Uraemic vascular damage and calcification in children on dialysis

Prevention vs damage limitation?

Rukshana Shroff

Great Ormond Street Hospital and Institute of Child Health, London, United Kingdom
Cardiovascular disease in children – does it happen?
Outline

• CVD in childhood CKD
  – epidemiology
  – when does it begin?

• What is the nature of the vascular damage?
  - Risk factors
  - Clinical studies

• Is there direct evidence of vascular damage and calcification?
  – Clinico – pathological correlations

The role of Ca and P in vascular injury
Mortality in childhood-onset CKD

Oh et al, Circulation, 2002
CVD is the most common cause of death in childhood CKD

**US**
- 38% deaths were from CVD  
  *Chavers et al, KI 2002*

**Dutch cohort study**
- 24% deaths due to CVD / cerebrovascular disease  
  *Groothoff et al, KI 2002*

**ANZDATA**
- 57% deaths on HD and 43% on PD are due to cardiac causes  
  *McDonald et al, NEJM 2004*
There is an independent and graded association between GFR and CVD

Go et al, NEJM; 2004
Metabolic disturbances in early CKD

GFR 90

↑ FGF-23

↓ Vitamin D

↑ PTH

↑ Ca x P

Dialysis

Levin et al, KI; 2007
Arterial Medial Calcification in CKD

Atherosclerosis

Arteriosclerosis

Adapted from London et al, NDT 2002
## Risk factors for vascular injury

### ‘Traditional’ risk factors
- Hypertension
- Diabetes
- Hypercholesterolemia
- Dyslipidemia
- Insulin resistance
- Obesity
- Smoking
- Male gender
- Family history of CVD

### Risk factors in CKD
- Abnormal Ca & Phosphate
- Hyperpapathyroidism
- ? Vitamin D analogues
- Chronic fluid overload
- Inflammation
- Oxidative stress
- Hyperhomocysteinemia
- Albuminuria
- Malnutrition
- Perturbation in physiological inhibitors (fetuin-A, OPG)
- Abnormal FGF-23 levels
Clinical studies in children – key papers

Patients with calcification were:

- older
- longer dialysis vintage
- Higher P & Ca xP
- Higher Ca intake from binders

Goodman et al, NEJM, 2000
<table>
<thead>
<tr>
<th>Authors / Journal</th>
<th>Number of dialysis pts</th>
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<td>cIMT, CAC</td>
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<td>- Mean calcitriol dose</td>
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<td>Mitsnefes / JASN 2005</td>
<td>16</td>
<td>cIMT, distensibility</td>
<td>- dialysis duration</td>
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<td></td>
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<td>- mean PTH levels</td>
</tr>
<tr>
<td>Shroff / JASN 2007</td>
<td>85</td>
<td>cIMT, PWV, CAC</td>
<td>- dialysis duration</td>
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<td>Civilibal / Ped Nephrol 2007</td>
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<td>cIMT, FMD, ECHO</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- total &amp; LDL cholesterol</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- mean calcitriol dose</td>
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**PTH levels and vascular outcome**

<table>
<thead>
<tr>
<th></th>
<th>Low PTH levels</th>
<th>High PTH levels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calcium</strong></td>
<td>× ↔ Phosphorus</td>
<td>→ Phosphorus</td>
</tr>
<tr>
<td><strong>Phosphorus</strong></td>
<td>× ↔ Calcium</td>
<td>→ Calcium</td>
</tr>
<tr>
<td><strong>Ca + PO₄ deposition in soft tissues</strong></td>
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<td></td>
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<table>
<thead>
<tr>
<th></th>
<th>CKD 3 - 4</th>
<th>Dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>KDIGO</td>
<td>unknown</td>
<td>2 - 9 times ULN</td>
</tr>
<tr>
<td>European guidelines</td>
<td>Normal range</td>
<td>2 – 3 times ULN</td>
</tr>
</tbody>
</table>
Is high PTH a risk factor for CVD?

**Inclusion criteria**
- Children on dialysis who are:
  - 5 – 18 years old
  - Dialysis for ≥ 6 months
  - CKD Stage IV for ≥3 years

**Exclusion criteria**
- Underlying inflammatory disease eg vasculitis
- Diabetes mellitus
- Uncontrolled hyperlipidaemia
- Uncontrolled hypertension
- Smokers

**Based on mean time-integrated PTH levels –**

Group I - PTH ≤ 2x ULN  [n = 41]
Group II - PTH > 2x ULN  [n = 44]

Matched for confounders

*Shroff et al, JASN 2007*
Increased cIMT is associated with high PTH levels

Shroff et al, JASN 2007
Arterial stiffness is associated with high PTH levels

Shroff et al, JASN 2007
Vascular calcification is associated with high PTH levels

Calcification present in 17/85 (20%) patients

<table>
<thead>
<tr>
<th>PTH &lt;2 ULN n = 41</th>
<th>PTH &gt;2 ULN n = 44</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 (12%)</td>
<td>12 (27%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Calcification score Median (range)</strong></td>
<td><strong>Calcification score Median (range)</strong></td>
<td></td>
</tr>
<tr>
<td>7.8 (0 – 98)</td>
<td>85.3 (0 – 2039)</td>
<td>0.001</td>
</tr>
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Shroff et al, JASN 2007
Vitamin D as a predictor of cardiovascular damage?
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Bimodal effect of 1,25 dihydroxy D

Shroff et al, JASN 2008
The anti-inflammatory effect of Vit D influence calcification

$$p < 0.0001$$

$$r = -0.53$$

1,25 dihydroxy Vit D (pmol/L)

hs-CRP (mg/L) (log axis)

Calcification score (log axis)

<table>
<thead>
<tr>
<th>hs-CRP (mg/L)</th>
<th>1,25(OH)₂D (pmol/L)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>low</td>
<td>&gt;10</td>
<td>8</td>
</tr>
<tr>
<td>normal</td>
<td>&gt;10</td>
<td>14</td>
</tr>
<tr>
<td>high</td>
<td>&gt;10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>&lt;10</td>
<td>6</td>
</tr>
</tbody>
</table>
A biphasic dose–response curve for vitamin D on vascular health

Vit D deficiency
- Hyperparathyroidism
- Rickets
- Bone pain, fractures
- Vascular disease
- HT & LVH
- Increased mortality

Vit D overdosing
- Adynamic bone disease
- Hypercalcaemia
- Vascular calcification

Zittermann A; Curr Opin Lipidol; 2007
Is there direct evidence of vascular damage and calcification in CKD vessels?

Shroff et al, Circulation, 2008
Shroff et al, JASN, 2010
*Ex vivo* changes in intact human arteries from children with CKD
Ca accumulation begins pre-dialysis

- **Normal**
  - n = 6

- **Pre-dialysis**
  - n = 10

- **Dialysis**
  - n = 24

Ca load in the vessel wall (µg/ml)

- p = 0.0005
- p = 0.02
The vessel Ca load correlates with the serum Ca x P product

\[ p = 0.007 \]
\[ r^2 = 0.41 \]
The vessel Ca load increases only with time on dialysis.

For the time in CKD IV-V before starting dialysis (yrs):
- Ca load in the vessel: $p = 0.30$, $r^2 = 0.03$
- $n = 34$

For the time on dialysis (years):
- Ca load in the vessel: $p = 0.041$, $r^2 = 0.29$
- $n = 24$
Ca load correlates with the carotid IMT in dialysis patients

Pulse wave velocity
In 2/31 patients

Coronary calcification on CT scan
In 2/31 patients

Ca load in the vessel (µg/µL)

Carotid Intima Media Thickness (mm)

$\bullet$ Pre-dialysis $n = 9$

$\blacktriangledown$ Dialysis $n = 22$

$p = 0.01$

$r^2 = 0.42$
Dialysis vessels have VSMC loss

![Graph showing the number of VSMCs per unit area for Normal and Dialysis groups.](image)

- Normal: n = 4
- Pre-dialysis: n = 8
- Dialysis: n = 10

![Images of tissue sections showing VSMCs in Normal and Dialysis conditions.](image)
Dialysis vessels have maximum fetuin-A deposition

<table>
<thead>
<tr>
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<th>Pre-dialysis</th>
<th>Dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>n = 4</td>
<td>n = 6</td>
</tr>
<tr>
<td>% fetuin-A positive areas /unit area</td>
<td>1.2 ± 1.1</td>
<td>8.5 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>16.2 ± 5.6</td>
<td>15.0 ± 2.7</td>
</tr>
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</table>

p = 0.03
Circulating calcification inhibitors as biomarkers of cardiovascular damage?
Fetuin-A decreases with time on dialysis

- **Healthy controls**: 0.41 ± 0.13
- **Dialysis patients**: 0.84 ± 0.3

- **p = 0.002**
- **r² = 0.32**

Number of subjects:
- **Healthy controls**: n = 75
- **Dialysis patients**: n = 61

- **p < 0.0001**

Time on dialysis (years) vs. Fetuin-A levels (gm/L)
Fetuin levels influence vessel stiffness and calcification

Shroff et al, NDT, 2008
Mechanistic insights into the accelerated calcification in dialysis patients – role of Ca and P

1mM P + 1.8mM Ca
2mM P + 1.8mM Ca
2mM P + 2.7mM Ca
Dialysis vessels have time-dependent Ca accumulation

Incubation in 2mM P + 2.7mM Ca

Ca load in the vessel wall (µg/µL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-dialysis</td>
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<td></td>
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<tr>
<td>Dialysis</td>
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- Normal: n = 6
- Pre-dialysis: n = 10
- Dialysis: n = 24

Significance levels:
- p = 0.01
- p = 0.16
- p = 0.0007
Ca is more potent at inducing calcification than P

![Graph showing Ca load in the vessel wall (µg/mL) for different conditions: 1mM PO₄ + 1.8mM Ca, 2mM PO₄ + 1.8mM Ca, 3mM PO₄ + 1.8mM Ca, and 2mM PO₄ + 2.7mM Ca. The graph compares normal, pre-dialysis, and dialysis conditions. Significant p-values are indicated: p < 0.0001 for 3mM PO₄ + 1.8mM Ca vs. 1mM PO₄ + 1.8mM Ca, and p = 0.02 for 2mM PO₄ + 2.7mM Ca vs. 2mM PO₄ + 1.8mM Ca.](image)
Dialysis vessels have VSMC loss in high Ca + P media

\[ p = 0.03 \]

Number of VSMCs (per 0.25m$^2$ area of vessel)

- 1mM PO$_4$ + 1.8mM Ca
- 2mM PO$_4$ + 1.8mM Ca
- 2mM PO$_4$ + 2.7mM Ca

- Normal n = 4
- Pre-dialysis n = 8
- Dialysis n = 10

Dialysis – high Ca + P

[Image of histological section]
Ca induced apoptosis may be a prerequisite to calcification.
Clinico – pathological correlations

• Ca accumulation begins pre-dialysis and is accelerated on dialysis

• Dialysis vessels have lost protective mechanisms and appear to be ‘primed’ to calcify in high Ca and P conditions

• In the presence of a high P even a small increase in Ca can significantly increase calcification
Progression of vasculopathy
Conclusions

• Calcification begins early in CKD and progresses inexorably on dialysis

• Transplantation can only partially reverse the effects of dialysis on the vasculature

• Our currently available imaging techniques are not sensitive enough to detect early vascular calcification

**Prevention is key**
- Prevent mineral dysregulation
- Maintain normal vit D levels
- Pre-emptive renal transplantation
Acknowledgements

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