GLUTATHIONE PEROXIDASE 1 (GPX-1) PRO200LEU POLYMORPHISM AND DIABETIC NEPHROPATHY IN TYPE 1 DIABETES – A PRELIMINARY STUDY

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Abstract
Superoxide (ROS) production induced by hyperglycaemia seems to be the key-event in pathogenic pathways activation in diabetic nephropathy (DN). Glutathione peroxidase 1 (GPX-1) is a key-enzyme implied in defense against oxidative stress. The purpose of this study was to assess the association of Pro200Leu (known also as Pro198Leu) polymorphism in GPX-1 gene with risk to develop DN, in type 1 diabetes (T1D). We studied 238 patients with T1D, divided in group A (106 patients) with macroalbuminuria or ESRD (End Stage Renal Disease) and group B (132 patients) without microalbuminuria. Genotyping was performed using PCR-RFLP. Hardy-Weinberg equilibrium was observed in group B (p=0.564), in group A being a deviation which had not reached statistical significance (p=0.089). At the association test, ProPro [OR=2.614, 95% CI (1.163-5.872), p=0.017] was the risk genotype and LeuLeu genotype was protective [OR=0.383, 95% CI (0.170-0.860), p=0.017] for overt DN. The distribution of alleles in the two groups was significantly different. These differences were concordant with data from the association test, both for Pro allele [ORpro = 1.590, 95% CI (1.082-2.335), p=0.017] and for Leu allele [ORleu = 0.629, 95% CI (0.428-0.924), p=0.017]. Pro allele of Pro200Leu polymorphism was associated with the high risk for advanced DN in patients with type 1 diabetes in Romania. The results are hard to be interpreted, as this is the first report of this polymorphism in DN, and the frequencies of alleles differ from those in the literature. Further studies are necessary in order to replicate this association, and to explain the intervention mechanism of this polymorphism in DN.

Keywords: diabetic nephropathy, type 1 diabetes, risk factor, glutathione peroxidase 1 (GPX-1), Pro198Leu, polymorphism, oxidative stress, antioxidant enzymes.

Introduction
Diabetic nephropathy is an important complication of diabetes mellitus, being the main cause of end stage renal disease (1). There have been described over 20 clinical risk factors for this disease (2,3). Although the pathogeny of this disease is not enough understood, certain processes are surely involved: the oxidative stress with formation of glycation end products and endothelial dysfunction, cytokine release and growth factors, arterial hypertension, insulin resistance. Moreover, the genetic susceptibility seems to play a role in the appearance of this affection (4).

Hyperglycaemia, at the relevant levels for diabetes mellitus, leads to the generation of reactive oxygen species (Reactive Oxygen Species – ROS), over the neutralization capacity
of the body. This activates four pathways implied in the development of diabetic complications: NF-kB, PKC, polyol pathway and formation of glycation end products – AGE (5,6). The increase of oxidative stress determines the uncoupling of eNOS (endothelial nitric oxide synthase) with decrease NO production, the increase of RNS production (Reactive Nitrogen Species), endothelial dysfunction, increased release of cytokine and growth factors (ANG II, ET-1, TGF\(_{\beta}\) etc.), NAD(P)H activation with oxidative stress accentuation (7).

The mechanical stress due to arterial hypertension stimulates ROS production by the vascular cells (8,9,10) and alters the response capacity of endothelium to stimuli (11,12,13,14). The synergic action of renin-angiotensin system activation (RAS) (15) with ROS production at the level of vascular and mesangial cells (16,17) alters the capacity of vascular relaxation (18,19) probably mediated by nitric oxide, leading to intraglomerular hypertension, which predisposes to renal hypertrophy mediated by the local supraexpression of TGF\(_{\beta}\), fibronectin (20,21) and inhibitor for metalloproteinases (22,23).

The degree of insulin resistance, at type 1 diabetes diagnosis, seems to be the most important predictor of diabetic nephropathy development (24,25,26). The insulin resistance described in patients with nephropathy (27,28) is in inverse proportion to GFR diminution (29). ROS involvement in insulin resistance (30) is sustained by the association of oxidative stress markers with diabetes mellitus (31,32) and by the experiments in vitro which show that cell treatment with agents that induce ROS (33), or with high doses of hydrogen peroxide (34) may induce insulin resistance (35,36,37).

Oxidative stress is connected to all the important processes involved in the development of diabetic nephropathy, in type 1 diabetes. This is why the defense against oxidative stress is very important in disease pathogeny. In mammals, glutathione peroxidase family (GPX) is the main system of antioxidative defense (38). GPX-1 is the most abundant GPX isoenzyme, being responsible for 96% of the GPX antioxidative activity at the renal level (39), its expression being increased in conditions of hyperglycemia, in contrast with the other important antioxidant enzymes (CAT, SOD1, SOD2, Thioredoxine, Thioredoxin reductase) which are not expressed in a different way (40). Selenium deficiency significantly decreases the GPX-1 activity (41), leading to the increase of oxidative stress and acceleration of renal affections in conditions of hyperglycaemia (42). In contrast to this, the selenium supplementation is accompanied by the increase of GPX-1 expression and the diminution of renal injury in patients with diabetes (43,44).

Familial aggregation of nephropathy histological lesions in patients with diabetes (45), concordance of 83% of diabetic nephropathy in twins with diabetes (46) and ethnical differences related to incidence of diabetic renal disease (47,48) sustain the genetic predisposition for this complication of diabetes. The level of expression and GPX-1 activity is also genetically inherited (49). Within this context it is attractive the investigation of the relationship among polymorphisms, possible functional, of GPX-1 and the susceptibility for diabetic nephropathy in patients with type 1 diabetes.

Human GPX-1 gene (GeneID: 2876) is located 3p21.3, has 2 exons and by alternative splicing results more ARNm transcripts, respectively 2 isoforms, one of 201 aa and the other of 98 aa (48) Pro200Leu polymorphism (c.599C>T, rs1050450), previously described as Pro198Leu (599C/T) (49) has as consequence the Leu – Pro nonconservative substitution. T mutant allele (Leu) was associated with the decrease of enzyme activity with 9% - 13% (50,51) and probably with the limitation of the increase of gene expression as response to selenium (52,53) in spite of these controversies, this polymorphism was associated with the increased risk of appearance of macrovascular complications in diabetic patients (54,55), and with components of metabolic syndrome (56).
The purpose of this study is to investigate if Pro200Leu polymorphism is associated with advanced stages of diabetic nephropathy in type 1 diabetes.

**Materials and methods**

The study included 238 unrelated patients with type 1 diabetes. The enrolment in the study has been made by obtaining the informed consent of patients, in compliance with the declaration of Helsinki. The patients have been divided in group A (106 patients) with advanced stages of diabetic nephropathy - macroalbuminuria or ESRD (End Stage Renal and group B with 132 patients, who, also had diabetes with an evolution for at least 20 years, they do not have kidney affectation. The diagnosis of type 1 diabetes was confirmed by determining the C-peptide (< 0.3 nmol/l). In addition, for all the patients, the treatment with insulin has been initiated in the first 12 months when the diagnosis was made, and the beginning was through ketoacidotic coma. The patients have been included in the group with diabetic nephropathy if they showed a glomerular filtration rate of ≤ 59 ml/min/1.73 m² and albuminuria > 300 mg/l in the first morning urine. Those from the control group showed a glomerular filtration rate of >90 ml/min/1.73 m² and less than 120 ml/min/1.73 m².

The genomic DNA was extracted from the patients’ peripheral venous blood, using Promega Wizard Isolation Kit. The genotyping of Pro200Leu polymorphism (rs1050450) was made using the PCR-RFLP technique. It was amplified a fragment of 186 bp, using the following primers, previously used by Sutton (57) (genotyping conditions are given in Table 1).

**Table 1. Genotyping conditions for Pro200Leu polymorphism**

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Primers</th>
<th>Restriction conditions</th>
<th>Electrophoresis</th>
</tr>
</thead>
</table>
| Pro200Leu    | \( F_{GPX-1} \): 5’-TGT GCC CCT ACG CAG GTA CA - 3’  
\( R_{GPX-1} \): 5’- CCC CCG AGA CAG CAG CA - 3’ | Apal (37°C) | Polyacrylamide 8% |

The mixture used for PCR reaction contained: 1 µl genomic DNA, PCR Buffer (2X) 5 µl, dNTP 0.04 µl, primers F and R of 0.04 µl each, Taq polymerase 0.1 µl, water 3.78 µl, working in a final volume of 10 µl. PCR reaction had a stage of initial denaturation of 2 minutes at 72 degrees 95°C followed by 35 cycles – 40sec at 94°C, 40sec at 60°C, 40sec at 72°C and ended by a final elongation of 1 minute at 72 degrees. Amplicons of size 186 bp have been verified through electrophoresis in 2% agarose gel, and then restricted, using 5U of Apal (Fermentas) enzyme in each reaction. The enzymatic digestion lasted 3 hours at 37°C. Restriction fragments have been visualized under UV light, after the electrophoresis in 8% polyacrylamide gel and colouring with ethidium bromide.

The first stage of the statistical analysis of genotype distribution in the two groups was the testing concerning the deviation from Hardy-Weinberg equilibrium using „Pearson's chi-square test”, being calculated also the “inbreeding coefficient” for the population in the two groups. Then were calculated Odds ratio (OR) and 95% confidence intervals (95%CI) starting from the contingency tables, in order to assess the association between GPX-1 genotypes and diabetic nephropathy. At the end of the analysis, with the help of weighted contingency tables, was made the Cochran – Armitage test (58,59), test \( \chi^2 \), for raising the awareness, having to be doubled by the trend testing, due to the small number of patients. After OR calculation, was made the analysis through multivariate logistic regression, model in which we introduced the adjustment for age, gender and smoker status. The calculations for the case-control study have been made using PLINK software. In all the cases the values P considered significant from the statistical point of view were > 0.05.

**Results and discussions**
The clinical data of patients enrolled in the study are rendered in Table 2. The mean age of patients in group with cases of diabetic nephropathy was of 36.8 years (SD=+/- 2.5), and that in the control group was of 38.3 years (SD=+/- 1.8), at t test being a significant difference in favour of those in the control group, who were older, on the average, with 1.5 years. There has not been any significant difference between the two groups, with regard to gender distribution or the smoker status.

**Table 2.** Clinical data of patients enrolled in the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cases n=106 (%)</th>
<th>Group B n=132 (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (SD)</td>
<td>36.8 (+/-2.5)</td>
<td>38.3 (+/-1.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>46 (43.3)</td>
<td>54 (40.9)</td>
<td>0.6993</td>
</tr>
<tr>
<td>Women</td>
<td>60 (56.7)</td>
<td>78 (59.1)</td>
<td></td>
</tr>
<tr>
<td>Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever smoker</td>
<td>36 (33.6)</td>
<td>35</td>
<td>0.2129</td>
</tr>
<tr>
<td>Never smoker</td>
<td>70 (66.03)</td>
<td>97</td>
<td></td>
</tr>
</tbody>
</table>

PCR-RFLP analysis revealed the existence of the three genotypes of Pro200Leu polymorphism. The presence of the allele 593C (200Pro) leads to the appearance of a restriction situs for Apa I enzyme at the level of amplicon, this being split into two fragments of 66 bp, respectively 81bp. The presence of allele 593T (200Leu) does not lead to the appearance of the restriction situs, the amplicon remaining at the size of 147bp.

When analyzing the Hardy-Weinberg equilibrium was noticed that there is no significant deviation (p = 0.564) from the equilibrium condition for group B (control without diabetic nephropathy) while for the group with patients having nephropathy (B) there is a slight deviation but which does not reach the statistical signification threshold (p = 0.0898). (Table 3.)

**Table 3.** Pro200Leu genotype distribution, allelic frequency, inbreeding coefficient and testing for the deviation from Hardy-Weinberg equilibrium

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of genotypes obtained in the study</th>
<th>Number of genotypes expected in the study</th>
<th>Frequency of genotypes in the study (%)</th>
<th>Frequency of allele Leu (+/- SD)</th>
<th>Inbreeding coefficient</th>
<th>Statistical signification p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group – with type 1 diabetes, without diabetic nephropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu/Leu</td>
<td>69</td>
<td>52.27</td>
<td>67.65</td>
<td>0.72 (+/- 0.028)</td>
<td>0.0501</td>
<td>0.564</td>
</tr>
<tr>
<td>Leu/Pro</td>
<td>51</td>
<td>53.69</td>
<td>53.69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>12</td>
<td>9.09</td>
<td>10.65</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group with nephropathy – with type 1 diabetes and diabetic nephropathy

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of genotypes obtained in the study</th>
<th>Number of genotypes expected in the study</th>
<th>Frequency of genotypes in the study (%)</th>
<th>Frequency of allele Leu (+/- SD)</th>
<th>Inbreeding coefficient</th>
<th>Statistical signification p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu/Leu</td>
<td>44</td>
<td>41.50</td>
<td>39.86</td>
<td>0.61 (+/- 0.036)</td>
<td>0.1647</td>
<td>0.0898</td>
</tr>
<tr>
<td>Leu/Pro</td>
<td>42</td>
<td>39.62</td>
<td>50.28</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>20</td>
<td>18.86</td>
<td>15.86</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The inbreeding coefficient for control group, without nephropathy, was very small (0.0501) while that for the group with nephropathy was higher (0.1647) (Table 3). The genotype distribution analysis shows that LeuLeu is more frequent in group B – patients with diabetic renal disease as compared to those from group A – patients without renal affectation (52.27 % vs 41.50%), and ProPro genotype is more frequent in group B – patients with
diabetic nephropathy (18.86% vs 9.09%). ProLeu genotype has a similar distribution in the two groups (38.63% vs 39.62%). As shows the difference between allelic frequencies, 200Pro variant seems to be associated with an increased risk of diabetic nephropathy (OR=1.590, 95%CI=1.082-2.335) while 200Leu variant seems protective (OR=0.629, 95%CI=0.428-0.924).

Table 4. The results of the association test of Pro200Leu polymorphism with diabetic nephropathy in type 1 diabetes

<table>
<thead>
<tr>
<th></th>
<th>Difference of allelic frequency</th>
<th>Heterozygosity</th>
<th>Homozygosity</th>
<th>Allelic positivity</th>
<th>Odds Ratio corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu risk allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Pro]&lt;&gt;[Leu]</td>
<td>0.629</td>
<td>0.494</td>
<td>0.383</td>
<td>0.430</td>
<td>0.644</td>
</tr>
<tr>
<td>95% C.I.</td>
<td>0.428-0.924</td>
<td>0.217-1.126</td>
<td>0.170-0.860</td>
<td>0.200-0.926</td>
<td></td>
</tr>
<tr>
<td>Chi²</td>
<td>5.61</td>
<td>2.86</td>
<td>5.62</td>
<td>4.83</td>
<td>5.03</td>
</tr>
<tr>
<td>p</td>
<td>0.017</td>
<td>0.090</td>
<td>0.017</td>
<td>0.027</td>
<td>0.024</td>
</tr>
<tr>
<td>Pro risk allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Leu]&lt;&gt;[Pro]</td>
<td>1.590</td>
<td>1.291</td>
<td>2.614</td>
<td>1.543</td>
<td>1.567</td>
</tr>
<tr>
<td>95% C.I.</td>
<td>1.082-2.335</td>
<td>0.740-2.253</td>
<td>1.163-5.872</td>
<td>0.922-2.585</td>
<td></td>
</tr>
<tr>
<td>Chi²</td>
<td>5.61</td>
<td>0.81</td>
<td>5.62</td>
<td>2.73</td>
<td>5.03</td>
</tr>
<tr>
<td>p</td>
<td>0.017</td>
<td>0.367</td>
<td>0.017</td>
<td>0.098</td>
<td>0.024</td>
</tr>
</tbody>
</table>

When doing the test of allelic positivity, if we consider 200Pro allele as reference (OR=1), then the presence of a 200Leu allele seems to be significantly protective (p=0.024) for the appearance of diabetic nephropathy (OR=0.43, 95% C.I. = 0.200-0.926). 200Leu allele in homozygotic form seems to be protective (OR=0.383, 95% C.I. = 0.170-0.860), reaching the statistical significance (p=0.017), while in heterozygotic form Leu allele seems to be also protective (OR=0.49, 95% C.I. = 0.217-1.126), but does not succeed in reaching the statistical significance (P=0.090). On the other hand, the homozygotes for Pro allele have a risk of nephropathy (OR=2.614, 95% C.I. = 1.163-5.872) significantly from the statistical point of view (p=0.01781). To the same effect, the heterozygotes for 200Pro allele have a lower risk of nephropathy (OR=1.291).

Nevertheless, after the correction was applied, necessary due to the small number of patients and homozygotes for 200Pro allele, made with the help of Cochran – Armitage test, the results are statistically significant, the corrected values being: OR_{Pro} = 1.567 (p=0.02496) and OR_{Leu} = 0.644 (p=0.02496).

After the multivariate logistical regression, model in which we introduced the adjustment for age, gender and smoker status OR_{adjustedProLeu} = 0.729 (95% C.I. = 0.343-0.942), OR_{adjustedPro} = 2.947 (95% C.I. = 1.329-5.951) and the presence of a Leu allele has a OR_{adjustedLeu} = 0.735 (95% C.I. = 0.352-0.998), which emphasizes the possibility for the 200Pro „wild type” allele to confer protection to its bearers.

From our data, this is the first study to investigate the association of Pro200Leu polymorphism, GPX-1 gene and diabetic nephropathy in type 1 diabetes. Our results show a possible contribution of Pro200Leu polymorphism to genetic susceptibility of diabetic nephropathy in patients with type 1 diabetes in Romania. In our study the statistical significance threshold was reached for LeuLeu homozygote genotype (OR=0.383, p=0.017), for 200Leu allele in the test of allelic positivity (OR=0.43, p=0.02) and for ProPro homozygote genotype (OR=2.614, p=0.01). The significant differences of allelic frequencies...
and significant values in Cochran – Armitage test confirm the trend that suggests that there is a probability of 98% for Pro allele to associate with advanced stages of diabetic nephropathy.

As it can be noticed in Table 4, in the group with diabetic nephropathy there is a tendency of deviation from H-W equilibrium, which does not reach the statistical significance (p=0.089), and the inbreeding coefficient is relatively increased (0.1647), although one of the conditions of including in the study was that the patients not to be related. One of the possible explanations which we can give to these results is the fact that over the group of patients with diabetic nephropathy operated a selection factor.

Our results are difficult to interpret, for many reasons: this is the first report of polymorphism in diabetic nephropathy, the existence of some modifying factors, both of oxidative stress and of GPX-1 expression and activity, data about GPX-1 role and about the functional role of this polymorphism in counteracting the oxidative stress of different physiological or pathological processes is controversial, GPX being related to senescence, apoptosis, RAS, insulin resistance or atherosclerosis.

The main modifying factors of GPX-1 gene expression are represented at the levels of selenium, age, gender, smoking and alcohol consumption. The selenium regulates GPX-1 expression (60), probably through the intermediary of SECIS elements (selenocysteine insertion sequence) and of UGA codon (61). The deficiency of selenium determines the decrease of GPX-1 activity up to 40%,(62) and selenium supplementation in population with reduced levels of this element increases the activity of this enzyme (63). The age is accompanied by the decrease of GPX-1 activity and expression (64) and of the decrease of renal function (65). The gender may influence the levels of oxidative stress, the estrogens being able to raise GPX-1 level through MAPK and NF-kB (66) and through p53 and p21, independently of the level of oxidative stress (67). At the same time men present a faster decline of renal function as compared to women (68) and higher values of urinary albumin excretion (69). Smoking is associated with lower activities of GPX-1 in men (70) and vegetable and fruit consumption increase its enzymatic activity (71). Alcohol consumption triggers lipid peroxidation and its influence over the enzymatic activity of GPX-1 is being controversial (50, 53). As we have shown after the multivariate logistic regression, model in which we introduced the adjustment for age, gender and smoker status, the obtained results remained concordant, which confirm the intervention of this polymorphism in the susceptibility for diabetic nephropathy and show that these factors significantly influence the relation between polymorphism and diabetic nephropathy. A limitation of the study was the fact that there had not been data about Selenium, sexual hormones levels and about alcohol consumption.

Oxidative stress may cause the senescence of cells by telomere shortening (72) which had reached a critical length lead to p53 activation which may induce the stopping of cellular cycle and apoptosis through the intermediary of p21(cyclin-dependent kinase inhibitors (CDK2)) or of Bax protein (73). The increase of oxidative stress may also cause release of cytochrome C from mitochondria into cytosol, which can be related to Apaf1 with activation of caspase-9 and apoptosis (74). The senescence process is associated with the increase, independently of Se levels, of GPX-1 expression and enzymatic activity, but not of the other antioxidant enzymes (64). Antioxidative stress, senescence and apoptosis of tubular cells (75) and podocytes (76) precocious events of DN, lead to the increase of albuminuria, renal dysfunction and premature death in animal models (66).

The hyperglycemia activates the local renin-angiotensin system, resulting the formation of ANG II (77), which leads to the activation of NADPH oxidase (78) probably at level p22phox and Nox4, triggering the endothelial dysfunction. GPX-1 superexpression is
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protective against endothelial dysfunction due to hyperglycemia (79) hyperhomocysteinemia (80, 81) and atherosclerosis (82).

C-abl activation and Arg or p53, by ROS leads to the increase of GPX-1 expression (83), by binding P53 to „consensus binding site” located in MnSOD and GPX-1 promoter, trying to compensate the increased levels of ROS. If GPX-1 overexpression induced by p53 is accompanied by an adequate clearance of H2O2 then the IKKα activity is inhibited with the decrease of NF-kB levels (84). If this GPX-1 activation is not accompanied too by the increase of CAT activation then will produce the accentuation of oxidative stress and apoptosis (74). Yet, there exist reports that show that GPX-1 overexpression as response to oxidative stress seems to be protective against the apoptosis induced by ROS (85), both in lethal acute stress and in moderate oxidative stress (86) but is not protective against early diabetic nephropathies in animals (87).

Insulin resistance is associated with dephosphorylation of receptor for insulin (88) and decrease of Akt activation (89). Insulin stimulation produces an explosion of intracellular ROS, which inhibits phosphatases (90), favouring the phosphorylation of β subunit of insulin receptor (91) and of Akt (92). GPX-1 superexpression accelerates the ROS decrease, diminishing phosphatase inhibition and consecutively of insulin receptor phosphorylation, leading to insulin resistance (93). This theory is confirmed by studies made on patients (94) and animal models (93) which show that GPX-1 overexpression is associated with hyperglycemia, hyperinsulinemia, accumulation of adipose tissue and increase of leptin levels, in spite of the insulinomimetic effect of selenium, which is rather probable to be mediated through the intermediary of selenoproteins (93). The GPX-1 superexpression induced by the vascular pressure leads the increased production of hydrogen peroxide, which, in the absence of expression increase and activities of the other scavenger enzymes, triggers the activation of pro-atherogenic genes (95).

The impossibility of increasing GPX-1 expression is correlated with accelerated atherosclerosis, related to age (96) and with the impossibility of defense against mild or lethal oxidative stress (86). The decreased activity of GPX-1 is one of the main powerful predictor factors of cardiovascular events (97,98) and patients with diabetic nephropathy have a cardiovascular risk of approximately 10 times higher in non-microalbuminuric patients (99).

GPX-1 implication in the above mentioned processes is also confirmed by the studies of association concerning Pro200Leu polymorphism. As we have shown in the introduction, the presence of T allele (Leu) was associated with the decrease of enzyme activity with 9% - 13% or the limitation of expression decrease as response to selenium, while other studies had no effect. The same allele is a risk factor for: calcification of coronary arteries (55), ischemic heart disease, cerebral vascular disease, peripheral arterial disease (54) in patients with type 2 diabetes; central obesity and hypertriglyceridemia in patients with metabolic syndrome (38); hypertension in the Japanese population (100). The same allele is also associated with survival of very old patients, bearers of Pro allele, presenting an excess of mortality at middle and old age (101).

In our study the variant which confers risk of diabetic nephropathy is 200Pro variant (OR<sub>ProPro</sub>=2.614, and OR<sub>Pro</sub>=1.543). It is rather unusual, as we would have expected that patients with a better antioxidant defense be protected of this complication of diabetes. Yet, taking into consideration the unusual great frequency of Leu allele in both groups (0.72 vs. 0.61 in general population), it seems that the facts are more complex. Due to the fact that in our group of patients with nephropathy 93 patients (87%) were dialyzed patients, corroborated with the deviation from Hardy-Weinberg equilibrium which does not reach the statistical significance of the group with nephropathy and with the increased inbreeding
coefficient in this group (0.1647) which is not explained by a „selection bias” (the inclusion condition verified was that the patients are not related) we conclude that at the level of the group with nephropathy it is possible that operated a selection factor, which determines the decrease of 200Pro allele.

This speculation concerning a possible excess mortality in patients with 200Pro may be explained by the fact that metabolic syndrome and insulin resistance are the most powerful predictor factors, both in nephropathy and in other cardiovascular complications, including cardiovascular mortality (24, 25) and this allele is associated with a higher level of gene expression, GPX-1 overexpression being associated with insulin resistance and metabolic syndrome (93, 94). But there are data that contradict this speculation – the results obtained on patients with metabolic syndrome which show that it is associated with 200Leu allele and data concerning the association of Leu allele with other cardiovascular complications with increased mortality risk (54,55) possible due to the impossibility of GPX-1 expression and activity increase (96). On the other hand it seems that bearers of Leu allele present an advantage of survival hard to be explained in general population (102).

It seems that the equilibrium of redox status is very delicate and not enough known. Also its relationship with diabetes and its complications might be even much more difficult to be understood, being possible that the patients with Leu genotype to be, as a matter of fact, the survivors who have a lower antioxidant defense as adjustment of GPX-1 levels, which are not accompanied by a concomitant increase of the other scavenger enzymes (103,104) that lead to the chronic increase of levels of hydrogen peroxide and, eventually, to an excess mortality. As GPX-1 is involved in many complex physiopathological processes it’s hard to intuit through which of these mechanisms this polymorphism, probably functional, exercise its effect.

Conclusions

Our data show that 200Pro allele of Pro200Leu polymorphism in GPX-1 gene seem to be associated with the increase risk of advanced diabetic nephropathy, further studies being necessary in order to replicate these results, to explain the mechanism of action of this polymorphism over GPX-1, ROS levels and the mechanism involved in the pathogeny of diabetic nephropathy in type 1 diabetes.

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References

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