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Metal Deposition in Calcific Uremic Arteriolopathy

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Abstract

Background—Calcific uremic arteriolopathy (CUA) is an often fatal disease that affects patients with end stage renal disease. Although animal studies support a role for metals in the pathogenesis of CUA, metal accumulation in human tissue has not been previously evaluated.

Objective—To evaluate metal deposition in CUA.

Methods—Twelve histologically proven cases of CUA were identified from the UAMS dermatopathology database. Five skin biopsies from chronic kidney disease patients exposed to gadolinium contrast but without CUA were used as controls. Quantification of metals including iron, aluminum and gadolinium in the lesional skin was performed using inductively coupled mass spectrometry.

Results—Seven patients had documented exposure to gadolinium-based contrast in the preceding 2 years before CUA. Three of them had concurrent nephrogenic systemic fibrosis. Highly significant quantities of iron ($p=0.03$), and aluminum ($p=0.0002$) were detected in CUA specimens compared to controls. Significant amounts of gadolinium were present in several CUA biopsies.

Limitations—Observational, retrospective study design and small sample size.

Conclusion—Tissue iron and aluminum content is increased in CUA. A significant amount of gadolinium is also present in some CUA specimens. Based on animal studies which strongly implicate metals in the pathogenesis of CUA, our data suggest that metal deposition should be considered in the pathogenesis of human CUA.

Keywords

Calcific uremic arteriolopathy; Calciphylaxis; Nephrogenic systemic fibrosis; metals; gadolinium

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INTRODUCTION

Calcific uremic arteriolopathy (CUA) popularly known as calciphylaxis is a disorder of small-vessel calcification with a spectrum of presentation ranging from painless cutaneous plaques or erythematous lesions to extremely painful nodules and necrotic skin ulcers. It is a disease of great morbidity affecting up to 1-4% of patients with end stage renal disease.¹⁻³ The reported incidence rate of CUA varies from 4.5 per 1000 patient-years in the dialysis population⁴ to 4.5 per 1 million people per year in the general population.⁵ Recent observations suggest that the incidence of CUA is increasing over the last decade.⁴ While several putative risk factors have been implicated to explain its pathogenesis and increasing incidence, the exact mechanisms of CUA remain poorly understood.

Traditionally, hyperparathyroidism and deranged calcium-phosphorus metabolism (chronic use of calcium salts/vitamin D, elevated calcium phosphorus product ($ca \times p > 70$) and elevated alkaline phosphatase),^{2,6,7,8} have been identified as classic risk factors for CUA. Nevertheless, CUA has also been reported in the absence of these metabolic derangements,⁸ and even in patients with low parathyroid hormone levels/adynamic bone disease.⁹ To explain this apparent discrepancy, a multitude of other risk factors have been postulated, including: race, obesity,^{1,10,11} diabetes mellitus,¹² peritoneal dialysis,² low serum albumin,^{1,9,13} pro-coagulant state (warfarin usage, Protein C or S deficiency),¹³ erythropoietin therapy,¹¹ inflammation (elevated C-reactive protein),¹⁴ and deficiency of calcification inhibitory protein Fetuin -A/ alpha2-heremans schmid glycoprotein.¹⁵ As many patients with chronic kidney disease and end stage renal disease have many of these other risk factors, yet never develop CUA, other unidentified risk factors that are involved in CUA pathogenesis may exist.

Animal studies strongly suggest a role for metals in the pathogenesis of CUA,¹⁶⁻¹⁸ yet such an association in humans has not been definitively established. Initial case control studies have reported absence of an association between intravenous iron therapy and human CUA.^{11,19} More recently, Weenig et al. noted a fourfold increase in the risk of developing CUA with elevated serum aluminum levels ($>25\text{ng/ml}$).²⁰ Similarly, co-existence of CUA with nephrogenic systemic fibrosis,^{3,21,22} a systemic disorder affecting patients with renal insufficiency exposed to gadolinium contrast and who develop gadolinium-induced iron mobilization and aluminum accumulation,²²⁻²⁴ suggests a role for metals in CUA pathogenesis. The purpose of this study is to examine if there is excess tissue deposition of metals including aluminum, iron and gadolinium in human calciphylaxis. To examine this, we utilized inductively-coupled mass spectrometry (ICP MS) to quantify gravimetrically the deposition of such metals in tissue and to compare this data to skin biopsies from chronic kidney disease controls exposed to gadolinium contrast but without NSF or calciphylaxis.

METHODS

Approval from the institutional review board of the University of Arkansas for medical sciences (UAMS) was obtained to conduct this study. Cases were identified from the UAMS dermatopathology database (n=12 from January 2000 to December 2007) based on the presence of both clinical (painful nodules or ulcers with erythema and induration) and histological (medial calcification, intimal hyperplasia, and necrosis) evidence of CUA. Demographic data was recorded in all cases. All available data on the etiology of end stage renal disease, mode of renal replacement therapy, history of co-morbid conditions including diabetes mellitus, liver disorders, hypercoagulable state (antiphospholipid antibody syndrome, protein C/ S deficiency) and history of exposure to gadolinium- based contrast agents were recorded. Additionally, in 5 patients who were primarily seen at UAMS, medical records were reviewed to obtain clinical and laboratory data including serum iron,

total iron binding capacity, ferritin, transferrin saturation, serum albumin, C - reactive protein, calcium, phosphorous and erythrocyte sedimentation rate in the preceding three month period before diagnosis. The time interval between gadolinium- based contrast exposure and CUA diagnosis was also recorded. For metal analysis, in addition to 12 CUA samples, 5 skin biopsies from patients with chronic kidney disease and exposure to gadolinium contrast but without CUA or NSF were selected and were used as controls. De-identified paraffin-embedded skin biopsies were submitted for gadolinium, iron and aluminum quantification. Quantitative measurement via inductively coupled plasma mass spectrometry (ICP-MS) on 20 tissue blocks was performed identical to previously published techniques.²³ Unpaired student t test was performed to evaluate the significance of metal accumulation in CUA compared to controls.

RESULTS

Demographic, clinical and laboratory data

A total of 12 patients with histological diagnosis of CUA were identified. Three patients were also diagnosed with NSF concurrently using standard clinical and histological criteria. Among all CUA patients, seven had a documented history of exposure to gadolinium-contrast enhanced MRI preceding CUA diagnosis. The median time from gadolinium contrast exposure to CUA diagnosis was 12 weeks (range 4 weeks to 2 years). The type and dosage of gadolinium contrast could not be ascertained in many patients. Median age at presentation was 46 years with a predominance of females (9 females, 3 males). Median body mass index was elevated at 35.45 kg/m² (Range 21.8 to 48.3 kg/m²). None of the patients included in the current study were on aluminum-based phosphate binders. Although we could confirm that 4 patients had received oral or parenteral iron supplementation prior to the diagnosis of CUA and one did not receive any iron supplementation, data were not available in the rest.

Among those in whom historical laboratory studies were available (n=5) we noted: low serum albumin levels (median; 2.8 g/l), an elevated C- reactive protein levels (median: 21.8 units/l), an elevated calcium phosphorous product (62.3) and an elevated parathyroid hormone levels (median: 363.5 pg/ml). A few patients also manifested an elevated serum ferritin (median: 623.9 ng/dl) and low total iron binding capacity (median: 190.3 µg/dl) consistent with systemic inflammation. All other relevant clinical and laboratory data are summarized in Tables 1&2, respectively.

Quantification of gadolinium and other metals in CUA tissues (Table 3)

Representative histologic sections of CUA in NSF patients are demonstrated in FIGURE 1. Compared to controls (mean, 280.4 µg/g; SD, 139.5 µg/g), significantly higher quantities of iron (mean, 889.3 µg/g; SD, 361.5 µg/g) were present in CUA affected-skin tissues (p=0.03). Tissue iron content was increased in both isolated CUA (median 704 µg/g; range 485-1369 µg/g) and concurrent CUA/NSF cases (median 1097 µg/g and range 974-1299 µg/g). Similarly, tissue aluminum content was elevated in all CUA specimens (mean, 1493.3 µg/g; SD, 1960.1 µg/g) compared to controls (mean, 99.2 µg/g; SD, 45.5 µg/g). Although, the increase in tissue aluminum content was comparable between isolated CUA (median: 874 µg/g; range: 69-7277 µg/g) and concurrent CUA/NSF cases (median: 1965 µg/g and range: 241-1988 µg/g), the highest aluminum content was found in a patient with CUA who did not have concurrent NSF (aluminum 7277 µg/g). Significant amounts of gadolinium were detected in all three concurrent NSF/CUA cases (median 61 µg/g; range 1.9-271 µg/g). Surprisingly, appreciable quantities of gadolinium (> 1 µg/g) were present in 3 isolated CUA cases (median 6 µg/g; range 2.9-22 µg/g) as well. None of the control skin biopsies

contained significant quantities of gadolinium. Using Wilcoxon rank-sum test, there was no significant difference in iron and aluminum count between survivors and non-survivors.

DISCUSSION

This report details several novel and important observations that may yield additional insights into the pathogenesis of CUA and uremic vascular calcification. We present the first evidence that there is significant tissue deposition of metals including iron and aluminum in the sampled skin obtained from CUA lesions. Second, we present evidence of gadolinium deposition in lesional tissues of some CUA patients who did not have concurrent NSF; albeit at lesser levels, this is still an important observation and highlights the importance of gravimetric quantification, as opposed to simple qualitative analysis. Third, we confirm the co-existence of NSF and CUA in some patients and furthermore, demonstrate gadolinium deposition in the skin of these patients with concurrent disease.

The source of excess tissue iron in CUA is unknown as many of our patients were not on any iron supplementation. As erythrocyte extravasation is rare in CUA, it is also unlikely to explain iron accumulation.²⁵ It is possible that systemic inflammation liberates catalytic iron at the level of tissue,²⁶ and in gadolinium-contrast exposed patients, gadolinium could have triggered iron dysmetabolism (iron mobilization and decrease in total iron binding capacity) and systemic inflammation,²⁴ with resultant iron accumulation. Similarly, possible mechanisms and source of excess aluminum accumulation in the tissues of CUA patients is unknown. Increased dietary intake in the context of poor urinary excretion could have contributed to tissue aluminum load.²⁷ In some patients, parenteral nutrition,^{10,28} or medications,²⁹ could also be an important source of aluminum accumulation. Other speculative sources of aluminum elevation in tissue include: *a*) systemic inflammation and consequent low serum transferrin such as is in CUA patients may result in competition for transferrin binding sites between iron and aluminum, resulting in aluminum deposition,³¹ or *b*) hyperparathyroidism may result in high bone turnover and mobilization of aluminum from bone stores (even from aluminum deposits from long ago).³² In fact, the latter mechanism could explain the observed strong association of high parathyroid hormone levels with CUA. Pathology in CUA is characterized by *a*) endothelial injury with mural thrombosis; *b*) intimal fibrosis/proliferation and *c*) vascular osteogenesis and calcification.^{7,10,33} CUA biopsies evaluated in our study had several fold higher tissue iron, aluminum and gadolinium levels.²³ Traditionally, iron circulates bound to transferrin and ferritin, and little free iron is available in the circulation. Increased amounts of free and catalytically-active iron leads to generation of reactive oxygen species,²⁶ and oxidative stress that are capable of inducing lipid peroxidation and endothelial injury. Oxidative stress can also promote a phenotypic switch of vascular smooth muscle cell (VSMC) from a contractile to osteogenic phenotype; a response associated with an increased expression and transactivity of Runx2, a key transcription factor of osteogenic differentiation.³⁴ Osteogenic transformation of VSMC is suspected to be an important pathogenic mechanism in the development of CUA,²⁸ and vascular calcification.³⁵ Indeed, medial calcification in CUA has been shown to be accompanied by increased expression of osteopontin,^{7, 28} and bone sialoprotein. In addition, on electron microscopy these CUA specimens revealed the presence of matrix vesicles identical to those observed in human bone.^{7,28} Aluminum could potentiate iron-mediated oxidative stress,³⁶ and induce osteogenic differentiation of stromal cells.³⁷ Furthermore, aluminum and gadolinium may induce *de novo* bone formation (osteogenesis) by stimulating a G-protein coupled cation sensing mechanism producing an osteoblast phenotype upon the undifferentiated progenitor cells,³⁸⁻⁴⁰ Lastly, iron by itself has been implicated as a potential initiator of the nucleation process for crystal deposition,^{16,39} and this may also be involved in the precipitation of calcium and

phosphorous in CUA lesions. These factors and pathways are represented graphically in FIGURE 2.

Animal studies also provide evidence for the potential role of iron and gadolinium in CUA. Selye was first to propose *two-hit hypothesis* of CUA where metals such as iron could act as challengers (*second hit*) that precipitate CUA in an appropriate host environment (which he called as sensitization or *first hit*).¹⁷ Subsequently, Gabbiani et al demonstrated that CUA lesions could be incited in dihydrotachysterol (DHT) sensitized rats by injecting iron chloride subcutaneously.⁶ Similarly, supporting a role for gadolinium in CUA pathogenesis, in 1962, Selye, in his pioneering experiments, demonstrated that trichlorides of lanthanum and cerium caused local calcification when injected into the subcutaneous tissue of rats (*second hit*) after sensitization with agents such as parathyroid hormone (PTH), vitamin D, or a diet high in calcium and phosphorus (*first hit*). Sensitization and challenging applied sequentially resulted in not only local but also systemic calcification and scleroderma-like skin changes.^{17,18} Subsequently, McClure et al. tested the calcergenic effect of lanthanides (including GdCl₃) in the mouse, and obtained a similar reaction pattern.⁴¹ Johansson et al also have demonstrated that intravenous injections of lanthanum chloride (LaCl₃) produces hypercalcemia and hyperphosphatemia, a milieu proven to be conducive to the deposition of calcium phosphate.⁴² While these reports, in aggregate, indicate that calcification and lesions of CUA may be induced by injection of metal salts into the skin, a more detailed understanding of the mechanism of CUA *in vivo* in humans including an analysis of tissue content of metals has never been performed previously. Thus, our finding of increased metal content in tissue affected by CUA is the first quantifiable evidence of metal deposition in human CUA. Our data, when interpreted in light of these experiments in animal models, provides suggestive evidence for a role of metals, particularly iron and lanthanides, in the pathogenesis of CUA in humans.

Exposure to gadolinium-based contrast agents during renal insufficiency has been highly associated with NSF, a systemic fibrosing disorder associated with high mortality.⁴³ We recently demonstrated that exposure to gadolinium contrast in this same setting induces iron-mobilization and systemic inflammation upon dosing,²⁴ and that there is tissue deposition of gadolinium, aluminum and iron in NSF patients.²³ Interestingly, Edsall et al. reported histological findings of CUA in the same skin biopsies of those diagnosed with NSF; our study confirms and strengthens this observation.²¹ Additionally, we present evidence that appreciable amounts of gadolinium are present, not only in concurrent CUA/NSF cases, but also in some of our isolated CUA cases without clinically or histologically diagnosed NSF. Co-existence of CUA with NSF along with detection of significant gadolinium in some isolated CUA biopsies raises the possibility that these two conditions share a common pathway of pathogenesis and that gadolinium, aluminum and iron may play a role in disease evolution. This observation is supported by two recent findings: **1)** gadolinium and iron preferentially accumulate in large,²³ and small vessels in NSF,⁴⁴ and **2)** there is vascular accumulation of calcium and phosphorus in NSF lesions.³⁰ Although it is unlikely that gadolinium universally participates in CUA pathogenesis, and indeed gadolinium deposition was not demonstrated in all CUA cases, when it is present, it is perhaps a very important event.

The major limitation of our study is its small sample size. Thus, preliminary findings from our study require confirmation in a larger study. A second limitation is that it is observational and retrospective in nature, precluding rigorous establishment of a cause and effect relationship. Third, detailed laboratory data such as iron studies, plasma aluminum levels and ionized calcium were not available in most patients. The fourth limitation is that gadolinium and aluminum deposition were not consistently detected in all CUA biopsies. It is possible that sampling error or exposure to other metals used in medicine, strontium,

lanthanum, played a role in these cases. Finally, there are only a few control cases from patients with stage 3 chronic kidney disease but without CUA or NSF. Thus, we do not know with certainty that iron and aluminum deposition to this degree does not occur in the skin of patients with advanced chronic kidney disease without CUA.

In spite of diligent research over the last four decades, CUA remains an enigmatic disorder. With a steadily increasing number of chronic kidney disease patients, we believe nephrologists might encounter this clinical entity more often. This underscores the importance of larger studies geared towards a better understanding of the pathogenesis of CUA. Findings from our study suggest a role for metal deposition in the pathogenesis of CUA. Future prospective studies are required to confirm these findings and to establish a cause and effect relationship between metal deposition and CUA and thus provide opportunities for prevention and treatment.

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Abbreviations

CUA	Calcific Uremic Arteriopathy
NSF	Nephrogenic systemic fibrosis

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Figure 1. Skin biopsy of a patient with concurrent NSF and calciphylaxis

The low power image shows a biopsy of a patient with concurrent nephrogenic systemic fibrosis characterized by increased activated fibroblasts and collagen in the deep dermis and extending along the interlobular septae (insert A) and calciphylaxis characterized by calcification of the intima of small vessels in the subcutaneous tissue (insert B). Insert A shows increased dermal cellularity, primarily consisting of reactive type fibroblasts. Insert B shows subcutaneous tissue that contains many small vessels with intimal calcification and some with complete occlusion of the lumen. Significant amounts of gadolinium (60 $\mu\text{g/g}$), iron (1097 $\mu\text{g/g}$) and aluminum (1988 $\mu\text{g/g}$) were present in this biopsy.

Figure 2.
Schematic description of pathogenic pathways in calciphylaxis.

Table 1

Demographic, clinical and histopathological data of CUA patients

Patient No.	Age	Sex	Race	BMI	Dialysis	Renal diagnosis	Outcome	Necrosis on Biopsy	Gadolinium exposure to CUA
1	26	F	B	NA	PD	SLE	S	+	2 years
2	49	F	B	48.3	HD	FSGS	S	+	3 months
3	46	F	B	40.8	NA	SLE	D	+	2 years
4	26	F	B	33.1	NA	SLE	S	+	2 years
5	47	F	W	32	HD	ARF	D	+	2 months
6	49	M	W	37.8	PD	DM	S	-	7 months
7	46	F	W	21.9	PD	PCKD	D	-	5 weeks
8	69	F	B	NA	HD	DM, HTN	NA	+	NA
9	69	F	B	NA	NA	Unknown	NA	-	Yes
10	80	F	W	NA	PD	HTN	NA	+	NA
11	62	M	NA	NA	NA	Unknown	NA	+	NA
12	55	M	W	NA	HD	SLE	NA	+	NA

BMI =Body Mass Index; NA= not available DM= Diabetes Mellitus; HTN=Hypertension; SLE= Systemic Lupus Erythematosus; CAD= Coronary artery disease; PCKD= polycystic kidney disease; FSGS= Focal segmental glomerulosclerosis; PD= Peritoneal dialysis; HD= Hemodialysis; S= Survival; D=Death

Table 2

Laboratory data of CUA patients (n=5)

Parameter	Value
Serum Calcium (mg/dl)	8.7 (7.2-10.2)
Serum Phosphorus (mg/dl)	6.2 (3-7.9)
Ca X P	62.3 (28.8-80.6)
Serum PTH (pg/ml)	363.5 (81.4- 785)
Serum albumin (g/dl)	2.8 (1.6-3.8)
TIBC (µg/dl)	190.5 (151-240)
Serum Ferritin (ng/dl)	623.9 (77-1129)
C-reactive protein (U/l)	21.8 (4.49- 30.3)

PTH: parathyroid hormone; TIBC: total iron binding capacity

Table 3

Gadolinium, Iron and Aluminum content in skin biopsies of CUA patients and controls.

Patient serial no.	Gadolinium ($\mu\text{g/g}$)	Iron [‡] ($\mu\text{g/g}$)	Aluminum [†] ($\mu\text{g/g}$)
1	6.1	737.5	181.2
2	2.91	703.9	874.5
3	0.95	1164.6	477.7
4	0.42	1320.8	481.9
5	22	484.9	69.3
6*	1.9	1299	241
7*	271	974	1965
8	0	300.3	1132.9
9*	60.5	1097.3	1987.6
10	0	651	2080.2
11	0	569.4	1151.8
12	0	1368.9	7276.8
Controls Serial no.			
1	0.9	228.5	122
2	0.6	362.8	104
3	0.35	465.6	86.4
4	0.06	251.6	153
5	0.15	99.7	30.8
Acid Blank (no gadolinium)	0.02	49.4	32.9

[‡]P= 0.03 versus controls.[†]P= 0.0002 versus controls

* Biopsies with concurrent CUA and NSF. Data are presented in μg of each metal per gram of dry tissue. In acid Blank, no metal was added and data are expressed in PPB parts per billion. Controls were skin biopsies from patients with chronic kidney disease (serum creatinine range: 1.4-1.9 mg/dl) exposed to gadolinium contrast but without calciphylaxis. Gd: Gadolinium; Fe: Iron; Al: aluminum.