The effect of insulin resistance on inflammatory response and oxidative stress in acute cerebral ischemia

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Abstract

OBJECTIVE: Insulin resistance (IR) has effects on inflammation and oxidative stress which have importance in acute stroke. Our aim was to investigate the relationships between IR, inflammation, oxidative stress and stroke severity in acute ischemic stroke patients.

METHODS: We examined the relationships between inflammation, oxidative stress and stroke severity in 75 acute stroke patients with and without IR. Serum levels of oxidative stress markers (nitric oxide (NO), malondialdehyde (MDA), glutathione (GSH)) were measured as well as the cytokines interleukin-6 (IL-6) and interleukin-10 (IL-10).

RESULTS: The levels of IL-10 (13.7±19.11 vs 51.20±89.32 pg/ml, \( p<0.00 \)) in IR group were significantly reduced. Patients with IR had higher levels of NO (30.26±17.63 vs 22.57±14.5 μmol/L, \( p=0.04 \)) and IL6 (27.44±57.13 vs 8.68±11.8 pg/ml, \( p<0.00 \)) and higher NIHSS scores (11.40±5.35 vs 8.81±5.76, \( p=0.04 \)) when compared with noninsulin resistant group. IL-10 was found negatively correlated with HOMA. Additionally, the parameters with positive correlations with HOMA were NIHSS, IL-6 and NO.

CONCLUSIONS: Inflammation and oxidative stress are more evident in acute stroke patients with insulin resistance which may cause worse stroke severity. Our data also suggest that IL-10 as an antiinflammatory cytokine can be much lower in insulin resistance in acute phase of ischemic stroke. However it can be elevated as an adaptive mechanism in metabolic syndrome as a chronic condition.
INTRODUCTION
The alterations in glucose metabolism including hyperglycemia associated with insulin resistance (IR) can occur in acute phase of ischemic stroke. Hyperglycemia without pre-existing diabetes mellitus (DM) has previously been shown in acute stroke (Ozkul 2010; Robinson 2004; Capes 2001). This can be attributed to several underlying mechanisms like stress-induced endocrine changes in acute ischemic stroke or a non-specific reaction to acute stress and tissue injury with the associated autonomic and metabolic alterations (Robinson 2004). Inflammation and oxidative stress, which have importance in the acute phase of ischemia, may also contribute to this pathogenesis. Many cells in the brain, including endothelial cells, perivascular macrophages, microglia, astrocytes and neurons, produce cytokines including interleukin-1beta (IL-1beta), tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) as a result of ischemia (Del Zoppo et al. 2001; De Simoni et al.2002; Perini et al. 2001; Akyol et al. 2006; Aschner et al. 1998; Fassenbender et al. 1994). Although both IL-6 and IL-10 are well known cytokines in metabolic syndrome and acute ischemic stroke, their associations with IR occurring as a stress response to acute cerebral ischemia have not been studied before. The another aspect is oxidative status in acute stroke patients with IR. DM brings an additive oxidative stress load to acute ischemic stroke patients (Guldiken et al. 2009). However the effect of IR on oxidative status in acute phase of ischemia is still unknown. Our aim was to examine the relationships between IR, inflammation, oxidative stress and stroke severity in acute ischemic stroke patients.

MATERIAL AND METHOD
We included in our study 75 nondiabetic acute cerebral ischemic infarct patients who were admitted to our neurology department. Patients with a previous history of a cerebrovascular event, cerebral hemorrhage, hemorrhagic infarct, or transient ischemic attack, and those with a history of any infectious or inflammatory disease, cancer, autoimmune disorder, hematological disorders and renal or hepatic diseases, and those who had used immunosuppressant or anti-inflammatory drugs in the previous two months were all excluded. Patients with undetermined etiology were not accepted and patients with etiological factors other than large artery disease, cardioembolism and small vessel occlusion were not included in our study. All blood samples were taken within the first 48 hours of ischemic stroke before the use of any kind of medication such as recombinant tissue plasminogen activator, pentoxifylline, antioxidant, anticoagulant, antiplatelet, or anti-inflammatory drugs that could influence the variables under study.

For each patient, a complete physical and neurological examination was performed; complete blood count, blood chemistry, sedimentation rate and coagulation tests, electrocardiography, pulmonary X-ray, brain computed tomography and/or cranial magnetic resonance imaging were carried out, and ischemic lesion was assessed. Risk factors (hypertension, hyperlipidemia, arrhythmia), body mass index (BMI), stroke duration, infarct localization (cortical/subcortical), hemispheric lateralization (right/left), Bamford clinical classification (Bamford et al. 1991) (total anterior circulation infarction (TACI), partial anterior circulation infarction (PACI), lacunar infarction (LACI) and posterior circulation infarction (POCI)), and the Trial of Org 10172 in Acute Stroke Treatment classification (TOAST) (Adams et al. 1993) (large-artery atherosclerosis, cardioembolism, small-vessel occlusion, stroke of other determined etiology, and stroke of undetermined etiology) were considered in both insulin-resistant (IR-G) and non-insulin resistant (NIR-G) groups. The severity of the neurological condition was rated on the National Institute of Health Stroke scale (NIHSS) at the time of admission. IR was estimated according to the homeostasis model assessment (HOMA, the product of fasting glucose (mmol/L) and insulin (units/mL) divided by the constant 22.5). HOMA values above 2.7 were considered as insulin resistant. The blood samples were centrifuged at +4 °C at 3000 rpm for 10 minutes, and after serum were separated, they were kept in –80 °C until they were analyzed. IL-6 concentrations were determined by chemiluminescent method in an IMMULITE 2000 hormone auto analyzer using commercial kits. In serum samples IL-10 levels were determined via commercial human Bender MedSystems (Bender MedSystems GmbH, Campus Vienna Biocenter 2, A-1030 Vienna, Austria) ELISA kit (catalogue number BMS215/2CE). The test results were calculated by bioelisa reader Elx800 using standart curve. NO (nitrite+nitrate) in samples was assayed by a modification of the cadmium reduction method by Navarro Gonzales (Navarro-Gonzales et al. 1998). The samples were analyzed spectrophotometrically using a microplate reader and quantified automatically against KNO3 standard curve, and the results were expressed as µM/L. To assess MDA production and hence lipid peroxidation Ohkowa method was used (Ohkawa et al. 1979). Total GSH measurements were performed by the method of Tietze (Tietze 1969). The GSH concentration was determined using standard aqueous solutions of GSH. Results were expressed as mg/dl.

Statistical analysis
The Statistical Package for the Social Sciences (SPSS) 15.0 (SPSS Inc. Chicago, IL, USA) was used or statistical analysis and statistical significance was defined as p<0.05. Results were given as mean±standard deviation. Comparisons of numeric values of all variables were performed using the Mann Whitney U-test or Student’s t-test. Chi square tests were used for analyzing categorical variables. Odds ratios and 95% confidence inter-
vals (CI) were calculated. $p<0.05$ values were accepted as statistically significant. We tested whether or not the variables which were detected in numeric scales in both groups had normal distribution by using the Kolmogorov Smirnov test. We used Spearman's test for correlation analysis of variables which had no normal distribution. The variables which had normal distribution were examined by Pearson correlation analysis.

RESULTS

This study included 75 acute ischemic stroke patients (28 female (37.3%), and 47 male (62.7%)). Their ages were between 44 and 85 (mean age 70.7±8.77) years. Insulin resistance was detected in 42 (56%) patients. There were no significant differences between the IR-G and NIR-G groups with regard to age, gender, BMI, stroke risk factors, stroke duration, infarct localization and etiological factors ($p>0.05$) (Table 1). IL-10 (13.17±19.11 vs 51.20±89.32 pg/ml, $p<0.00$) levels of IR-G patients were significantly lower. Other differences between the two groups were detected in levels of IL-6 (27.44±57.13 vs 8.67±11.8 pg/ml, $p<0.000$) and NO (30.26±17.63 vs 22.56±14.51 μmol/L, $p=0.04$) and NIHSS scores (11.4±5.35 vs 8.81±5.76, $p=0.04$) (Table 2, Figure 1).

The inflammatory cytokine IL-6 was correlated with NIHSS ($r=0.522$, $p<0.00$), fasting glucose ($r=0.436$, $p<0.00$), insulin ($r=0.452$, $p<0.00$) and HOMA ($r=0.544$, $p<0.00$). There were also correlations between IL-10 and insulin ($r=–0.445$, $p<0.00$) and HOMA ($r=–0.448$, $p<0.00$). NO was correlated with fasting glucose ($r=0.326$, $p=0.004$), insulin ($r=0.271$, $p=0.019$), HOMA ($r=0.326$, $p=0.004$), and NIHSS ($r=0.380$, $p=0.001$) whereas MDA only correlated with fasting glucose ($r=0.314$, $p=0.006$). No correlations were found between GSH and the other parameters studied. Other correlations were found between NIHSS scores and HOMA ($r=0.388$, $p=0.001$), insulin ($r=0.261$, $p=0.024$), and fasting glucose ($r=0.416$, $p<0.00$) (Table 3).

DISCUSSION

The main finding of this study is the existence of distinctive inflammatory status and oxidative stress in acute ischemic stroke patients with IR when compared with the NIR-G. IL-6 and IL-10 are well known cytokines and their roles in the pathophysiology of stroke have been studied in literature (Perini et al. 2001; Oto et al. 2008; Homi et al. 2010). Although IR has effects on inflammation and oxidative stress which has been shown in metabolic syndrome as a chronic disease, the relationship between cytokines and IR in acute phase of stroke has not been studied yet. In this study we found increased IL-6 and decreased IL-10 levels in IR-G patients who also had higher NIHSS scores showing stroke severity.

In the literature, elevated serum IL-6 levels are associated with increased cardiovascular risk in obese and diabetic patients, and contribute to the low-grade inflammation that accompanies the metabolic syndrome (Spranger et al. 2003; Bastard et al. 2000; Fon-

| Tab. 1. Demographic data and stroke risk factors of insulin resistant (IR-G) and non-insulin resistant (NIR-G) acute ischemic stroke patients. |
|-----------------|-----------------|-----------------|-----------------|
| Age 70.47±8.65 | 71.18±9.03      | >0.05           |
| Gender: Male (%) | 26 (61.9)      | 21(63.6)       | >0.05           |
| Body Mass Index | 26.23±2.42     | 26.05±2.99     | >0.05           |
| Hypertension (%) | 31(73.8)       | 29 (87.9)      | >0.05           |
| Heart failure (%) | 5 (11.9)      | 4 (12.1)       | >0.05           |
| Cardiac arrhythmia (%) | 6 (14.3) | 5(15.2)       | >0.05           |
| Hyperlipidemia (%) | 23 (54.8)   | 17 (51.5)     | >0.05           |
| Stroke duration (hours) | 15.05±11.04 | 14.9±9.6     | >0.05           |
| Right hemisphere infarcts (%) | 18(42.9) | 17 (51.5) | >0.05           |
| Cortical infarcts (%) | 33 (78.6)  | 21 (63.6)     | >0.05           |
| TOAST | Large-artery atherosclerosis | 11 (26.2) | 16(48.5) | >0.05 |
| Cardioembolism | 5 (11.9) | 6(18.18)       | >0.05           |
| Small-vessel occlusion | 27(64.3) | 22 (66.7) | >0.05           |

Values are mean ± standard deviation
Insulin resistance, inflammation and oxidative stress in acute ischemic stroke

In a recent study inflammatory state can be seen in obese children as early as six years of age (Stoppa-Vaucher et al. 2012). IL-6 and IL-10 are important physiological contributors to central insulin and leptin action, linking it to hypothalamic endoplasmic reticulum stress and inflammation (Ropelle et al. 2010). Circulating levels of IL-10 have been found to be increased in obese women (Esposito et al. 2003). This may be the result of a compensatory mechanism. Higher IL-10 levels in obese patients may represent an attempt to inhibit continued proinflammatory cytokine production, which fails in the case of metabolic syndrome. IL-10 affects peripheral glucose metabolism by increasing insulin sensitivity and protecting skeletal muscle from obesity-associated macrophage infiltration and increases in inflammatory cytokines that have deleterious effects on insulin signaling and glucose metabolism (Hong et al. 2009).

IL-10 has anti-inflammatory effects by inhibiting monocyte/macrophage synthesis of IL-6 and TNF-alpha by blocking gene transcription. It also downregulates the release of intercellular adhesion molecule-1 (ICAM-1) and matrix metalloproteinase (MMP), which all increase in ischemic stroke (Silvestre et al. 2001) and inhibits the production of numerous inflammatory cytokines, including TNF-alpha, IL-1, IL-6, and IL-8 (Fiorentino et al. 1991; de Waal et al. 1991). Hence it protects the host from the harmful effects of an unbalanced inflammatory response. Elevated levels of IL-10 early post-insult are protective and may be beneficial to counter-regulate the early inflammatory response. These cytokines are important not only in chronic diseases like metabolic syndrome but also in acute inflammatory response like acute ischemic stroke. In this study we found elevated IL-6 and decreased IL-10 levels in the IR-G. Both of these cytokines were also correlated with insulin and HOMA. Additionally IL-6 was positively correlated with levels of fasting glucose. Our data suggested that the inflammatory response against acute cerebral ischemia was more distinctive in insulin resistant patients.

The acute phase response at the onset of stroke, especially elevated IL-6 levels, also affects stroke severity (Akyol et al. 2006; Clark et al. 1998). Neurological deficit onset and recovery rates in individuals with higher levels of IL-6 were clearly poorer in literature (Clark et al. 1998; Vila et al. 2000; 2001). Likewise our data suggested that elevated IL-6 levels reflect severe neurological deficit. This may be the result of inflammatory response in acute ischemia causing a procoagulant tendency (Ozkul et al. 2010; Stoppa-Vaucher et al. 2012). During inflammation-induced activation of coagulation, the function of anticoagulant pathways can be impaired (Levi et al. 2008; 2010, Oláh et al. 2005; Bernard et al. 2010) which may worsen stroke severity.

In the literature there are conflicting data about IL-10 and its relationship to neurologic deficits in acute stroke. In a recent study including 135 acute ischemic stroke patients, elevated IL-10 was strongly and independently positively correlated with severe neurological deficit (Tab. 2). The levels of cytokines, oxidative stress markers and NHSS scores of insulin resistant (IR-G) and non-insulin resistant (NIR-G) groups.

<table>
<thead>
<tr>
<th>Mean±SD</th>
<th>IR-G</th>
<th>NIR-G</th>
<th>CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIHSS</td>
<td>11.40±5.35</td>
<td>8.81±5.76</td>
<td>0.019</td>
<td>5.15</td>
</tr>
<tr>
<td>Cytokines</td>
<td>IL-6 (pg/dl)</td>
<td>27.44±57.13</td>
<td>8.67±11.8</td>
<td>-1.42</td>
</tr>
<tr>
<td></td>
<td>IL-10 (pg/dl)</td>
<td>13.17±19.11</td>
<td>51.20±39.32</td>
<td>-70.18</td>
</tr>
<tr>
<td>Oxidative stress markers</td>
<td>NO (μmo/L)</td>
<td>30.26±17.63</td>
<td>22.56±14.51</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>MDA (μmol/L)</td>
<td>42.89±38.45</td>
<td>41.37±31.18</td>
<td>-14.9</td>
</tr>
<tr>
<td></td>
<td>GSH (mg/dl)</td>
<td>44.33±16.43</td>
<td>46.5±18.71</td>
<td>-10.28</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (SD). CI means confidence interval.

Tab. 3. Statistically significant correlations between studied parameters.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Correlated variables</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>NIHSS Fasting glucose</td>
<td>0.416</td>
<td>0.00</td>
</tr>
<tr>
<td>NIHSS Insulin</td>
<td>0.261</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>NIHSS HOMA</td>
<td>0.388</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>NIHSS IL-6</td>
<td>0.522</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>IL-6 Fasting glucose</td>
<td>0.436</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>IL-6 Insulin</td>
<td>0.452</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>IL-6 HOMA</td>
<td>0.544</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>NO NIHSS</td>
<td>0.380</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>NO Fasting glucose</td>
<td>0.326</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>NO Insulin</td>
<td>0.271</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>NO HOMA</td>
<td>0.326</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>MDA Fasting glucose</td>
<td>0.314</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>IL-10 Insulin</td>
<td>-0.445</td>
<td>0.00</td>
</tr>
<tr>
<td>IL-10 HOMA</td>
<td>-0.448</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>
impairment (Chang et al. 2010). However, there were data in the literature also suggesting an association between lower IL-10 and early neurological deterioration (Vila et al. 2003). In experimental studies, animals with IL-10 gene deficiency exhibited larger infarcts, increased neutrophil infiltration, and raised levels of TNF-alpha, matrix metallopeptidase-2 and 9 (Malefyt et al. 1991; Silvestre 2001 et al). IL-10 also regulates apoptotic proteins detected in cerebrospinal fluid after brain ischemia, and therefore has neuroprotective effects (Tarkowski et al. 1999). We found lower levels of IL-10 in the IR-G which had statistically increased NHSS scores, reflecting higher stroke severity. The conflicting data of such clinical studies may be the result of different study designs, small study groups, comorbid diseases or underlying diseases like undiagnosed insulin resistance. Our data suggested that inflammatory response against acute cerebral ischemia is more prominent in patients with IR than in noninsulin resistant patients.

Endothelium-derived NO is an important endogenous mediator of cerebral blood flow protection (Rudic et al. 1999). It acts as a neuroprotective with a vasodilator effect in the early phases of ischemic stroke. However, the overproduction of NO by induced and neuronal NO synthases results in elevated levels of peroxynitrite, which ultimately causes cellular injury in the latter phase (Rudic et al. 1999). In animal studies it was also shown that NOS-II knockout mice had 30% smaller infarcts and better neurological outcome following middle cerebral artery occlusion than their wild type litter mates (Huang et al. 1996). Likewise, several studies in the literature found a better clinical outcome in patients with lower NO serum levels (Ozkul et al. 2007; Castillo 2000).

Oxidative stress may cause a complex dysregulation of cell metabolism and has a role in the pathogenesis of insulin resistance and beta-cell dysfunction (Pitocco et al. 2010). It has been studied previously in acute cerebral ischemic patients with DM. Their data suggested that oxidative stress and counterbalancing antioxidant capacity are more pronounced in diabetics than in non-diabetic acute stroke patients. Although DM brings an additive oxidative stress load to acute ischemic stroke (Guldiken et al. 2009), the association of oxidative stress and insulin resistance in these patients has not been studied in literature. The role of NO, MDA and GSH as oxidative stress markers was investigated in our study, and we found higher NO levels in the IR group with higher stroke severity, although other parameters did not differ.

NO levels were also correlated with insulin, fasting glucose levels and HOMA. Another positive correlation was found between MDA and fasting glucose. Not only DM but also IR can cause an additive oxidative stress in acute ischemic stroke. Patients with more severe IR have a tendency to higher NO levels and NHSS scores. Our data suggested that inflammation and oxidative stress are more evident in acute stroke patients with insulin resistance which may cause higher stroke severity. The previous studies showed that IL-10 as an anti-inflammatory cytokine can be elevated as an adaptive mechanism in metabolic syndrome which is a chronic inflammatory condition (Esposito et al. 2003; Hong et al. 2009). However we found decreased IL-10 levels in IR due to acute inflammatory response like acute ischemic stroke. The significant associations of IR, inflammation and oxidative stress may be relevant to stroke severity and hence should be considered in novel treatment strategies of acute stroke patients.

CONCLUSION

In the acute phase of ischemic stroke, insulin resistance may cause additive oxidative stress and inflammatory response. The increased IL-10 levels which can be detected in metabolic syndrome as a compensatory mechanism seems to be impaired in acute phase of cerebral ischemia. We found higher IL-6 and lower IL-10 levels in insulin resistant acute stroke patients who had more severe neurological deficits than in the NIR-G. Although NO levels were significantly higher in IR-G, no significant differences between the two groups were detected in levels of other studied oxidative stress markers.

In conclusion, not GSH, MDA but the relationships between insulin resistance and NO, IL-6 and IL-10 may be relevant to stroke severity. We believe that in future clinical researches with larger study groups are needed to determine the exact role of IR in the pathophysiology of acute cerebral ischemia. Further studies may help us to use novel treatment strategies in clinical practice for acute ischemic stroke patients with IR.

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