

# Advanced Oxidation Protein Products and Advanced Glycation End Products in Children and Adolescents With Chronic Renal Insufficiency

Katarína Šebeková, MD, DSc,<sup>★</sup> Kristína Klenovicsová, MD, PhD,<sup>†</sup>  
Juliana Ferenczová, MD, PhD,<sup>‡</sup> Juraj Hedvig, MD, PhD,<sup>‡</sup>  
L'udmila Podracká, MD, PhD,<sup>‡</sup> and August Heidland, MD, PhD<sup>§</sup>

**Objective:** Advanced oxidation protein products (AOPPs) represent dityrosine-containing cross-linked protein modifications formed mainly via myeloperoxidase reaction, supposed to accelerate the uremia-associated atherogenesis and renal fibrosis.

**Design, Subjects, and Main Outcome Measures:** In a cross-sectional study, we investigated the accumulation of AOPPs and advanced glycation end product (AGE)-specific fluorescence corrected for albumin in children and adolescents with chronic renal failure (CRF,  $n = 42$ ), end-stage renal disease (ESRD,  $n = 12$ ), kidney transplanted patients (Tx,  $n = 16$ ), and age-matched healthy controls ( $n = 38$ ).

**Results:** AOPP levels were 2.4-fold higher in the CRF and ESRD patients, and 1.6-fold higher in the transplanted subjects when compared with the controls ( $P < .001$ ). In comparison with healthy controls, AGE levels rose 2-fold in the CRF, 7-fold in the ESRD, and 5-fold in the kidney transplanted children and adolescents, ( $P < .001$ ). Patients with cardiovascular affliction presented with higher AGE levels than those without diagnosed cardiovascular disease ( $P < .02$ ). In patients with stabilized renal function, AOPP and AGE levels did not change significantly during 12 months.

**Conclusion:** Pattern of accumulation of AOPP and AGE in children and adolescents with chronic renal disease differs. Accelerated rise in AOPP levels in some children and adolescents in predialysis stage of chronic renal insufficiency, inadequate to deterioration of renal function, might require further attention.

© 2012 by the National Kidney Foundation, Inc. All rights reserved.

<sup>★</sup>Institute of Molecular BioMedicine, Comenius University, Bratislava, Slovakia.

<sup>†</sup>Department of Clinical and Experimental Pharmacotherapy, Slovak Medical University, Bratislava, Slovakia.

<sup>‡</sup>Department of Pediatrics and Adolescents, P.J.Šafárik University, Košice, Slovakia.

<sup>§</sup>Department of Internal Medicine, University of Wuerzburg, Wuerzburg, Germany.

**Funding Support:** This study was supported by the Agency of the Ministry of Education of the Slovak Republic for the Structural Funds of the European Union, Operational Program Research and Development (Contract No. 034/2009/2.1/OPR&D).

Address reprint requests to Katarína Šebeková, MD, DSc, Institute of Molecular BioMedicine, Comenius University, Sasinkova 4, 811 08 Bratislava, Slovakia. E-mail: [kata.sebekova@gmail.com](mailto:kata.sebekova@gmail.com)

© 2012 by the National Kidney Foundation, Inc. All rights reserved.

1051-2276/\$36.00

doi:10.1053/j.jrn.2011.10.022

ADVANCED OXIDATION PROTEIN products (AOPPs) are dityrosine-containing and cross-linking protein products formed mainly during oxidative burst of monocytes via myeloperoxidase reaction.<sup>1,2</sup> Plasma AOPPs are mainly carried by albumin ("Alb," hereafter).<sup>1</sup> In the adults with chronic renal insufficiency (CRI), AOPPs accumulate proportionally with the decline in renal function.<sup>1,3,4</sup>

AOPPs are not innocent end products of activation of macrophages: they are biologically active molecules capable of mediating oxidative stress and respiratory burst in monocytes.<sup>1</sup> They are capable of inducing/accelerating renal injury: in the tubular cells, via the CD36 pathway,<sup>5</sup> and in vascular endothelial cells, via a receptor for advanced glycation

end-products (RAGE)-mediated signaling pathway.<sup>6</sup> In the rat model of streptozotocin-induced diabetes, they promote renal inflammation through activation of renal NADPH oxidase.<sup>7</sup> In the remnant kidney model, AOPPs induce renal fibrosis via a redox-sensitive inflammatory pathway.<sup>8</sup> Acting as potent high-density lipoprotein receptor antagonists, AOPPs may contribute to the abnormal composition of high-density lipoprotein, and thus to high cardiovascular risk factor in patients with CRI.<sup>9</sup>

Decrease in renal function is also associated with the accumulation of advanced glycation end products (AGEs),<sup>10</sup> formed either via Maillard reaction or, alternatively, under the conditions of enhanced oxidative and carbonyl stress.<sup>11</sup> AGEs are supposed to be involved directly or indirectly in pathogenesis of various diseases, among others are chronic renal failure and its complications.<sup>10</sup>

At least 2 independent groups studied the accumulation of AGEs in children and adolescents with CRI.<sup>12,13</sup> To our best knowledge, the accumulation of AOPPs in children and adolescents has not been so far reported.

We studied the pattern of AOPP accumulation in children and adolescents with deteriorated renal function and compared it with that of the AGE. We determined AGE-specific fluorescence of plasma, as it reflects a group of substances, not a single one: accumulation of AGEs and AOPPs has been simultaneously studied in adult patients with CRI,<sup>14</sup> and formerly, we compared AGE accumulation pattern in children and adolescents and adults with CRI.<sup>12</sup>

## Material and Methods

The study was carried out according to the Declaration of Helsinki, after the approval of the protocol by the Ethics Board of PJŠ University Medical Faculty (Košice, Slovakia). Written informed consent was obtained from the participants or their legal representatives.

### Patients

We investigated 42 children and adolescents (12 females/30 males) with chronic kidney disease (CKD) who thus far did not require dialysis, 12 children and adolescents (8 females/4 males) with end-stage renal disease (ESRD) on renal replacement therapy with dialysis, and 16 children

and adolescents (9 females/7 males) who had successful kidney transplantation 5.6  $\pm$  5.3 years ago. Age range of the subjects was 1 to 24 years. The underlying diseases were congenital anomalies of the kidney and urinary tract ( $n = 12$ ), hereditary nephropathy ( $n = 18$ ), and acquired nephropathy ( $n = 40$ ).

Eight patients were hemodialyzed and 4 were treated by peritoneal dialysis for a mean of 3.0  $\pm$  2.6 years. Transplanted patients were treated with triple combination of prednisone/cyclosporine A or tacrolimus/mycophenolate mofetil. Twenty-two CKD patients with nephrotic syndrome received prednisone. Twenty-four patients (8 from each group) presented with uremia-associated cardiovascular disease (CVD, 42% suffered from hypertension; 7% and 16%, from systolic and diastolic dysfunction, respectively; 35% had left ventricle hypertrophy; and in 2 patients, intravascular calcification had been diagnosed). In 8 ESRD patients and 7 transplanted patients, AGE and AOPP levels were reevaluated after 12 months of follow-up.

Thirty-eight healthy age- and sex-matched children and adolescents served as the control group. Basic characteristics of the cohorts are given in Table 1.

## Methods

Blood was withdrawn from fasting subjects, except for hemodialyzed patients whose blood was sampled before dialysis. Plasma creatinine, Alb, and C-reactive protein (CRP) were determined by standard laboratory methods. Creatinine clearance was calculated.<sup>15</sup> Plasma samples were frozen at  $-80^{\circ}\text{C}$  until analysis of AOPPs (photometrically, according to Witko-Sarsadt et al.),<sup>1</sup> and AGE-specific fluorescence according to Munch et al.<sup>16</sup>

## Statistical Analyses

Data were tested for normality and equality of variance and were compared using analysis of variance with post hoc Scheffe test, or Kruskal-Wallis test with Mann-Whitney  $U$  test, as appropriate. Paired data were compared using Student  $t$  test or Wilcoxon signed ranks test. Spearman or Pearson correlation coefficients were calculated. Multivariate analysis (general linear model) was performed. Chi-square was used to compare categorical data. Data are given as median, mean  $\pm$  SD.  $P < .05$  was considered as significant.

**Table 1.** Basic Characteristics of the Subjects

	Controls (n = 38)	CKD (n = 42)	ESRD (n = 12)	Kidney Tx (n = 16)	P (ANOVA; Kruskal– Wallis)
Age (years)	14.0; 13.8 ± 4.9	13.5; 13.5 ± 7.1	14.5; 13.4 ± 4.3	16.5; 16.7 ± 4.0	n.s.
Subjects presenting with CVD	0	8	8*	8*	.009 <sup>chi</sup>
Creatinine clearance (ml/minute/1.73 m <sup>2</sup> )	112; 111 ± 17	102; 97 ± 35 <sup>†</sup>	12; 13 ± 4 <sup>‡</sup>	78; 85 ± 22 <sup>‡,§</sup>	.001
Albumin (g/L)	48; 47 ± 5	46; 43 ± 9	41; 37 ± 7 <sup>‡</sup>	45; 47 ± 9 <sup>†</sup>	.001
C-reactive protein (mg/L)	ND	2.1; 2.5 ± 2.8	1.9; 5.4 ± 7.2	0.5; 1.2 ± 1.5 <sup>§</sup>	.027
AOPP/albumin (μmol/g)	1.2; 1.7 ± 1.6	2.1; 4.1 ± 4.8 <sup>¶</sup>	3.4; 4.0 ± 2.1 <sup>¶</sup>	1.9; 2.8 ± 2.2	.001
AGE/albumin (AU/g)	3.3; 3.5 ± 1.0	6.0; 6.9 ± 3.2 <sup>§,¶</sup>	22.2; 22.6 ± 6.9 <sup>‡</sup>	13.4; 16.4 ± 9.7 <sup>‡,§</sup>	.001

CKD, patients with chronic renal disease treated conservatively; ESRD, patients with end-stage renal disease on dialysis; Tx, transplantation; CVD, cardiovascular disease; AOPP, advanced oxidation protein products; AGE, advanced glycation end products; AU, arbitrary units; n.s., not significant; chi, chi-square (frequencies calculated only between the patients' groups); ND, not determined; ANOVA, analysis of variance.

Data are given as median; mean ± standard deviation.

\**P* < .05 versus CKD group.

<sup>†</sup>*P* < .01 versus ESRD.

<sup>‡</sup>*P* < .01 versus controls.

<sup>§</sup>*P* < .05 versus ESRD.

<sup>¶</sup>*P* < .05 versus controls.

Statistical program SPSS v.16 (IBM SPSS Inc., USA) was used.

## Results

### Cohort Characteristics

The groups were comparable by age. Both genders were presented proportionally (*p*<sub>chi</sub>: n.s.) (Table 1). ESRD patients presented with the lowest creatinine clearance values. Renal function of CKD and transplant patients did not differ significantly. ESRD patients displayed the lowest albuminemia (*P* < .01 vs. controls) and the highest CRP levels (*P* < .05 vs. transplant patients) among the groups.

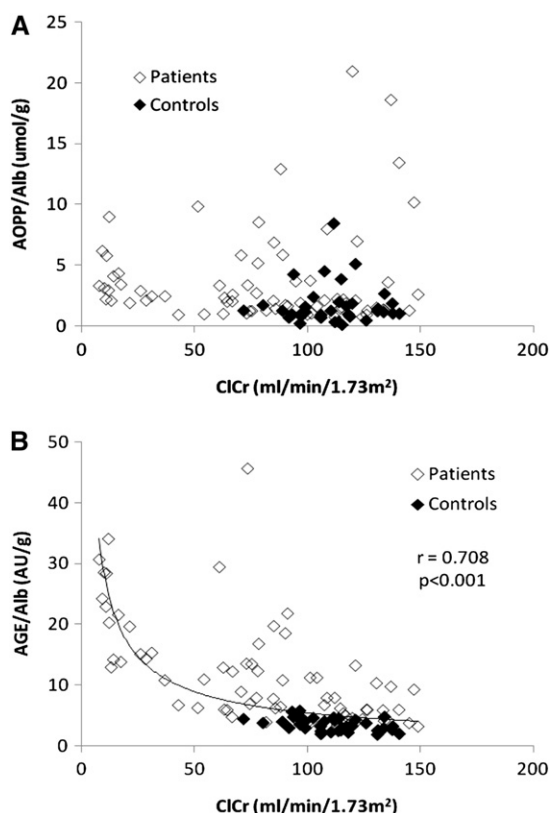
### AOPP and AGE Levels

Because the groups differed significantly in albuminemia, AOPP levels and AGE-specific fluorescence of plasma were corrected for Alb (Table 1). Plasma AOPP/Alb ratio was 2.5-fold higher in CKD and ESRD patients in comparison with healthy controls (*P* < .05). Plasma AGE/Alb ratio was 2-fold higher in CKD patients and 6.5-fold in the ESRD patients if compared with the controls (*P* < .05 and *P* < .01, respectively). Kidney transplanted patients presented with 1.6-fold elevated AOPP/Alb and 5-fold higher AGE/Alb ratios if compared with the controls. Thus, in

the transplant patients, the improved renal function was reflected by approximately 30% decline in both, AOPP/Alb ratios and AGE/Alb ratios, in comparison with levels observed in the ESRD patients. This decline was significant only in case of AGE/Alb ratio because of high interindividual variability in AOPP/Alb ratio. AOPP/Alb ratio showed no significant relationship with age, CRP, or creatinine clearance (Fig. 1A). AGE/Alb ratio increased with age (*r* = 0.203, *P* = .035) and showed a significant inverse relationship with creatinine clearance (Fig. 1B). Although the AGE/Alb and AOPP/Alb ratios correlated significantly (*r* = 0.506; *P* = .001), AOPPs/Alb showed no significant correlation with the age or renal function.

### Impact of Presence of CVD

Patients manifesting CVD and those without signs of CVD were comparable by age (16.0; 14.7 ± 5.2 vs. 14.0; 14.0 ± 6.7 years, *P* = 0.67). Subjects presenting with CVD had significantly higher plasma AGE/Alb ratio in comparison with those not affected with CVD (12.7; 15.1 ± 9.2 vs. 7.4; 10.1 ± 7.8 AU/g; *P* < 0.019), higher CRP concentration (2.5; 4.4 ± 5.6 vs. 1.4; 1.9 ± 2.2 mg/L; *P* < .037), and lower creatinine clearance (65; 61 ± 43 vs. 93; 90 ± 39 mL/minute/1.73 m<sup>2</sup>, *P* < .006). General linear



**Figure 1.** (A) Relationship between plasma AOPP to Alb ratio and CrCl in the children and adolescents. Relationship not significant. (B) Relationship between plasma AGE-specific fluorescence to Alb ratio and CrCl in the infants and adolescents. AOPP, advanced oxidation protein product; Alb, albumin; CrCl, creatinine clearance; AGE, advanced glycation end product.

model (CVD entered as a cofactor; CRP and creatinine clearance, as covariates) suggested that the higher AGE/Alb ratio in patients with CVD is solely on account of their more deteriorated renal function ( $F = 14.5$ ;  $P < .001$ ,  $R^2 = 0.37$ ). The groups did not differ significantly in AOPP/Alb ratio or albuminemia (data not given).

### Follow-up Study

Pre- and post-follow-up creatinine clearance, albuminemia, concentration of CRP, AOPP/Alb, and AGE/Alb ratios did not differ significantly at baseline and after follow-up in either group (data not given). In the ESRD patients, creatinine clearance changed by  $-2.1 \pm 3.1$  mL/minute/1.73 m<sup>2</sup>/year, and in the transplanted patients, by  $-6.6 \pm 15.9$  mL/minute/1.73 m<sup>2</sup>/year

(non significant [n.s.]). Changes in AOPP/Alb ( $-1 \pm 2$  vs.  $2 \pm 5$  μmol/g/year, respectively; n.s.), and in AGE/Alb ( $-2 \pm 5$  vs.  $-3 \pm 8$  AU/g/year, respectively; n.s.) did not differ significantly.

### Discussion

To the best of our knowledge, this is the first report on the accumulation of AOPP in children and adolescents with CRI. Our data suggest that in children and adolescents, pattern of AOPP accumulation differs from that in the adults,<sup>3,14,17</sup> and from that of the accumulation of AGEs.

In the adults, AOPPs accumulate proportionally to decline in renal function.<sup>3,4</sup> Circulating AOPPs rise by about 1.7-fold in predialysis patients and by about 2.5-fold in dialyzed patients, when compared with healthy controls.<sup>3,4</sup> We confirmed that AOPPs accumulate also in children and adolescents with decreased renal function. However, this rise is not proportional to residual renal function. Conservatively treated children and adolescents displayed comparable rise in AOPPs as compared with those on dialysis (about 2.5-fold in comparison with healthy controls). Kidney transplanted adults with normal renal function showed only insignificant 10% elevation of AOPP, whereas those with posttransplant CRI displayed about 1.4-fold higher levels.<sup>3,14,17</sup> Transplanted children and adolescents with satisfactory renal function presented with 1.6-times higher AOPPs than healthy controls. These differences could not be explained by differences in albuminemia or microinflammation. However, we observed a great interindividual variability in AOPP/Alb ratio. On the other hand, our follow-up study showed that in dialyzed and kidney-transplanted children and adolescents with stabilized renal function, plasma AOPP and AGE levels do not change significantly over 12-month period. This data fit to those of Furuya et al.<sup>3</sup> in the adults on peritoneal dialysis, showing that AOPP and AGE levels rise over 12-month period significantly only in patients in whom residual renal function deteriorates.<sup>3</sup> To the best of our knowledge, data from regular serial measurement of AOPPs over time in patients or healthy controls are not available. These data, particularly in correlation with other markers of inflammation, could elucidate whether elevated AOPPs reflect an acute or chronic event.

Moreover, in children and adolescents AOPP accumulation showed a different pattern from that of the accumulation of AGEs. In contrast to AOPPs, AGEs rose proportionally with declining renal function. Thus, our data seem to support the conclusions of Kalousova et al.<sup>18</sup> that AGEs may serve more as a marker of chronic damage, whereas AOPPs may better reflect acute changes such as microinflammation and/or oxidative stress.

Studies in adults with CKD show close association of elevated AOPPs with occurrence of atherosclerosis. AOPP levels independently predict occlusive atherosclerotic cardiovascular events in predialysis patients.<sup>19</sup> In predialysis and dialyzed patients, AOPPs strongly correlate with carotid artery intima-medial thickness.<sup>4,14</sup> In the nondiabetic peritoneally dialyzed patients, AOPPs are independent predictors of endothelial dysfunction.<sup>14,20</sup> In our study, presence of CVD was not reflected by elevated AOPP levels, despite the observed rise in AGE/Alb ratio and CRP levels. Because we studied children and adolescents, the majority of our patients with CVD suffered from hypertension and left ventricular hypertrophy, and calcifications were revealed only in 2 patients. We suppose that in our patients, CVD did not progress to overt atherosclerosis, which is associated with rise in AOPPs. Even the incipient atherosclerosis might have not been manifested long enough to alter the AOPP levels significantly. This assumption is supported also by the results of multivariate analysis, suggesting that decline in renal function was the single determinant of elevated AGE/Alb ratios in our CVD patients. However, we expect that other noninvasive methods, such as pulse wave velocity measurement or carotid artery intima-medial thickness, could provide more exact information on the potential association of elevated AOPPs with CVD in these patients.

Our study shows that the pattern of accumulation of AOPPs in children and adolescents differs from that of AGEs. Because our study is cross-sectional in nature, it would be preliminary to conclude that the magnitude and the pattern of AOPP accumulation differ in the children and adolescents from those in the adults. To confirm or exclude this assumption, more studies in children and adolescents are needed. High interindividual variability of AOPPs in our CKD children and adolescents, together with a relative stability of the

levels over time if deterioration of renal function is slow, raises the question whether AOPPs might serve as potential indicators of clinical situation requiring attention. AOPPs are suggested as important mediators of renal fibrosis<sup>8</sup> and the uremia-associated accelerated atherogenesis.<sup>4,14,19</sup>

## Practical Application

Accelerated rise in AOPP levels in some children and adolescents in predialysis stage of CRI, inadequate to deterioration of renal function, might require further attention. It remains to be elucidated whether AOPP determination could serve as a practical and clinically relevant tool to estimate or forecast the risk of accelerated renal fibrosis and/or atherogenesis.

## References

1. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int.* 1996;49:1304-1313.
2. Witko-Sarsat V, Friedlander M, Nguyen KT, et al. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol.* 1998;161:2524-2532.
3. Furuya R, Kumagai H, Odamaki M, Takahashi M, Miyaki A, Hishida A. Impact of residual renal function on plasma levels of advanced oxidation protein products and pentosidine in peritoneal dialysis patients. *Nephron Clin Pract.* 2009;112:c255-c261.
4. Yang XB, Hou FF, Wu Q, et al. Increased levels of advanced oxidation protein products are associated with atherosclerosis in chronic kidney disease. *Zhonghua Nei Ke Za Zhi.* 2005;44:342-346.
5. Iwao Y, Nakajou K, Nagai R, et al. CD36 is one of important receptors promoting renal tubular injury by advanced oxidation protein products. *Am J Physiol Renal Physiol.* 2008;295:F1871-F1880.
6. Guo ZJ, Niu HX, Hou FF, et al. Advanced oxidation protein products activate vascular endothelial cells via a RAGE-mediated signaling pathway. *Antioxid Redox Signal.* 2008;10:1699-1712.
7. Shi XY, Hou FF, Niu HX, et al. Advanced oxidation protein products promote inflammation in diabetic kidney through activation of renal nicotinamide adenine dinucleotide phosphate oxidase. *Endocrinology.* 2008;149:1829-1839.
8. Li HY, Hou FF, Zhang X, et al. Advanced oxidation protein products accelerate renal fibrosis in a remnant kidney model. *J Am Soc Nephrol.* 2007;18:528-538.
9. Marsche G, Frank S, Hrzenjak A, et al. Plasma-advanced oxidation protein products are potent high-density lipoprotein receptor antagonists in vivo. *Circ Res.* 2009;104:750-757.
10. Heidland A, Šebekova K, Schinzel R. Advanced glycation end products and the progressive course of renal disease. *Am J Kidney Dis.* 2001;38(4 suppl. 1):S100-S106.
11. Miyata T, Kurokawa K, van Ypersele de Strihou C. Relevance of oxidative and carbonyl stress to long-term uremic complications. *Kidney Int.* 2000;76(suppl):S120-S125.

12. Šebeková K, Podracká L, Blažíček P, Syrová D, Heidland A, Schinzel R. Plasma levels of advanced glycation end products in children with renal disease. *Pediatr Nephrol.* 2001;16:1105-1112.
13. Misselwitz J, Franke S, Kauf E, John U, Stein G. Advanced glycation end products in children with chronic renal failure and type 1 diabetes. *Pediatr Nephrol.* 2002;17:316-321.
14. Kocak H, Gumuslu S, Ermis C, et al. Oxidative stress and asymmetric dimethylarginine is independently associated with carotid intima media thickness in peritoneal dialysis patients. *Am J Nephrol.* 2008;28:91-96.
15. Schwartz GJ, Brion LP, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. *Pediatr Clin N Am.* 1987;34:571-590.
16. Munch G, Keis R, Wessels A, et al. Determination of advanced glycation end products in serum by fluorescence spectroscopy and competitive ELISA. *Eur J Clin Chem Clin Biochem.* 1997;35:669-677.
17. Antolini F, Valente F, Ricciardi D, Baroni M, Fagugli RM. Principal component analysis of some oxidative stress parameters and their relationships in hemodialytic and transplanted patients. *Clin Chim Acta.* 2005;358:87-94.
18. Kalousová M, Zima T, Tesar V, Lachmanová J. Advanced glycation end products and advanced oxidation protein products in hemodialyzed patients. *Blood Purif.* 2002;20:531-536.
19. Descamps-Latscha B, Witko-Sarsat V, Nguyen-Khoa T, et al. Advanced oxidation protein products as risk factors for atherosclerotic cardiovascular events in nondiabetic predialysis patients. *Am J Kidney Dis.* 2005;45(1):39-47.
20. Kocak H, Gumuslu S, Sahin E, et al. Advanced oxidative protein products are independently associated with endothelial function in peritoneal dialysis patients. *Nephrology (Carlton).* 2009;14:273-280.