 m^2 and albuminuria > 300 mg/l in the first morning urine sample and the control group include patients with GFR between 90 and 120 ml/min/1,73 m^2 and without microalbuminuria.

The genomic DNA was extracted from the peripheral venous blood of patients (2ml), using a classical protocol and then stored at -20°C. The Pro213Ser (rs4987310) polymorphism genotyping has been achieved by PCR-RFLP technique. A fragment of 186 bp was amplified, using the following primers: F 5′ - TGA TTC AGT GTG AGC CTT TG - 3′ and R 5′ - CTT GAC AGG TTG GTT CTG - 3′ [12]. The PCR mix included: genomic DNA 1 µl, PCR Buffer (2X) 5 µl, dNTP 0.04 µl, F and R primers 0.04 µl each, Taq ***polymerase*** 0.1 µl, water 3.78 µl, working within a final volume of 10 µl. PCR reaction was performed using a Perkin Elmer Gene Amp PCR system 2400. PCR program had an initial denaturation stage of 2 minutes at 72 degrees 95°C followed by 35 cycles - 1min. at 90°C, 1 min. at 60°C, 50 sec. at 72°C and a final elongation of 2 minutes at 72 degrees. The quality of amplicons has been checked by means of electrophoresis (agarose 2%, TBE 1x, 5V/cm). Five µl of each amplicon was restricted using 5U of Hph I enzyme (New England Biolabs, Inc. Beverly, USA) for three hours at 37°C. The restriction fragments have been viewed in UV light, after electrophoresis in 12% ***polyacrylamide*** gel and ethidium bromide staining.

The statistic analysis of genotypes spread within the two groups included the testing for deviation from Hardy-Weinenberg equilibrium, using "Pearson's chi-square test". The inbreeding coefficient was also calculated for the studied population. The Odds ratio (OR) and the 95% confidence interval (95% CI) starting from the contingency tables were calculated in order to evaluate the association between LECAM-1 genotypes and DN. Calculations for the control case-study were achieved using the DeFinetti (http://ihg.gsf.de) informatics program. The p value considered as being statistically significant was < 0.05.

RESULTS

PCR-RFLP analysis has showed the presence of all three genotypes of P213S polymorphism in the L-selectin gene. The presence of T (213Ser) allele generates a restriction site for Hph I enzyme and the amplicon digestion generates two fragments of 142 bp and 44 bp. When C (Pro213) allele is present, the restriction situs is not created, the amplicon is not digested and it maintains its size of 186 bp (Figure 1).

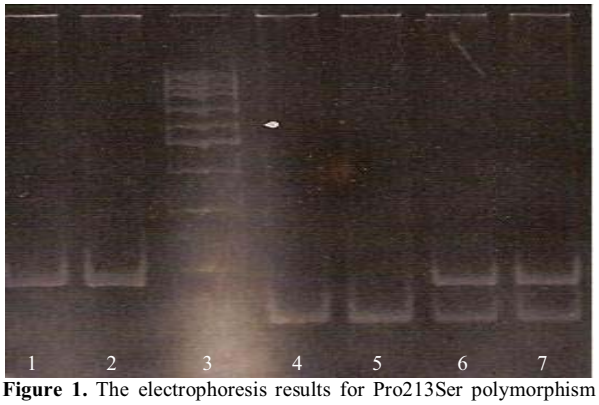

Figure 1. The electrophoresis results for Pro213Ser polymorphism (lines 1, 2: ProPro genotype; lines 3: DNA leader 100 bp - Fermantas, lines 4, 5: SerSer genotype, lines 6, 7 ProSer genotype).

Testing Hardy-Weinberg equilibrium has shown that there was no significant deviation of P213S genotypes distribution from the balance condition, either for the control group (X^2 = 0.06, p=0.81) or for the nephropathy group (X^2 = 0.29, p=0.59). There is a very low inbreeding coefficient calculated by us which represents an argument for validating the groups selected for our research. Genotypes spread analysis indicates that ProSer and SerSer are more frequent in our nephropathy group compared with controls without diabetic renal disease (ProSer: 36.36% vs. 24.09% and SerSer: 4.13 % vs 2.40%). Also, the Pro allele frequency is lower in the nephropathy group compared with controls (78% vs. 86%) (Table 1).

Calculation of OR, starting from the allelic frequency, has shown that the Ser allele entails a risk for diabetic renal disease (OR=1.69, 95% C.I.=1.002-2.881), while the Pro allele provides protection (OR=0.58, 95% C.I = 0.347-0.998).

When it is performed the allelic positivity test, considering the 213Pro as the reference allele, the presence of a single 213Ser allele entails a significant statistic risk (p = 0.03) for advanced stages of DN (OR = 1.88, 95% C.I = 1.028 - 3.465). Under homozygous form, the 213Ser allele seems to additionally increase the risk for DN (OR = 2.11), while the 213Pro allele seems to be protective (OR = 0.47), but the results do not reach the cut-off of the statistic significance (Table 2).

However, after the application of the required correction, due to the small number of patients and the

Table 1. Genotypes spread, alleles frequency and testing deviation from Hardy-Weinberg equilibrium.

Date / Genotype	Number of genotypes achieved during the research	Number of expected genotypes within the research	Frequency of genotypes within the research	P Allele frequency (+/- SD)	Inbreeding coefficient	Statistic significance -p
Control group – patients with type 1 DM and without DN						
ProPro	61	60.73	0.735	0.86 (+/- 0.028)	0.0258	0.814
ProSer	20	20.53	0.241			
SerSer	2	1.73	0.024			
Nephropathy group - patients with type 1 DM and DN						
ProPro	72	73.02	0.595	0.78 (+/- 0.026)	-0.0488	0.591
ProSer	44	41.95	0.364			
SerSer	5	6.02	0.041			

Table 2. Test results for association of Pro213Ser polymorphism in L-selectin gene with DN in the type 1 diabetes mellitus.

	Allelic frequency difference	*Heterozygous*	Homozygous	Allelic positivity	Corrected Odds Ratio
Ser - risk allele					
	[Pro]<->[Ser]	[ProPro]<->[ProSer]	[ProPro]<->[SerSer]	[ProPro]<->[ProSer+SerSer]	
Odds Ratio	1.699	1.864	2.118	1.887	1.634
95% C.I.	[1.002-2.881]	[0.994-3.496]	[0.397-11.307]	[1.028-3.465]	-
Chi^2	3.93	3.81	0.80	4.25	3.99
p	0.04	0.05	0.37	0.03	0.04
Pro - risk allele					
	[Ser]<->[Pro]	[SerSer]<->[ProSer]	[SerSer]<->[ProPro]	[ProPro+ProSer]<->[SerSer]	
Odds Ratio	0.588	0.880	0.472	0.573	0.602
95% C.I.	[0.347-0.998]	[0.157-4.929]	[0.088-2.520]	[0.108-3.025]	-
Chi^2	3.93	0.02	0.80	0.44	3.99
p	0.04	0.88	0.37	0.50	0.04

Note: Ser risk allele – Pro allele is considered OR=1; corrected OR– following application of the correction for small batches and Armitage test in order to set the trend; Pro risk allele – Ser allele is considered OR = 1.

small number of homozygotes for the 213Ser allele, by Armitage's test (achieved by means of DeFinetti program), the results are statistically significant. The corrected OR_{Ser} = 1.634 (p=0.04) and OR_{Pro} = 0.602 (p=0.04) emphasizes the possibility for the 213Ser mutant allele to be the risk allele, and the "wild type" – Pro allele to be protective for advanced stages of DN.

DISCUSSIONS

According to our knowledge, this is the first research which investigates the association between Pro213Ser polymorphism, in the L-selectin gene and DN in type 1 DM. The results show a possible contribution of this polymorphism to the increased risk for overt DN or ESRD in patients with type 1 DM. Due to the relatively limited number of patients, only the allelic positivity test and the ***heterozygous*** condition for the 213Ser allele have reached the statistic significance for the risk elevation. Regarding the SerSer homozygous condition, despite an OR = 2.11, the statistic significance could not be reached, probably due to the small number of homozygotes (two in the control group and five in the nephropathy group). The significant differences between the alleles frequencies following applied correction, suggests that there is >95% probability to achieve similar results to those of our research. The trend suggests that the Ser allele increases the risk of overt DN or ESRD development in type 1 DM patients.

Our results are difficult to explain and correlate with the already existing data in the literature. The functional significance of the Pro213Ser polymorphism is not accurately known, but it seems to have an impact upon two very important ***pathophysiological*** processes: 1 – the cells interaction during the intrauterine development; 2 – adherence between leucocytes and endothelial cells during the inflammation process.

The proper expression of L-selectin is crucial to normal placenta implant and development [16, 20] and thus interferes with the intrauterine development [7, 32]. In addition the mutant LECAM-1 213Ser allele was associated with a low birth weight [5]. Since within some populations the low birth weight is a risk factor for congenital ***oligonephronia*** and the DN [6, 26], it is possible that mutations or the abnormal expression of genes codifying for adherence molecules, including L-selectin, to interfere with intrauterine development and DN in patients with type 1 DM.

The high serum levels of L-selectin have been associated with different diseases in which the microinflamation has an important part – atherosclerosis, type 1 DM or DN in type 2 diabetic patients [15]. The increase of serum levels of L-selectin occurs as a consequence of its quick cleavage on the leucocytes surface in the neighbouring area of the trans-membrane domain, at the levels 346Lys and 347Ser. Thus the link between leucocytes and endothelial cells can be quickly destroyed and they can roll at the endothelium level [1]. In this fashion, when inflammation occurs, the L-selectin expression

decreases on the leucocytes surface, entailing increase of the serum soluble L-selectin level.

The relationship between the Pro213Ser polymorphism and different diseases has been also studied, and there have been found possible associations of the 213Pro allele with DN in patients with type 2 DM [12]. Also, certain researches could not find evidence in the favour of allelic association with diabetic retinopathy in type 2 DM [13] or with nephropathies having other aetiology except DM [30].

The 213Pro allele was reported to be the risk allele for the DN in type 2 diabetic patients. In our study, the risk allele seems to be 213Ser allele. Therefore, it is possible that the involvement of L-selectin in the predisposition to these two affections to be achieved by distinct mechanisms – in the first case by microinflamation / atherosclerosis and in the second, by means of the oligonephronia due to impaired intrauterine development, followed by vulnerability to outer deleterious agents.

As a conclusion, our results suggest a possible association between the P213S polymorphism and advanced stages of DN in type 1 diabetic patients. Additional research will help us clarify the mechanisms by which this polymorphism intervenes in chronic complications of diabetes.

Acknowledgements. This work was financed by the National University Research Council and National Ministry of Education and research (Project IDEI - CNCSIS 2150).

REFERENCES

[1] Alegre, M., Fallarino, F., Zhou, P., Frauwirth, K., Thistlethwaite, J., Newell, K., Gajewski, T., Bluestone, J., (2001): transplantation and the CD28/CTLA4/B7 pathway. Transplantation Proceedings, 33: 209-211.

[2] Aljada, A., Friedman, J., Ghanim, H., Mohanty, P., Hofmeyer, D., Chaudhuri, A., Dandona, P., (2006): Glucose ingestion induces an increase in intranuclear nuclear factor kappaB, a fall in cellular inhibitor kappaB, and an increase in tumor necrosis factor messenger RNA by mononuclear cells in healthy human subjects. Metabolism, 55: 1177-1185.

[3] Borch-Johnsen, K., Nargaard, K., Hommel, E., Mathiesen, E.R., Jensen, J.S., Deckert T, Parving H.H., (1992): Is diabetic nephropathy an inherited complication?. Kidney International, 41: 719-722.

[4] Chen, H.Y., Cui, B., Wang, S., Zhao, Z.F., Sun, H., Zhao, Y.J., Li, X.Y., Ning, G., (2007): L-selectin gene polymorphisms in Graves' disease. Clinical Endocrinology, 67: 145-151.

[5] Derzbach, L., Bokodi, G., Treszl, A., Vásárhelyi, B., Nobilis, A., Rigó, J.Jr., (2006): Selectin polymorphisms and perinatal morbidity in low-birthweight infants. Acta Paediatrica, 95: 1213-1217.

[6] Fagerudd, J., Forsblom, C., Pettersson-Fernholm, K., Saraheimo, M., Wadén, J., Rönnback, M., Rosengård-Bärlund, M., Björkesten, C.G., Thorn, L., Wessman, M., Groop, P.H., FinnDiane Study Group., (2006): Low birth weight does not increase the risk of nephropathy in Finnish type 1 diabetic patients. Nephrology Dialysis Transplantion, 21: 2159-2165.

[7] Genbacev, O.D., Prakobhol, A., Foulk, R.A., Krtolica, A.R., Ilic, D., Singer, M.S., Yang, Z.Q., Kiessling, L.L., Rosen, S.D., Fisher, S.J., (2003): Trophoblast L-selectin-mediated adhesion at the maternal-fetal interface. Science, 299: 405-408.

[8] Gruden, G., Setti, G., Hayward, A., Gnudi, L., Viberti, G., (2002): Rosiglitazone prevents stretch-induced MCP-1 production in human mesangial cells. Diabetologia, P93.

[9] Ihm, C.G., Park, J.K., Hong, S.P., Lee T.W., Cho, B.S., Kim, M.J., Cha, D.R., Ha, H., (1998): A high glucose concentration stimulates the expression of monocyte chemotactic peptide 1 in human mesangial cells. Nephron, 79: 33-37.

[10] Jones, W.M., Watts, G.M., Robinson, M.K., Vestweber, D., Jutila M.A., (1997): Comparison of E-selectin-binding glycoprotein ligands on human lymphocytes, neutrophils, and bovine γδT cells. The Journal of Immunology, 159: 3574-3583.

[11] Jutila, M.A., Kurk, S., Jackiw, L., Knibbs, R.N., Stoolman, L.M., (2002): L-selectin serves as an E-selectin ligand on cultured human T lymphoblasts. The Journal of Immunology, 169: 1768-1773.

[12] Kamiuchi, K., Hasegawa, G., Obayashi, H., Kitamura, A., Ishii, M., Yano, M., Kanatsuna, T., Yoshikawa, T., Nakamura, N., (2002): Leukocyte - endothelial cell adhesion molecule 1 (LECAM-1) polymorphism is associated with diabetic nephropathy in type 2 diabetes mellitus. Journal of Diabetes Complications, 16: 333-337.

[13] Kamiuchi, K., Hasegawa, G., Obayashi, H., Kitamura, A., Ishii, M., Yano, M., Kanatsuna, T., Yoshikawa, T., Nakamura, N., (2002): Intercellular adhesion molecule-1 (ICAM-1) polymorphism is associated with diabetic retinopathy in Type 2 diabetes mellitus. Diabetic Medicine, 19: 371-376.

[14] Kato, S., Luyckx, V.A., Ots, M., Lee, K.W., Ziai, F., Troy, J.L., Brenner, B.M., MacKenzie, H.S., (1999): Renin-angiotensin blockade lowers MCP-1 expression in diabetic rats. Kidney International, 56: 1037- 1048.

[15] Kretowski, A., Gillespie, K.M., Bingley, P.J., Kinalska, I., (2000): Soluble L-selectin levels in type I diabetes mellitus: a surrogate marker for disease activity?. Immunology, 99: 320-325.

[16] Kruse, A., Merchant, M.J., Hallmann, R., Butcher, E.C., (1999): Evidence of specialized leukocyte-vascular homing interactions at the maternal/fetal interface. European Journal of Immunology, 29: 1116-1126.

[17] Lin, J., Glynn, R.J., Rifai, N., Manson, J.E., Ridker, P.M., Nathan, D.M., Schaumberg, D.A., (2008): Inflammation and progressive nephropathy in type 1 diabetes in the diabetes control and complications trial. Diabetes Care, 31: 2338-2343.

[18] Maruya, E., Saji, H., Seki, S., Fujii, Y., Kato, K., Kai, S., Hiraoka A, Kawa K, Hoshi Y, Ito, K, Yokoyama S, Juji T, (1998) Evidence that CD31, CD49b, and CD62L are immunodominant minor histocompatibility antigens in HLA identical sibling bone marrow transplants. Blood, 92: 2169-2176.

[19] Mastej, K., Adamiec, R., (2008): Neutrophil surface expression of CD11b and CD62L in diabetic microangiopathy. Acta Diabetologica, 45: 183-190.

[20] Milstone, D.S., Redline, R.W., O'Donnell, P.E., Davis, V.M., Stavrakis, G., (2000): E-selectin expression and function in a unique placental trophoblast population at the fetal-maternal interface: regulation by a trophoblast-restricted transcriptional mechanism conserved between humans and mice. Developmental Dynamics, 219: 63-76.

[21] Mohanty, P., Hamouda, W., Garg, R., Aljada, A., Ghanim, H., Dandona, P., (2000): Glucose challenge stimulates reactive oxygen species (ROS) generation by leucocytes. Journal of Clinical Endocrinology & Metabolism, 85: 2970–2973.

[22] Ord, D.C., Ernst, T.J., Zhou, L.J., Rambaldi, A., Spertini, O., Griffin, J., Tedder, T.F., (1990): Structure of

the gene encoding the human leukocyte adhesion molecule-1 (TQ1, Leu-8) of lymphocytes and neutrophils. Journal of Biological Chemistry, 265: 7760-7767.

[23] Pawluczyk, I.Z., Harris, K.P., (1998): Cytokine interactions promote synergistic fibronectin accumulation by mesangial cells. Kidney International, 54: 62-70.

[24] Picker, L.J., Warnock R.A., Burns A.R., Doerschuk C.M., Berg E.L., Butcher E.C., (1991): The neutrophil selectin LECAM-1 presents carbohydrate ligands to the vascular selectins ELAM-1 and GMP-140. Cell, 66: 921-933.

[25] Pigott, R., Dillon, L.P., Hemingway, I.H., Gearing, A.J., (1992): Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. Biochemical and Biophysical Research Communications, 187: 584-589.

[26] Rossing, P., Tarnow, L., Nielsen, F.S., Hansen, B.V., Brenner, B.M., Parving, H.H., (1995): Low birth weight. A risk factor for development of diabetic nephropathy?. Diabetes, 44: 1405-1407.

[27] Sassy-Prigent, C., Heudes, D., Mandet, C., Bélair, M.F., Michel, O., Perdereau, B., Bariéty, J., Bruneval, P., (2000): Early glomerular macrophage recruitment in streptozotocin-induced diabetic rats. Diabetes, 49: 466-475.

[28] Seaquist, E.R., Goetz, F.C., Rich, S., Barbosa, J., (1989): Familial clustering of diabetic kidney disease: evidence of genetic susceptibility to diabetic nephropathy. New England Journal of Medicine, 320: 1161-1165.

[29] Toivonen, A., Kulmala, P., Rahko, J., Ilonen, J., Knip, M., (2004): Soluble adhesion molecules in Finnish schoolchildren with signs of preclinical type 1 diabetes. Diabetes/Metabolism Research and Reviews, 20: 48-54.

[30] Ugochukwu, C.C., Okpala, I., Pantelidis, P., Inusa, B., Ibegbulam, O., Onyekwere, O., (2008): L-selectin gene polymorphisms and complications of sickle cell disease. International Journal of Laboratory Hematology, 30: 312-316.

[31] Utimura, R., Fujihara, C.K., Mattar, A.L., Malheiros, D.M., Noronha, I.L., Zatz, R., (2003): Mycophenolate mofetil prevents the development of glomerular injury in experimental diabetes. Kidney International, 63: 209-216.

[32] Zhou, Y., Damsky, C.H., Fisher, S.J., (1997): Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome?. Journal of Clinical Investigation, 99: 2152-2164.

[33] Zollner, O., Lenter, M.C., Blanks, J.E., Borges, E., Steegmaier, M., Zerwes, H.G., Vestweber, D., (1997): L-selectin from human, but not from mouse neutrophils binds directly to E-selectin. Journal Cell Biology, 136: 707-716.