CLINICAL IMPORTANCE OF INSULIN RESISTANCE AFTER RENAL TRANSPLANTATION IN PATIENTS ON TRIPLE IMMunosUPPRESSIVE THERAPY WITH CYCLOSPORINE, CORTICOSTEROIDS AND MYCOFENOLAT MOFETIL*

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A b s t r a c t: Introduction: Post-transplant diabetes mellitus and impaired glucose tolerance are confirmed complications after solid organ transplantation associated with the use of glucocorticoids and calcinuerin inhibitors in maintenance immunosuppression. Insulin resistance (IR) is also an independent factor for cardiovascular morbidity and mortality among renal allograft patients. The aim of our work was to investigate the clinical importance of elevated IR in renal transplant recipients on standard triple-drug immunosuppression in correlation with immunosuppressive therapy and certain independent factors such as body mass index (BMI), time after transplantation, lipid disorders, etc.

Methods: 36 allograft pts with different periods after transplantation without previous glucose disorders were included in the study. An oral glucose tolerance test (OGTT) was made to distinguish pts with or without glucose disorders. The basal values of glucose (G) and insulin (I) were used to calculate indexes of IR and beta-cell function according to the homeostasis equations. Impaired fasting glucose (IFG), impaired glucose tolerance (IGT), impaired post prandial hyperglycemia (IPPH) and diabetes mellitus (DM) were also analysed.

Results: The mean value of the IR index was 2.57 ± 1.20. It was elevated in 31 pts (86%) The IR showed a positive correlation with: I₀ (p < 0.01), I₂ (p < 0.05), β cell

* The part of the paper entitled “Insulin resistance as a surrogate marker for chronic allograft nephropathy” was presented at the traditional symposium – Nantes Advances in Transplantation, June 2007.
function (p < 0.05) and CsA (p < 0.01). The fasting I, G, and BMI were shown as independent risk factors for IR (p < 0.01, p < 0.01, and p < 0.05 respectively). There were 12 pts with different glucose disorders (IFG, IGT, DM) and 24 pts without. The pts with glucose disorders showed an elevated IR index (91%) more frequently compared with (41.67%) decreased β-cell function.

**Conclusion:** IR is frequent among renal recipients with and without glucose disorders. IR is an independent risk factor for atherogenesis. Higher CsA trough levels are associated with higher Insulin values and indexes of IR. The defect in insulin action is more a prominent mechanism in post-transplant glucose disorders than the impaired insulin secretion.

**Key words:** insulin resistance, post-transplant diabetes mellitus, renal transplantation.

**Introduction**

Modern immunosuppressive therapy enables better graft and patient survival after solid organ transplantation. Nevertheless, a high cardiovascular patient morbidity and mortality strongly correlate with it and its side-effects. One of the main reasons in this pathology is glucose metabolism disorders, increased insulin resistance (IR) and post-transplant diabetes mellitus (PTDM). It is well known that modern immunosuppressive therapy has a diabetogenic effect and the IR and the insulin deficiency are involved in the pathogenesis of the PTDM, but the relative importance of the two different mechanisms is not sufficiently clear. [1] The measurement of IR is an early and very strong predictor of diabetes type 2. [2] Even in the absence of hyperglycaemia or diabetes, the IR represents an important risk factor for cardiovascular diseases and early death [2]. Obesity, excessive caloric intake without any physical activity, is also important. On the other hand, IR can appear independently of obesity, but it always gets worse with it. [3] Even as many as 25% of the general population can be insulin-resistant due to genetic factors. IR and hyperinsulinaemia change the proportion of the body lipids by increasing triglycerides and LDL and decreasing the "good" cholesterol HDL. It can increase the risk of hypercoagulability, initiate inflammatory changes and increase natrium retention which leads to higher blood pressure.

Pts after renal transplantation are insulin-resistant compared to a control group with similar demographic characteristics. [2] Several factors connected with IR are present in these pts such as lifelong immunosuppressive therapy, especially corticosteroids and cyclosporine A (CsA), hypertension, anti-hypertensive medications, obesity, etc.

According to ADA (American Diabetic Association – 1998), IR is defined as an impaired metabolic response to either exogenous or endogenous insulin. Some researches explain that IR is a pathological condition in which the
target cell reacts inadequately to the normal level of the circulating insulin. [4] This indicates that the normal basal values of glucose and insulin do not exclude increased IR, i.e. decreased insulin sensitivity. This is the reason why IR should be measured. The measurement of IR and hyperinsulinaemia can be done using many methods. The most important include interventions such as intravenous application of glucose and insulin which enable direct evaluation of the insulin answer after glucose load. These so-called "clamp" techniques are expensive, difficult to perform, potentially dangerous and need specially-trained technicians. There are more surrogate methods to measure IR or hyperinsulinaemia by measuring fasting values of G and I or determining of the I, C-peptide and glucose responses after being physiologically loaded with G, which can be done by OGTT. The indexes obtained with these methods are less complex than the direct measurements, but they correspond well with the results from the more invasive studies. [5] In any case, there is no consensus on whether any one special IR or insulin sensitivity index is more adequate than others. [6]

The aim of the study was to determine the frequency of IR in renal allograft patients with and without glucose disorders, to correlate IR indexes with the doses of immunosuppressive medications and other risk factors such as time after transplantation, age, lipid parameters and to evaluate the relative role of IR (impaired insulin action) and the deficit β-cell function (defects in insulin release) in the pathogenesis of new onset glucose metabolic disorder.

Materials and Methods

A group of 36 renal allograft pts (mean age 35.56 ± 9.47, range 14–53, 24 male and 12 female) with a good graft function (average GFR 59.44 ± 14.45 ml/min), 35.17 ± 27.87 months after the transplantation was included in the study. All the pts were under triple maintenance immunosuppressive therapy including Mycophenolat mofetil (MMF), Cyclosporin A (CsA) and Prednisolone (Pred) in the appropriate doses.

The including criteria were: minimum age 14 years, absence of previous clinical DM, normal glucose levels in the pre-testing period and a satisfactory graft function. Pts with a history of recent rejection episodes, steroid use for other comorbidity conditions, or DM established before testing of the glucose metabolism were excluded. All examinations in the study were performed in accordance with the World Medical Associations and Helsinki Declaration. All patients gave informed consent to participate.

Oral Glucose Tolerance Test. An OGTT with 75 gr. glucose (according to recommendations of WHO) was performed in all pts with normal fasting glucose levels. The classification of the glucose disorder was made according the criteria of ADA – 2003. [7] (pts with G0 6.1–6.9 had IFG; G0 < 7
and G₂ 7.8–11.1 had IGT; and G₀ > 7 and G₂ > 11.1mmol/l had DM.). The values of G and I were determined in all pts before and 2 hours after glucose loading. The concentration of I in the plasma was measured with MEIA (Micro-particle Enzyme Immunoassay) in an automatic analyzer (Abbott).

**Insulin Resistance and β–cell function homeostasis.** For a quantitative estimate of IR and β-cell function homeostatic model assessment was used (Matthews DR. et al), according to which the normal value of HOMA-IR is considered to be 1, and for HOMA-β-cell is 100%. The evaluations were made using these formulas:

\[
HOMA - IR = \frac{(G_{\text{mg}} / \text{dl} \times 0.05551) \times \mu lU / ml}{22.5}
\]

\[
HOMA - \beta = \frac{20 \times \mu lU / ml}{(G_{\text{mg}} / \text{dl} \times 0.05551) - 3.5}
\]

**Renal function and BMI:** For evaluation of the renal function the Cockcroft-Gault formula was used. The body mass index (BMI) and other analyses within the framework of regular biochemical monitoring were made among all pts.

**Statistics.** For statistical data processing, descriptive statistical methods were used: average values, standard deviation and percentage. The following analytical methods were used: Spearman–ratio for correlation of certain risk factors, multiple regression analysis for independent risk factors, Student’s –T– test for untied samples, \( \chi^2 \)-test and Fisher exact test for proportions.

**Results**

The results of all examined parameters in the whole group, as well as glucose disorders after OGTT, are presented in Table 1.

Table 1 – Таблица 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Go – fasting glucose</td>
<td>5.28 ± 0.74 mmol/l</td>
</tr>
<tr>
<td>G₂ – after loading glucose</td>
<td>6.64 ± 1.89 mmol/l</td>
</tr>
<tr>
<td>GD – Glucose disorders</td>
<td>12 pts (33%)</td>
</tr>
<tr>
<td>IGT – Impaired glucose tolerance</td>
<td>7 pts (20%)</td>
</tr>
</tbody>
</table>

Clinical importance of insulin resistance…

IFG – Impaired fasting glucose  2 pts (5%)
IPPH – impaired postprandial hyperglycemia  2 pts (5%)
DM – *diabetes mellitus*  1 pts (3%)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Io – fasting insulin</td>
<td>10.65 ± 5.0 µIU/ml</td>
</tr>
<tr>
<td>I2 – after loading insulin</td>
<td>40.56 ± 22.60 µIU/ml</td>
</tr>
<tr>
<td>HOMA-IR – Insulin resistance</td>
<td>2.57 ± 1.20</td>
</tr>
<tr>
<td>HOMA-ß cell function</td>
<td>144.19 ± 94.78%</td>
</tr>
<tr>
<td>BMI – body mass index</td>
<td>25.61 ± 4.47 kg/m²</td>
</tr>
<tr>
<td>CsA – trough level</td>
<td>133.19 ± 79.72 ng/ml</td>
</tr>
<tr>
<td>GFR (Cockroft–Gault)</td>
<td>59.44 ± 14.45 ml/min</td>
</tr>
<tr>
<td>PRED – daily dose</td>
<td>7.85 ± 2.94 mg/day.</td>
</tr>
<tr>
<td>MMF – daily dose</td>
<td>2 gr/24 h</td>
</tr>
</tbody>
</table>

**OGTT and Glucose metabolism disorders.** Before glucose loading, no patient showed confirmed glucose disorder. The average value of fasting glucose levels was 5.28 ± 0.74 mmol/l, and G after loading was 6.64 ± 1.89 mmol/l. The basal insulin values were 10.65 ± 5.0 µIU/ml and after loading 40.56 ± 22.60 µIU/ml. After the OGTT, 12 pts (33.3%) showed glucose disorder: 7 pts with IGT (impaired glucose tolerance), 2 pts with IFG (impaired fasting glucose), 2 pts with IPPH (impaired postprandial hyperglycaemia G2 from 7–7.8 mmol/l), and 1 with DM (*diabetes mellitus*).

**HOMA-IR.** The average value of HOMA-IR was 2.57 ± 1.20 and of HOMA-ß 144.19 ± 94.78%. Values of HOMA-IR higher than 1.3 are considered to be pathological, and were elevated in 31 out of 36 pts (86%).

The correlation of the majority of variables that have an impact on HOMA-IR and HOMA-ß values were made in the whole group (Spearman).

HOMA-IR positively correlates with I₀ (\( \rho = 0.873^{**}, p < 0.01 \)), and I₂ (\( \rho = 0.352^*, p < 0.05 \)), with HOMA-ß (\( \rho = 0.651^{**}, p < 0.01 \)), as well as with the level of CsA (\( \rho = 0.439^{**}, p < 0.01 \)).

A positive correlation of IR was also noted with BMI, G₀, G₂ and with daily doses of corticosteroids, but that correlation did not reach statistical significance. Table 2.

Multiple regression analyses were used to determine independent risk factors for IR and showed that G₀ (beta = 0.491, \( p < 0.01 \)), HOMA-ß (beta = -0.712, \( p < 0.01 \)), I₀ (beta = 0.747, \( p < 0.01 \)) and BMI (beta = 0.066, \( p < 0.05 \)) were ss. associated with HOMA-IR. Table 3.
Table 2 – Таблица 2

Correlation (Spearman) of the majority of variables with HOMA-IR and HOMA-β

<table>
<thead>
<tr>
<th>Variables</th>
<th>HOMA-IR</th>
<th></th>
<th>HOMA-β cell function</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ρ</td>
<td>p</td>
<td>ρ</td>
<td>p</td>
</tr>
<tr>
<td>Io</td>
<td>0.873</td>
<td>&lt; 0.01</td>
<td>0.692</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>I2</td>
<td>0.352</td>
<td>&lt; 0.05</td>
<td>0.293</td>
<td>n.s.</td>
</tr>
<tr>
<td>Go</td>
<td>0.491</td>
<td>0.01</td>
<td>-0.582</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>G2</td>
<td>0.186</td>
<td>n.s.</td>
<td>0.019</td>
<td>n.s.</td>
</tr>
<tr>
<td>HOMA-β cell</td>
<td>0.651</td>
<td>&lt; 0.01</td>
<td>1.000</td>
<td>n.s.</td>
</tr>
<tr>
<td>CsA-level</td>
<td>0.439</td>
<td>&lt; 0.01</td>
<td>0.149</td>
<td>n.s.</td>
</tr>
<tr>
<td>BMI</td>
<td>0.191</td>
<td>n.s.</td>
<td>0.108</td>
<td>n.s.</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.000</td>
<td>n.s.</td>
<td>0.651</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Time of transplantation</td>
<td>-0.330</td>
<td>n.s.</td>
<td>-0.110</td>
<td>n.s.</td>
</tr>
<tr>
<td>TC</td>
<td>-0.151</td>
<td>n.s.</td>
<td>-0.061</td>
<td>n.s.</td>
</tr>
<tr>
<td>PRED-dose</td>
<td>0.013</td>
<td>n.s.</td>
<td>0.098</td>
<td>n.s.</td>
</tr>
<tr>
<td>LDL</td>
<td>-0.110</td>
<td>n.s.</td>
<td>-0.064</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

TC – Total Cholesterol; BMI – Body Mass Index; CsA – Cyclosporin A

Table 3 – Таблица 3

Multivariate linear regression analysis of independent risk factors for IR (s.s.)

<table>
<thead>
<tr>
<th>Variables</th>
<th>IR β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Go</td>
<td>0.491</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>HOMA β-cell</td>
<td>-0.712</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Io</td>
<td>0.747</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>BMI</td>
<td>0.066</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Go – fasting glucose, Io – basal insulin

**B-cell function.** Normal function of the β-cell is considered to be from 90–120%. HOMA-β cell function > 120% was shown in 19 out of 36 pts or 52.78%. Decreased secretion ability of the β-cell, i.e. HOMA-β < 90%, was noted in 12 pts out of the whole group (33.3%).

HOMA-β function showed a positive statistically significant (ss) correlation with Io (ρ = 0.692**, p < 0.01) and with HOMA-IR (ρ + 0.651**, p < 0.01).
A positive but statistically non-significant correlation existed between HOMA-ß cell function and I\textsubscript{2}, BMI, CsA levels, as well as the daily prednisone doses.

**Glucose disorders.** According to the glucose disorders after OGTT, the whole group of patients was divided into two different subgroups: group A – with different glucose disorders (n = 12, mean age 39 ± 6.6 years, 10 males and 2 females, mean time of transplantation 26.4 ± 24.48 months) 7 pts with IGT (20%), 2 pts with IFG (5%), 2 pts with impaired postprandial hyperglycaemia (5%), and 1 with DM (3%), and group B – without glucose disorders (n = 24, mean age 33.8 ± 10.29 years, 10 females and 14 males, and mean time of transplantation 39.5 ± 28.91 months). Table 4

<table>
<thead>
<tr>
<th>Analysis</th>
<th>G\textsubscript{0}</th>
<th>G\textsubscript{2}</th>
<th>I\textsubscript{0}</th>
<th>I\textsubscript{2}</th>
<th>HOMA-IR (index)</th>
<th>HOMA-ß (index)</th>
<th>CsA</th>
<th>k.s. mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n = 12)</td>
<td>5.78 ± 0.94</td>
<td>8.19 ± 2.54</td>
<td>12.43 ± 5.61</td>
<td>39.07 ± 16.93</td>
<td>3.14 ± 1.33</td>
<td>128.61 ± 77.84</td>
<td>157.58 ± 96.43</td>
<td>8.3 ± 4.17</td>
</tr>
<tr>
<td>B (n = 24)</td>
<td>5.03 ± 0.46</td>
<td>5.87 ± 0.73</td>
<td>9.76 ± 4.53</td>
<td>41.18 ± 25.27</td>
<td>2.29 ± 1.05</td>
<td>153.18 ± 102.77</td>
<td>121 ± 68.98</td>
<td>7.6 ± 2.15</td>
</tr>
</tbody>
</table>

**Discussion**

IR or decreased insulin sensitivity is not a disease or a specific diagnosis, but it is strongly associated with cardiovascular diseases, hypertension, DM type 2, obesity or non-alcoholic liver steatosis. Renal transplant recipients have a high prevalence of increased IR which is confirmed by other authors. Our group of pts showed an IR prevalence of 86%. The reason for this high percentage could be found in some other dependent factors as: glucose metabolism disorders, hypertension and anti–hypertensive therapy, lipid disorders, increased BMI and immunosuppressive therapy. Calcineurin inhibitor CsA as a main immunosuppressant increases insulin secretion as well as IR, which is confirmed
in the literature. [9] Hjelmesaeth et al. confirmed that neither the daily dosage nor the CsA concentration in the blood has a significant impact on IR. [2] Our results showed a positive correlation between IR and CsA (Fig. 1) and I0 and CsA (Fig. 2). IR inversely correlated with the time after transplantation which can be explained by the improvement of the renal function but also by the lowering of the immunosuppressive medications. CsA probably increases the risk for PTDM through insulin secretion disorder or deterioration of IR, which are more pronounced earlier after transplantation. Thus, we noticed a shorter (but ns) after-transplantation time in the group with glucose disorders. The same author also proved that high doses of prednisolone are independent predictors of IR after transplantation. Our study also confirmed a positive, but statistically nonsignificant correlation between the daily doses of prednisolone and IR, which can be explained by the relatively low average daily steroid dose at the time of performing the OGTT. A multiple regression analysis showed that fasting insulin concentration and BMI were independently associated with indexes of IR, which is a similar finding to the results of Oterdoom LH, 2005. [10]

It is known that IR and insulin deficiency are involved in the pathogenesis of glucose disorders, but the relative importance of the two mechanisms is still under debate. Hagen et al. showed that among the pts who developed

PTDM 6 years after the first examination, the insulin secretion was significantly
decreased, before and after loading with glucose. [1] Our material showed that
in the group of pts with glucose disorders, IR was a pre-dominant mechanism
and it was present in 91.6% of the pts, while the decreased secretion ability of
the ß–cell as a mechanism was present in 41.67% of the pts, which is ss. less.

In the glucose disorder group, the average value of the IR index was ss.
higher than within the group without glucose disorder, while the average value
of the index for the ß–cell function was ns. higher in group B than in group A.
According to Weir, PTDM related to steroid use has been assumed to be
function of IR with a concomitant relative deficiency in insulin production. He
also showed that pts treated with CsA (independent of steroids) were associated
with impaired glucose tolerance, hyperinsulinaemia and IR rather than di-
ninished insulin secretion. [11, 12] However, delineating whether IR, diminished
insulin secretion, or both, is the primary cause of posttransplant glucose disor-
ders in the clinical setting will have significant therapeutic implication [13].

Conclusion

Renal transplant recipients with and without glucose disorders have a
high frequency of increased IR, which is predominant especially in pts with
verified glucose disorders. IR is a strong predictor of diabetes and should be
calculated. Fasting I, G and BMI, as independent risk factors for IR after renal
transplantation, should be also taken into consideration. A simple OGTT can
discover more subtle disorders of the glucose metabolism such as IGT, IFG and
postprandial hyperglycemia. Both impaired insulin secretion and IR are present
in allograft pts with posttransplant glucose disorders, but a defect in insulin
action seems to be a prerequisite. By discovering the increased IR and the initial
glucose disorders in time, cardiovascular morbidity and mortality can be decre-
ased among these pts either with cessation or modification of immunosuppres-
sive therapy, appropriate diet and way of life.

REFERENCES

6-year prospective study on new onset diabetes mellitus, insulin release and insulin


Приложение. Онд. дюл. мед. наука, XXIX/1 (2008), 129–139


Резиме

**Клиничко Значење на Инсулинската Резистенција по Ренална Трансплантација Каж Пациенти На Тројна Имуносупресивна Терапија Со Циклоспорин, Кортисо̀н и Микофенолат Мофетил**

Петковска Л., Ивановски Н., Димитровски Ч., Серафимоски В.
Целта на нашиите испитувања беше да се утврди застапеноста на ИР кај пациенти со и без гликозни нарушувања по трансплантација на бубрег, да се направи корелацијата со елементите на имуносупресивната терапија, како и да се проценат релативната важност на ИР и инсулинската секреција при создавање на посттрансплантационите гликозни нарушувања.

Без испитани 36 пацијенти со различно време по трансплантација без претходни гликозни нарушувања. За проценка на гликозните нарушувања беше направен OGTT. Базалните вредности на гликоза (Г0) и инсулин (И0) беше користени за пресметување на индексите за ИР и бета-клеточната функција.

ИР среќно изнесува 2,57 ± 1,20 и беше над референтните вредности кај 31 пациент (86%). Вредностите за ИР позитивно корелираа со: И0 (p < 0,01), И2 (p < 0,05), HOMA-ß (p < 0,05), како и со нивата на CsA (p < 0,01). Базалнот И, Г и BMI се пакажаа како независен ризик фактори за ИР (p < 0,01, p < 0,01 и p < 0,05 соодветно). Вредностите на Г0, Г2 и индексот за ИР (p < 0,05) беше повисоки во групата болни со гликозни нарушувања во споредба со групата без истите. Кај 11 од 12 пациенти со гликозни нарушувања (91,6%) се потврди ИР, додека намалена секреција на и од бета-клетката имаа 5 од 12 пациенти (41,67%), што се покажа со аномалки отколку зголемената ИР (Fisher exact test за пропорциии; p < 0,05).

ИР е многу застапена по реналната трансплантација, како кај пацијенти со, така и кај пациенти без гликозни нарушувања. ИР е независен ризик фактор за атерогенеза. Концентрациите на CsA позитивно корелираат со базалните вредности на И и индексите за ИР. Таа е доминантен механизам за гликозните нарушувања во посттрансплантационот период во споредба со намалената инсулинска секреција.

Ключни зборови: инсулинска резистенција, посттрансплантационен дијабетес мелитус, ренална трансплантација.