Levels of advanced glycosylation end-products (AGE) in sera of pregnant diabetic women: comparison between type 1, type 2 and gestational diabetes mellitus

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Summary. - The chronic hyperglycemia can lead to an increase of the advanced glycosylation end-products (AGE) levels on proteins and macromolecules. Abnormal levels of AGE in several tissues has been associated with the pathogenesis of late diabetic complications. In diabetic pregnant women, high AGE levels might influence the delicate maternal-fetal balance and therefore alter the pregnancy outcome. In this preliminary study, we have measured the AGE in sera of 44 diabetic women in two trimester. Sixteen sera from non diabetic pregnant women have been used as controls. The AGE have been analyzed by means of an ELISA method with an anti-RNase-AGE, produced in the Laboratory of Clinical Biochemistry of the Istituto Superiore di Sanità.

Diabetic patients type 1 and type 2, in good metabolic control, showed normal AGE levels at both trimester. Patients with gestational diabetes showed significantly high serum AGE levels (p < 0.05). A more extended study will give better insight on the association between AGE levels and a physiopathology of diabetic pregnancy.

Keywords: advanced glycosylation end-products, diabetes mellitus type 1, type 2, gestational diabetes mellitus, pregnancy, ELISA method.


Le pazienti diabetiche tipo 1 e tipo 2, in buon controllo metabolico, mostravano livelli di AGE nella norma in entrambi i trimestri esaminati. Le pazienti con diabete gestazionale mostravano livelli serico di AGE significativamente alti (p < 0.05). E' necessario ampliare la casistica per dimostrare una correlazione tra AGE e fisiopatologia della gravidanza nel diabete.

Parole chiave: prodotti finali della glicosilazione non enzimatica, diabete mellito tipo 1 e tipo 2, diabete gestazionale, gravidanza, metodo ELISA.

Introduction

Reducing sugars, such as glucose and ribose, react non-enzymatically with the free amino groups of proteins to initiate a post-translational modification process called non enzymatic glycosylation [1-3]. This reaction proceeds from reversible Schiff bases to stable, covalently bonded Amadori rearrangement products [4, 5]. Once formed, Amadori products undergo further rearrangement reactions to produce a heterogeneous group of protein-bound moieties called advanced glycosylation end-products or AGE. These reactions occur slowly, and only proteins with significant amounts of Amadori products accumulate substantial amounts of AGE in vivo. This occurs on proteins with long half-lives, such as collagen and myelin, a variety of experimental studies suggests that AGE may play an important role in the structural and functional alterations which occur in proteins during ageing [1, 2, 6-9]. Moreover, the chronic hyperglycemia which characterizes diabetes
mellitus can lead to an even greater increase of circulating and tissue levels of AGE on several proteins and other macromolecules, a phenomenon which is believed to be associated with the pathogenesis of late diabetic complications [10–12].

AGE have in common a yellow-brown pigmentation, a characteristic fluorescence spectrum, and the ability to form protein-protein cross-links. AGE are highly reactive and continue to react with nearby amino groups, over time, to produce both intra- and intermolecular cross-links [13, 14]. Although circulating AGE form in part by the in situ reaction of glucose with serum proteins, a large portion of these products can enter the plasma compartment as AGE-modified peptides (AGE-peptides) via catabolism of AGE-modified proteins [14].

The precise structural elucidation of the AGE which form in vivo remains a formidable problem [3]. These products are present in low abundance, are structurally heterogeneous, and are labile to chemical hydrolysis. This has been the main problem, so far, for the exact identification of AGE biochemical structure, since the isolation and identification of particular AGE often has necessitated the use of chemical hydrolysis procedures. This leaves open the possibility that naturally occurring AGE may comprise precursor products that are structurally distinct and that may be present in higher abundance than any of the individual AGE which have been isolated so far [15–17].

As an approach toward studying the formation of AGE in vivo, we have developed an immunochemical assay for proteins modified by advanced glycosylation. We reasoned that it might be feasible to synthesize an AGE-immunogen in vitro and produce antiserum to a AGE epitopes in vitro. Recent reports suggest that it is in fact possible to raise antiserum specific for AGE epitopes [18–20].

In diabetic pregnant women, high AGE levels might possibly influence the delicate maternal-fetal balance mechanism both in umbilical cord vessels and in the placenta and therefore alter the pregnancy outcome.

In this preliminary study, we have produced an antiserum suitable for the detection of AGE-modified proteins that occur in vivo and we have measured serum AGE levels in diabetic women at different stages of pregnancy and compared them with the metabolic control (blood glucose and glycosylated HbA1c).

Patients and methods

A total of 44 sera from patient with type 1, type 2 and gestational diabetes mellitus (GDM) and obtained at the 1st and 3rd trimester were studied. Sixteen sera from non diabetic pregnant women were used as controls. Blood glucose and HbA1c were measured by standard methods (glucose oxidase and ion exchange chromatography respectively). Serum AGE levels were determined by competitive ELISA, employing the anti-AGE anti-serum produced in the Laboratory of Clinical Biochemistry of the Istituto Superiore di Sanità, using RNase-AGE as immunogen and an hyperimmunization protocol as described by Makita et al. [21]. The ELISA technique was performed using BSA-AGE as the competitor antigen.

Results

The metabolic parameters observed in diabetic patients and control subjects are summarized in Table 1. No significant differences were observed; alterations in new-born status (macrosomia) were, only, observed in four women affected by type 1 diabetes.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>no.</th>
<th>Blood glucose (mg/100 ml)</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes, type 1 (1st trim.)</td>
<td>15</td>
<td>118 ± 7</td>
<td>6.9 ± 0.3</td>
</tr>
<tr>
<td>Diabetes, type 1 (3rd trim.)</td>
<td>15</td>
<td>107 ± 6</td>
<td>6.3 ± 0.5</td>
</tr>
<tr>
<td>Diabetes, type 2 (1st trim.)</td>
<td>3</td>
<td>117 ± 19</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>Diabetes, type 2 (3rd trim.)</td>
<td>2</td>
<td>93 ± 6</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>Gestational diabetes (3rd trim.)</td>
<td>3</td>
<td>85 ± 3</td>
<td>6.7 (no. 1)</td>
</tr>
<tr>
<td>Control subjects</td>
<td>16</td>
<td>98 ± 3</td>
<td>6.0 ± 0.2</td>
</tr>
</tbody>
</table>
The reproducibility of ELISA method is shown in Fig. 1. It can be seen that standard curves produced of different time intervals are superimposable.

The serum AGE levels measured in the different patients groups are shown in Fig. 2. There was a trend forward increasing AGE values from the 1st and 3rd trimester, both type 1 and type 2 diabetes, although this difference did not reach statistical significance. Moreover no difference was observed when diabetes values were compared with the control sera, excepted in the case of GDM/3rd trimester (p < 0.05).

Discussion and conclusions

Two were the aims of this work: the first to establish whether anti-AGE ELISA method was reproducible in time; the second whether AGE could be detected with this method in sera of pregnant women affected by different types of diabetes mellitus.

The reproducibility of the standard ELISA curves suggests that the method is reliable and accurate. It confirms a previous work by the authors [22] and it may therefore be used routinely for AGE detection in specific tissues and other biological fluids.

In this study, as part of a clinical trial, all patients with type 1 and type 2 diabetes were in good metabolic control, as demonstrated by the normal blood glucose and HbA1c values. As expected, therefore, the levels of serum AGE were not different from those of non-diabetic subjects. Thus the four cases of abnormal new-born weight may reflect intrinsic hormonal alterations associated with the diabetic status, rather than to be dependent upon metabolic and biochemical derangement.

The only group which showed raised AGE levels was that comprising cases of GDM. This is not surprising, since hyperglycemia in these patients may be present without particular signs throughout pregnancy and only be detected when an OGTT is performed during the third trimester. By contrast, type 1 and type 2 pregnancies are programmed far in advance and not permitted before a good metabolic control is reached.

A more extended study, including badly controlled diabetic (both type 1 and type 2 diabetes) pregnant women, will give better insight on the association between high serum AGE levels and an altered new-born status.

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REFERENCES


