Diabetes mellitus exacerbates advanced glycation end product accumulation in the veins of end-stage renal failure patients
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Vasc Med 2006; 11: 245
DOI: 10.1177/1358863x06072202

The online version of this article can be found at:
http://vmj.sagepub.com/cgi/content/abstract/11/4/245
Diabetes mellitus exacerbates advanced glycation end product accumulation in the veins of end-stage renal failure patients

N Nazratuna, AA Mahmooda, UR Kuppusamyb, T Sara Ahmadb and SY Tanè

Abstract: The excess accumulation of advanced glycation end products (AGEs) contributes to the chronic complications of type 2 diabetes mellitus (DM) and renal failure. Biopsy specimens (n = 184) of arterial (n = 92) and venous (n = 92) tissues were obtained (radial artery and cephalic vein) from end-stage renal disease (ESRD) patients with or without DM and normal healthy subjects (n = 12) requiring surgery (trauma patients). Immunohistochemical assessment of the blood vessels revealed the presence of pentosidine (AGE marker) in both veins and arteries in 72% of the ESRD patients. The percentage of arteries and veins that showed positive pentosidine staining in ESRD patients with type 2 DM alone was 100% and 92% respectively, in the non-diabetic ESRD patients it was <70% (for arteries and veins), and in the ESRD patients with hypertension as an additional co-morbidity to type 2 DM it was 70% and 82%, respectively. The veins of ESRD patients with DM showed a strong (+++) positive staining and very strong (+++++) positive staining was observed in the patients with DM and hypertension. Only mild (+) or moderate (+++) pentosidine staining intensity was observed in the arteries of ESRD patients without or with co-morbidities, respectively. The accumulation of AGE in the vein rather than the artery may be a better reflection of the extent of complications of ESRD.

Key words: AGE; artery; diabetes mellitus; ESRD; pentosidine; vein

Introduction

Renal failure is associated with the retention of a variety of toxic compounds responsible for the uremic syndrome. In this condition, there is a defective antioxidant production and increased susceptibility to plasma lipid oxidation, oxidative damage to proteins and reactive carbonyl formation derived from the non-enzymatic oxidation of glucose and proteins or lipids.1,2 The non-enzymatic glycation reaction between ketones or aldehydes with the amino groups of proteins contributes to the aging of proteins, leading to the formation of advanced glycation end products (AGEs) and to complications of several pathological conditions including diabetes, hypertension, atherosclerosis, Alzheimer’s disease and end-stage renal disease (ESRD).1-4 The causes of AGE accumulation in ESRD have not been fully delineated. Nevertheless, it has been speculated that renal insufficiency leads to AGE accumulation due to the decreased removal of AGE precursors and/or increased generation of AGEs through oxidative stress.2,4 AGEs and related compounds can modify proteins, including those in the vascular endothelium and lipoprotein, and thus could play a significant role in the development of vascular disease in patients with renal failure.2 AGEs constitute a heterogeneous class of structures and pentosidine is a well-known and characterized structure among them. Pentosidine is a fluorescent, bifunctional condensation product of arginine, lysine and ribose and is formed by sequential glycation and oxidation reactions known as the Maillard reaction.1,5 Its formation requires aerobic conditions, while an anti-oxidative state inhibits such a reaction.6 AGEs in blood exist in both free and protein-bound forms. The free and protein-bound forms are metabolized in the kidney and the products are easily excreted by the
normal kidney. However, in uremia, the kidney is unable to metabolize both forms of AGEs. Dialysis enables the free form to be filtered but has little effect on the protein-bound form, which are too large to pass through the dialysis membrane.

Previous reports were limited to studies on the expression of AGEs in plasma and accumulation in the arterial wall, kidney mesangium, glomerular and other basement membranes, primarily in Caucasian populations. Makita et al have demonstrated using a radioreceptor technique that ESRD worsens AGE accumulation in the arteries of diabetes patients. However, to date, there are still no reports on the AGE accumulation in the veins of ESRD patients with type 2 diabetes mellitus (DM) and/or hypertension. It is speculated that the AGE accumulation in the blood vessels could contribute to ESRD complications such as thrombosis. As the morbidity and mortality rate due to AGE-mediated vascular complications remains quite high for patients with ESRD, this study aims to compare the in situ localization of the AGEs, particularly pentosidine (using an immunohistochemical technique), between veins and arteries from ESRD patients with or without type 2 DM and hypertension. The data obtained in this study could give a better understanding of the clinical manifestation and the association between AGEs, vein thrombosis and atherosclerosis induced by diabetes in ESRD patients. This may lead to a better prognosis of these diseases.

Table 1  ESRD patient characteristics and percentage of blood vessels with positive pentosidine staining.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n)</th>
<th>ESRD patients (n)</th>
<th>Percentage of blood vessels with positive pentosidine staining (total or artery/vein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>92</td>
<td>72</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>21–68 (51 ± 13)</td>
<td>21–87 (55 ± 14)</td>
<td>–</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3</td>
<td>57</td>
<td>82</td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>35</td>
<td>50</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malay</td>
<td>3</td>
<td>45</td>
<td>68</td>
</tr>
<tr>
<td>Chinese</td>
<td>2</td>
<td>31</td>
<td>71</td>
</tr>
<tr>
<td>Indian</td>
<td>1</td>
<td>16</td>
<td>81</td>
</tr>
<tr>
<td>Haemodialysis treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of sessions/week</td>
<td>–</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Duration of dialysis (years)</td>
<td>–</td>
<td>1–6</td>
<td>–</td>
</tr>
<tr>
<td>Blood vessels, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artery</td>
<td>–</td>
<td>92</td>
<td>72</td>
</tr>
<tr>
<td>Vein</td>
<td>–</td>
<td>92</td>
<td>72</td>
</tr>
<tr>
<td>ESRD patients (artery/vein)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESRD alone</td>
<td>–</td>
<td>18</td>
<td>66/66</td>
</tr>
<tr>
<td>With type 2 DM only</td>
<td>–</td>
<td>13</td>
<td>100/92</td>
</tr>
<tr>
<td>With hypertension only</td>
<td>–</td>
<td>28</td>
<td>64/54</td>
</tr>
<tr>
<td>With type 2 DM and hypertension</td>
<td>–</td>
<td>33</td>
<td>70/82</td>
</tr>
</tbody>
</table>

The percentage was calculated based on the number of specimens that showed positive immunoreactivity (+ to ++++++) to pentosidine over the total number of specimens analysed in each categorized group (for arteries and veins respectively). A minimum of three sections per blood vessel specimen were stained and analysed to determine the intensity of the pentosidine staining.

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vessel), fixed using acetone and allowed to air dry until completely dehydrated. The tissue sections were then incubated with anti-pentosidine monoclonal antibody (0.25 mg/ml; DakoCytomation Denmark A/S) for 30 minutes in a humid chamber at room temperature and the adjacent sections (without incubation with primary antibody) were used as a negative control beside the normal healthy tissue sections. The sections were washed three times with 0.05 M tris buffered saline (TBS), pH 7.2–7.6, and incubated with enzyme-labelled secondary antibody (DakoCytomation) for 30 minutes at room temperature, and subsequently washed three times with TBS. This was followed by the detection with Fast-Red substrate chromogen. The slides were mounted using DAKO glycergel mounting medium. Normal mouse serum (68 000 mg/ml) was used as a negative control reagent. The slides were analysed by a pathologist and an observer in a blinded fashion and scored on a semi-quantitative scale (0, negative; +, mild; ++, moderate; ++++, strong; +++++, very strong) according to the degree of positive staining. Specimens that showed positive pentosidine staining scores of +, ++, ++++ and +++++ were considered as having AGE accumulation.

Data analysis

The percentage was calculated based on the number of specimens that showed positive immunoreactivity (+ to +++++) to pentosidine over the total number of specimens analysed in each categorized group, as stated in Table 1. A minimum of three sections per blood vessel specimen were stained and analysed to determine the intensity of the pentosidine staining. The results obtained in this study are reported based on observation.

Results

The positive staining was the result of specific binding of the antibody to the pentosidine which resulted in a red-coloured precipitate at the antigen site. Out of the 184 tissue biopsies from 92 ESRD patients, immunostaining with anti-pentosidine monoclonal antibodies was positive in 132 (72%) specimens whilst 52 (28%) showed negative staining for pentosidine. There was no evident anti-pentosidine monoclonal antibody reactivity/immunostaining in the arteries (Figure 1a) and veins (Figure 1b) from healthy individuals. There were no significant age- or sex-related differences in the distribution of pentosidine or between the types of vessels (arteries and veins) (Table 1). Mild (+) immunostaining was observed in the arteries (Figure 1c) and veins (Figure 1d) of ESRD patients. Interestingly, in several ESRD patients with or without type 2 DM, the AGE accumulation was significant in either artery or vein only (result not shown). Approximately 28% of the renal failure patients did not have detectable AGE accumulation in the vein and/or artery. It is noteworthy that 89% of these patients did not have type 2 DM (result not shown).

The percentage of arteries and veins that showed positive pentosidine staining in ESRD patients with type 2 DM alone was 100% and 92% respectively, in the non-diabetic ESRD patients it was <70% (for arteries and veins), and in the ESRD patients with hypertension as an additional co-morbidity to type 2 DM it was 70% and 82%, respectively (Table 1). Interestingly, the veins of ESRD patients with diabetes showed a strong (+++) positive staining (Figure 1f) and a very strong (++++) positive staining in the presence of DM and hypertension (Figure 1g). Meanwhile, most of the arteries (97%) had relatively the same staining intensity, which was mild (+) or moderate (++) (Figures 1c and 1e) regardless of the presence of the co-morbidities.

The percentage of blood vessels with positive pentosidine staining among the three ethnic groups, namely Malay (from the Malay Archipelago), Chinese (mostly from south China) and Indian (from Sri Lanka and south India) was 68%, 71% and 81%, respectively (Table 1). The percentage of patients in each ethnic group represents the percentage of ethnic distribution in the population living in Kuala Lumpur and the city suburbs. The Indian patients (75%) also had type 2 DM and 50% of them also had hypertension. Most of the high staining intensity (+++ or ++++) was observed in the veins of these patients.

Discussion

This study documents for the first time the expression of pentosidine in the vascular tissues of both veins and arteries in ESRD patients with or without type 2 DM in the presence of hypertension, using an immunohistochemical technique. Studies have shown that the accumulation of precursor carbonyl compounds of pentosidine can increase the generation of pentosidine in uremic conditions. Kuppusamy et al showed that antioxidant enzyme activities, namely superoxide dismutase, catalase and glutathione peroxidase, in ESRD patients were significantly lower and that the levels of AGEs were increased in the serum of ESRD patients. This could also be one of the main factors that contribute to the increased level of AGEs in the vascular tissues of ESRD patients.

Most of the ESRD patients with type 2 DM, regardless of the presence of hypertension, showed positive staining for pentosidine. This is probably due to the fact that the type 2 DM patients in this study developed hypertension and ESRD as a consequence of the disease (type 2 DM) progress and complications. This was particularly noted in the majority of Indian patients. It is not possible to suggest or speculate any genetic influence pertaining to this observation due to the limited scope of this study.

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The rate of AGE formation was reported to be much faster in diabetic patients than in non-diabetic individuals, possibly driven by the prolonged hyperglycemic states and increased oxidative stress. In ESRD, this condition can be worsened by the defect in the glomerular filtration rate and anemia. The excessive deposition of AGEs can cause irreversible tissue damage via inactivation of nitric oxide (essential in

Figure 1 Immunohistochemical detection of pentosidine in the blood vessels of ESRD patients and normal subjects. Light micrographs of negative immunostaining of artery (a: 10×) and vein (b: 40×) of a normal healthy individual; mild (+) staining in the artery (c: 10×) and vein (d: 10×) of a patient with ESRD alone; moderate (++) staining in the artery (e: 10×) and strong (+++) staining in the vein (f: 10×) of an ESRD patient with type 2 DM; very strong (++++) staining in the vein (g: 10×) of an ESRD patient with type 2 DM and hypertension.
maintaining normal vascular tone and blood pressure), generation of free radicals or by growth factor-mediated synthesis of extracellular matrix components and cell proliferation. These factors may also contribute to the increased susceptibility of diabetic patients to develop hypertension.\textsuperscript{10,12}

AGE accumulation can cause circulating matter such as platelets, immunoglobulins and apolipoproteins to adhere to the blood vessel wall and cause stenosis.\textsuperscript{10,12,13} AGEs can also perturb cellular function by binding to a variety of receptors such as macrophages and lead to the cell activation and release of cytokines. The modifications of these receptors by AGEs make it unrecognizable to its normal target. Thus, the scavenging macrophages, overwhelmed by the AGEs’ burden, become engorged and form foam cells, characteristic of early atherosclerosis. The site evolves from a fatty streak into histologically complex lesions characteristic of fully fledged atherosclerosis.\textsuperscript{10,12–14}

The present study showed that the intensity of the pentosidine staining was higher in the veins compared with the arteries, especially in the presence of type 2 DM. Tokgoz et al have reported that patients with ESRD have a higher tendency of getting vein thrombosis,\textsuperscript{15} and the concomitant presence of diabetes and renal dysfunction can enhance platelet activity and blood clot formation.\textsuperscript{16} AGEs can directly cause thrombosis by down-regulating endothelial cell expression on the surface anticoagulant thrombomodulin to increase the synthesis of procoagulant tissue factor.\textsuperscript{17–19} In addition, the AGE-stimulated rapid progression of amyloid could also lead to vein thrombosis.\textsuperscript{7,17,18} Further studies would need to be carried out to elucidate the exact role of AGE in the vein tissues.

The present study showed that the distribution of pentosidine in ESRD patients was the same for different age groups and sexes (both in veins and arteries), suggesting that the relationship between the ESRD-related complications and tissue levels of pentosidine were not confounded by these factors. The same trait has been studied before in the plasma of ESRD patients and similar results were reported.\textsuperscript{7,19}

Interestingly, a small percentage of ESRD patients (both with and without type 2 DM) did not have evident AGE accumulation in the artery and/or vein. This phenomenon may be explained by the new finding, soluble-receptor for advanced glycation end-product (sRAGE).\textsuperscript{20,21} The binding of AGE to the RAGE has been shown to cause the deterioration of various cell functions by increasing the intracellular oxidative stress and inflammation.\textsuperscript{20,21} However, recent studies have revealed that a truncated form of RAGE, sRAGE, which has the same ligand binding specificity as RAGE, can actually reverse the action of RAGE.\textsuperscript{20,21} However, to what extent sRAGE is present in an ESRD patient remains to be elucidated. Nevertheless, regular dialysis, good diabetic control and appropriate dietary intake of antioxidants as well as genetic predisposition (possibly involving deglycation enzymes) are some of the factors that may reduce or prevent AGE accumulation in blood vessels. Further investigation is required to understand fully the mechanism(s) involved in the ‘discrepancy’ of AGE accumulation in blood vessels.

Conclusion

Our results demonstrate for the first time the presence of pentosidine in both veins and arteries of ESRD patients using an immunohistochemical technique. The pentosidine accumulation in the vein was intensified in the presence of type 2 DM. The accumulation of AGE in the vein rather than the artery may be a better reflection of the extent of complications of ESRD. The findings in this study may contribute to a better understanding of the clinical manifestation and the association between AGEs, vein thrombosis and atherosclerosis induced by diabetes in ESRD patients.

Acknowledgements

This study was supported by the Intensification of Research in Priority Areas (IRPA) funding provided by the Ministry of Science, Technology and Environment (MOSTE) (grant number: IRPA 36-02-03-6020 (S&T)).

References


7 Suliman ME, Heimburger O, Barany P et al. Plasma pentosidine is associated with inflammation and malnutrition in end

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