Advanced glycation end-products and skin autofluorescence in end-stage renal disease: a review

Abstract: Chronic kidney disease (CKD), especially in its end stage, is marked by extremely high cardiovascular rates of morbidity and mortality; hemodialysis patients have a five-fold shorter life expectancy than healthy subjects of the same age. In CKD the metabolic products that accumulate in the body are so-called uremic toxins. These include advanced glycation end-products (AGE). AGE levels are markedly increased in CKD patients not only because of impaired excretion but also because of increased production. AGE formation has initially been described as a non-enzymatic reaction between proteins and glucose in the so-called Maillard reaction, but they are also more rapidly formed during oxidative stress and subsequent formation of reactive carbonyl compounds like (methyl)glyoxal. AGE accumulate in tissue where they cross-link with proteins, e.g., collagen, inducing tissue stiffening of blood vessels and skin. They may also interact with receptor of AGE (RAGE) and other receptors, which lead to activation of intracellular transduction mechanisms resulting in cytokine release and further tissue damage in CKD. The accumulation of AGE in the skin can be measured non-invasively using autofluorescence. The skin autofluorescence is a strong marker of cardiovascular mortality in CKD. The focus of this review is on the role of tissue and plasma AGE, and of skin autofluorescence as a proxy of tissue AGE accumulation, in the increase in cardiovascular disease in end stage renal disease (ESRD). This review will also present the possibility of reducing the AGE accumulation in ESRD patients using the following five methods: 1) use of low AGE peritoneal dialysis solutions; 2) use of advanced hemodialysis techniques; 3) use of AGE reducing drugs; 4) optimizing the nutrition of hemodialysis patients; and 5) renal transplantation.

Keywords: advanced glycation end-products; autofluorescence; cardiovascular disease; end-stage renal disease; hemodialysis; peritoneal dialysis; renal transplantation.

Introduction

Chronic kidney disease (CKD) encompasses a wide clinical spectrum of conditions, both in degree of renal function loss and in pathogenesis. The degree of renal function loss is currently divided in five CKD classes according to the level of the (estimated) glomerular filtration rate (eGFR). Mild CKD (CKD 1-2) is very common in the elderly but may also be present in some primary glomerular diseases with other more prominent features like proteinuria. More advanced loss of renal function finally resulting in end-stage renal disease (ESRD) or CKD 5 is less common, with atherosclerosis-associated glomerulosclerosis and diabetic kidney disease as the most prominent causes.

It is widely accepted that the focus of monitoring and treating CKD is on prevention of not only progressive loss of renal function, but also of the marked increase in cardiovascular disease (CVD) and death. The extremely high CVD rate in CKD 4-5 and dialysis patients forms the most impressive illustration: hemodialysis (HD) patients have a five-fold shorter life expectancy than healthy subjects of
the same age. The leading cause of death in patients with ESRD is CVD [1].

In CKD the metabolic products that accumulate in the body are so-called uremic toxins. These include advanced glycation end-products (AGE) [2]. AGE levels are not just markedly increased in CKD patients due to increased production; they are also due to impaired excretion [3]. Classically, AGE formation has been described as a non-enzymatic reaction between proteins and glucose in the Maillard reaction [4]. Glucose binds with proteins and forms chemically reversible early glycation products that undergo a slow and complex rearrangement eventually forming AGE. In addition to the formation from glucose-protein intermediates, AGE are also formed through lipid-derived intermediates, resulting in advanced lipoxidation products [5]. Furthermore, rapid formation of AGE via another pathway involving reactive carbonyl compounds like (methyl)glyoxal (so-called carbonyl stress) occurs during oxidative stress [6]. The glyoxalase system forms a defence mechanism against this pathway [7]. Finally, and of special interest in CKD, a source of AGE in humans is the intake of exogenous AGE from food and smoke [8]. When proteins with AGE linked to them are degraded to so-called glycation free adducts and glycation adduct residues of proteins, especially the former are subsequently excreted via the kidney [3]. In the case of renal failure, this excretion mechanism fails or is overridden. AGE will further accumulate in tissue where they cross-link with proteins, e.g., collagen, inducing tissue stiffening of blood vessels and skin. They may also interact with receptor of AGE (RAGE) and other receptors, via activation of intracellular transduction mechanisms resulting in cytokine release and further tissue damage in CKD [9].

The focus of this review is on the role of AGE, and of skin autofluorescence (SAF) as a proxy of tissue AGE accumulation, in the increase in CVD in more advanced stages of CKD Moreover, it will propose that the role of AGE and SAF is not restricted to the classical examples of AGE-associated CKD. For a long time, it has been well accepted that diabetic nephropathy is the classical model for demonstration of the pathogenic role of AGE. However, more recently it has become evident that AGE accumulation also has a role in progression of CKD and in CVD in non-diabetic kidney disease. This will be discussed in the following paragraphs.

Plasma advanced glycation end-products in chronic kidney disease

Galli et al. showed progressively higher levels of plasma pentosidine, assessed using high performance liquid chromatography comparing matched groups of healthy controls, CKD, and HD patients, respectively. Within the HD group, a negative correlation existed between the level of plasma pentosidine and dialysis frequency. They also proved that protein-leaking HD reduces the level of plasma pentosidine. In renal transplantation patients plasma pentosidine were similar to those in healthy controls [10].

Galli’s data are in line with those of other groups, showing that AGEs indeed accumulate in non-diabetic uremic patients, despite their normal serum glucose levels. In lower CKD classes, a relation between AGE levels, [Nε-carboxymethyl-lysine (CML)], and renal function is also evident, both in selected groups and in the community [11]. Among dialysis patients, both diabetics and non-diabetics have high plasma pentosidine and CML levels. Unfortunately, current HD techniques are only able to clear a portion of AGE from plasma [12]. Hou et al. propose that AGE and RAGE may contribute to amplification of inflammation in non-diabetic CKD [13]. Uribarri et al. showed that AGE intake contributes to the level of plasma AGE levels in CKD patients [14].

However, one should be aware that the impact and resulting damage of all these factors that accelerate plasma AGE levels increase are strongly dependent on the behavior of the molecules and tissues to which the AGE link. The degree of AGE accumulation and resulting damage will be more evident in tissues with slow turnover. In fact, in the commonly used plasma/serum compartment for taking AGE samples, AGE link to proteins with a high turnover rate. Several studies support that plasma/serum AGE may be a poor mirror of AGE dependent tissue damage [15–17]. Although the levels of plasma AGE are very high in ESRD patients, SAF as a mirror of dermal tissue AGE accumulation qualifies as a better marker of tissue damage than plasma AGE in these patients. Ueno et al. reported that in ESRD patients, both SAF and serum pentosidine correlated with carotid intima-media thickness, and SAF also inversely correlated with endothelial progenitor cells, while such a relation was absent for serum pentosidine. In multiple regression analysis, SAF, but not serum pentosidine and intima-media thickness, was related to endothelial progenitor cells [17]. Another example of this dissociation between AGE in plasma and long-lived tissues is a study by Hartog et al. in which plasma AGE and diastolic function were not related, while a strong relation existed with SAF. In this study the diastolic dysfunction of HD patients, assessed using ultrasound Doppler imaging, was related with the increase in SAF [15]. Furthermore in another study, serum CML did not correlate with CVD in a large group of HD and peritoneal dialysis (PD) patients, whereas SAF did [16].
The measurement of skin advanced glycation end-products using skin autofluorescence

SAF can be measured with the AGE Reader (DiagnOptics Technologies BV, Groningen, The Netherlands). The AGE Reader is a desk-top device that uses the characteristic fluorescent properties of certain AGE to quantify the level of AGE accumulation in the skin. In short, the AGE Reader illuminates a skin surface of 4 cm² guarded against surrounding light, with an excitation light source with a peak excitation of 370 nm (ultraviolet A). Emission light (fluorescence in the wavelength of 420–600 nm) and reflected excitation light (with a wavelength of 300–420 nm) from the skin is measured with a spectrometer. SAF is calculated as the ratio between the emission light and reflected excitation light, multiplied by 100 and expressed in arbitrary units (AU). In validation studies using skin biopsies taken from the site of SAF measurements, a strong correlation was found between SAF and the skin contents of the fluorescent AGE, pentosidine, as well as with the non-fluorescent AGE, CML, and Nε-(carboxyethyl)lysine (CEL) [18–20]. One of these validation studies was performed in HD patients [19]. Furthermore a combined analysis performed on the three studies showed that 76% of the variance in SAF may be explained by the associated pentosidine levels [21]. Meerwaldt et al. showed an intra-individual Altman error percentage of 5.03% with SAF measurements taken over one single day, and an Altman error percentage of 5.87% for seasonal variation [18]. Similar results were reported in stage III CKD patients by McIntyre et al. [22].

Skin autofluorescence in chronic kidney disease

As previously mentioned above, the behavior and levels of AGE in the human body are not only dependent on factors like glycemic and oxidative stress as accelerators of formation of AGE, but also on the presence of intact mechanisms for excretion of AGE free adducts and peptides, which are mainly excreted by the kidney. Thus, loss of renal function in progressive CKD strongly affects these excretion mechanisms, and partly explains the increase in plasma and tissue AGE levels in CKD. SAF increased as eGFR decreased and was related to CVD history in CKD patients [23]. In diabetic nephropathy, increased glycemic stress may contribute to higher plasma and tissue AGE levels, while in CKD, low-grade oxidative stress is commonly present and enhances AGE formation regardless of diabetic condition.

Skin autofluorescence in end-stage renal disease with renal replacement treatment

Additional important factors that increase SAF once renal replacement treatment has started are derived from factors associated with the renal replacement treatment itself, such as the dialysis vintage and the length and amount of glucose exposure in PD patients. McIntyre et al. reported that there is not a difference in the level of SAF in hemodialysis and peritoneal patients [24]. Previously, we found a connection between SAF and the presence of diabetes in HD patients [19, 25]. Furthermore, the level of SAF of diabetic HD patients is higher than that in diabetic patients with similar age that are not on dialysis [26]. This indicates that ESRD is a separate contributor of AGE accumulation in these patients. Further support has been previously published showing the correlation between SAF and HD vintage [19, 25].

Skin autofluorescence is a strong predictor of cardiovascular mortality in chronic kidney disease

The Diabetes Control and Complications Trial-Epidemiology of Diabetes Interventions and Complications (DCCT-EDIC) research, has revealed that the reduction in the risk of progressive nephropathy resulting from intensive therapy in patients with type 1 diabetes persist for at least several years after the end of treatment, despite increasing hyperglycemia. In addition, intensive therapy during the DCCT also reduced the risk of cardiovascular events by about 50% in type 1 diabetic patients 11 years after the end of the trial. In another DCCT-EDIC substudy on biochemically assessed AGE levels in skin biopsies, the higher levels of AGE were found to be independent predictors of worse renal and cardiovascular outcome [27]. These clinical studies strongly suggest that so-called ‘glycemic memory’ causes chronic abnormalities in diabetic vessels that are not easily reversed, even by subsequent, relatively good control of blood glucose. Among various biochemical pathways implicated in diabetic vascular complications, the process of formation and accumulation of AGE and their mode of action are most compatible with the theory ‘glycemic memory’ [28].
McIntyre et al. reported in a cohort of 1707 patients with CKD class 3 that a large number of cardiovascular and renal risk factors were associated with SAF, such as eGFR, hemoglobin, age, smoking, total cholesterol, diastolic blood pressure, c-Reactive Protein, waist to hip ratio, albuminemia, pulse wave velocity, diabetes and uremic acid [22]. In other studies, SAF also tends to be associated either to risk factors of cardiovascular mortality or to direct evidence of cardiovascular damage. Carotid artery intimal-medial thickness was correlated positively with SAF [29]. Skin autofluorescence was also inversely and independently associated with circulating endothelial progenitor cells in ESRD patients [17]. It has been reported that endothelial progenitor cells have the ability to repair cardiovascular damage [30]. In CKD children, tissue accumulation of AGE was observed, aggravated as eGFR declined and related to early cardiovascular changes and some biochemical CVD risk markers [31].

The clinically most relevant data on the impact of the accumulation of AGE on the cardiovascular system in ESRD patients come from studies that investigated the influence of SAF on cardiovascular morbidity and mortality. Our group was the first to shown that SAF was strong and independent predictor of overall and cardiovascular mortality in ESRD patients [19]. Increased SAF was also related to the presence of CVD in Asian (non-Caucasian) HD patients [23]. Also, a single point SAF measurement is a good predictor of mortality in diabetic patients. Diabetic patients have a higher risk for increased AGE accumulation and mortality than healthy persons [26]. Nevertheless, the presence of diabetes in HD patients did not confer an increased hazard in the multivariate Cox Regression analyses, which was supported by previous studies [19, 32]. The explanation for this phenomenon can be that the dialysis procedure interferes much more with the morbidity of the patients and the effect of diabetes is only marginal.

Jiang et al. recently reported on SAF levels a large cohort of 2388 maintenance dialysis patients (613 PD and 1775 HD) [16]. SAF was measured with the AGE Reader. In PD, SAF was strongly correlated with the duration of PD and glucose exposure dose, and independently associated with CVD. In multivariate analysis glucose exposure dose and SAF were the strongest risk factors for CVD in PD, after adjustment for age, gender, and other classic- or uremic-related risk factors. According to Receiver Operator Characteristic (ROC) curve for presence of CVD, the best cut-off point of SAF was determined to be 2.75 AU. Remarkably, this level is the same as used by Lutgers et al. in models for CVD risk reclassification for elderly type 2 diabetes patients [33].

Opportunities for reversibility of the advanced glycation end-products accumulation

Use of low advanced glycation end-products peritoneal dialysis solutions

During manufacturing, heat is commonly used for sterilization of the PD solutions causing most standard glucose-based PD solutions to contain AGE [34]. The duration of PD and the glucose exposure dose are independently associated with the level of SAF in PD patients. Moreover, as reported above, the glucose exposure dose and SAF are one of the strongest risk factors for CVD in PD [16]. The substitution of glucose with other substances such as icodextrin, a starch-derived branched water-soluble glucose polymer has led to even higher levels of SAF in the PD patients [35]. The problem of increased AGE accumulation as a consequence of the PD treatment might be resolved by use of PD solutions with neutral pH and low AGE content. The use of PD solutions with neutral pH and low AGE content results in lower AGE accumulation, less peritoneal membrane fibrosis and vascular sclerosis [36]. Such PD solutions are available for more than 10 years on the market; however, the first PD solution of this type has only been just recently approved by the FDA in the USA [34]. Evidence exist that use of PD solutions with neutral pH and low AGE content can result in significant improvement in patient and technique survival without any measurable change in peritonitis incidence [37].

Use of advanced hemodialysis techniques

The HD vintage in several studies has been proven to be a contributor to higher levels of SAF, suggesting that ESRD and the HD treatment itself can contribute to AGE formation [19, 25]. The use of different HD techniques to ameliorate the AGE accumulation in HD patients has been investigated for some time. A comparison of the removal of free plasma AGE and AGE peptides by low, high, and super flux HD showed that all modalities can effectively remove free plasma AGE during a single HD session. However, plasma protein-bound AGE did not decrease during a dialysis sessions, neither with high flux nor with low flux HD membrane. Super flux has been suggested to be a modality capable of reducing AGE peptides in the long-term [38–40]. Another study showed that long-term HD with protein-leaking membrane also reduces...
predialysis protein-bound and free plasma AGE levels [41]. Also, single dialysis session with on-line hemodiafiltration (HDF) results in lower plasma AGE than with those treated with conventional low and high flux HD. The long-term use of HDF provides lower predialysis plasma AGE levels compared with those treated with low and high flux HD [42].

Other factors can reduce the level of plasma AGE in HD patients. The material from which the dialyzer is made has an influence on the level of plasma AGE. The use of polysulfone membranes appear to result in lower levels of plasma AGE than using non-polysulfone membranes, such as modified or unmodified cellulose membranes [43]. Also, the use of Vitamin E-coated HD membrane resulted in reduction of plasma AGE levels [44]. The use of ultrapure HD fluid appears to result in lower plasma levels in HD patients. Several studies showed that ultrapure dialysate decreases plasma levels of AGE in HD patients [45, 46]. The reduction of plasma AGE by use of ultrapure dialysate is achieved regardless of dialyzer membrane type [47]. The mechanisms of the reduction of plasma AGE by use of ultrapure dialysate is still unknown. Furthermore, one study demonstrated that a daily HD (2 h, 6 times/week) regimen can effectively lower the levels of plasma AGE observed in a standard HD (4 h, 3 times/week) [48].

Therefore, we can conclude that reduction of the AGE accumulation can be achieved by using advanced HD techniques which use membranes that have large pores such as super flux, HDF or protein leaking HD. The reason is that these techniques are able to filter protein-bound AGE. Also, the use of more biocompatible membranes and ultra-pure HD fluid can reduce the AGE accumulation, most likely by reducing the level of inflammation and immune response. The use of more frequent HD regime such as daily or home dialysis can achieve better removal of plasma AGE and thus lower AGE accumulation. It would be important to obtain clinical data on the effects of such alternative HD techniques on CVD events and mortality. So far, such data are still lacking.

**Use of advanced glycation end-products reducing drugs**

The use of many herbal medicines and experimental drugs has shown a reduction of AGE accumulation [49–56]. Also, many conventional drugs are useful in the reduction of AGE. SAF levels can be reduced by using angiotensin receptor blockers and statins in PD patients [35]. A calcium channel blocker, azelidipine, has shown to be able to reduce the level of plasma AGE in hypertensive CKD patients [57]. In a single-center, randomized, 2-month, open-label, intention-to-treat, crossover study, sevelamer carbonate, a calcium-based phosphate binding drug, was used. Sevelamer blocks the absorption of ingested cytotoxic AGE in the gut. Sevelamer reduced the level of plasma AGE, lipids, and inflammation in diabetic CKD patients in absolute and relative terms when compared with the standard treatment of calcium carbonate [58].

It appears that there are many promising drug treatments and what is even better that already existing approved drug, like sevelamer can be used in the reduction of the AGE accumulation in patients with CKD.

**Optimizing the nutrition of hemodialysis patients**

Some controversy remains on the relationship between AGE intake and plasma AGE in humans [59–62]. Uribarri et al. [14] first showed that AGE intake correlates with circulating AGE levels in renal failure patients. However, a single point measurement of SAF did not correlate with the AGE intake of elderly healthy subjects [63]. The relationship between body mass index (BMI) and SAF was also studied. It was found that a single measurement of SAF had a positive correlation with BMI in healthy subjects [64], whereas BMI and SAF did not correlate in HD patients [19].

There is still controversy if a reduction in AGE accumulation can be achieved by reduction of AGE food intake in HD patients. Also, it appears that the nutrition state measured by BMI has some role in the accumulation of AGE in HD patients.

**Renal transplantation**

Renal transplantation in theory should be the ultimate treatment of CKD and therefore result in total reversibility of AGE accumulation. Indeed, the levels of protein-bound pentosidine in plasma in renal transplant patients are at the level of healthy subjects [10]. However, our findings showed that SAF of renal transplant patients are still increased when compared to patients with the same age. Also, the SAF was associated with several risk factors for CVD and chronic renal transplant dysfunction in patients with renal transplant [65]. This indicates that a complete reversibility may not easily be obtained because AGE accumulation in skin and other tissues with slow turnover is not
easily reversible. Moreover, on-going oxidative stress due to immunosuppressive treatment, episodes of rejection, and infections may be on-going stimuli for continued AGE accumulation. A latter study showed that SAF remains a very strong determinant of (still markedly increased) mortality and graft function loss [66]. Thus, renal transplantation is remarkably an incomplete solution, and a dire need for slow tissue AGE reversibility remains.

Clinical perspectives

SAF is a proven marker of CVD mortality in CKD. Nevertheless, clinical nephrologists often state that cardiovascular risk in CKD patients is so high that new risk markers will not change their already intensive treatment policy, and thus SAF or AGE markers have little to add. This may indeed be true for ESRD or HD/PD patients, but it is not valid for the quantitatively important groups of patients with CKD. Availability of tools to select persons in the very prevalent lower CKD classes but at the highest CVD risk is, in our opinion, of major importance in order to focus the conventional CVD preventive treatment. Secondly, newer approaches, such as illustrated by the sevelamer, may still make a difference even in CKD stage IV-V and HD/PD patients and renal transplant patients. Thirdly, the patients in CKD stage IV-V with the highest SAF levels may be those that qualify for priority renal transplant. And finally, the fact that SAF is a very simple tool to provide more insight into the role of AGE (dependent mechanisms) in development of CVD also counts.

Future prospects

More frequent and repeated measurement of SAF in CKD patients can give insight on many factors that influence the AGE accumulation in these patients. In earlier stages of CKD, more focus is needed on therapeutic options aiming at the reduction of AGE accumulation. An obvious possible line of further research can be the influence of drugs such as sevelamer on the accumulation of AGE measured by repeated SAF measurement. Also, the influence of different diets like low AGE diet or optimal nutrition level on the rate of AGE accumulation in early stages of CKD should be investigated. In ESRD, the influence of different renal substitution methods such as super flux HD, HDF, protein leaking HD, daily and home HD or use of ultrapure fluid on the rate of increase of SAF warrant further investigation.

Conclusions

AGE, especially those that are tissue-bound, are not just an important marker of patients’ outcome in CKD but also a major contributor to the underlying condition. Therefore, they should be monitored regularly and carefully. Today, there are many methods of tissue AGE reduction, like use of modern PD and HD techniques, AGE reducing drugs, dietary interventions and renal transplantation.

Conflict of interest statement

Authors’ conflict of interest disclosure: Andries J. Smit and Reindert Graaff are founders and shareholders of DiagnOptics Technologies BV, The Netherlands, which develops the AGE reader (http://www.diagnoptics.com). The other authors have no conflicts to declare.

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