Serum Phosphate as a Risk Factor for Cardiovascular Events in People with and without Chronic Kidney Disease: A Large Community Based Cohort Study

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Abstract

Background: Serum phosphate is a known risk factor for cardiovascular events and mortality in people with chronic kidney disease (CKD), however data on the association of these outcomes with serum phosphate in the general population are scarce. We investigate this relationship in people with and without CKD in a large community-based population.

Methods: Three groups from an adult cohort of the Quality Improvement in Chronic Kidney Disease (QICKD) cluster randomised trial (ISRCTN56023731) were followed over a period of 2.5 years: people with normal renal function (N = 24,184), people with CKD stages 1–2 (N = 20,356), and people with CKD stages 3–5 (N = 13,292). We used a multilevel logistic regression model to determine the association between serum phosphate, in these groups, and a composite outcome of all-cause mortality, cardiovascular events, and advanced coronary artery disease. We adjusted for known cardiovascular risk factors.

Findings: Higher phosphate levels were found to correlate with increased cardiovascular risk. In people with normal renal function and CKD stages 1–2, Phosphate levels between 1.25 and 1.50 mmol/l were associated with increased cardiovascular events; odds ratio (OR) 1.36 (95% CI 1.06–1.74; p = 0.016) in people with normal renal function and OR 1.40 (95% CI 1.09–1.81; p = 0.010) in people with CKD stages 1–2. Hypophosphatemia (<0.75 mmol/l) was associated with fewer cardiovascular events in people with normal renal function; OR 0.59 (95% CI 0.36–0.97; p = 0.049). In people with CKD stages 3–5, hyperphosphatemia (>1.50 mmol/l) was associated with increased cardiovascular risk; OR 2.34 (95% CI 1.64–3.32; p<0.001). Other phosphate ranges were not found to have a significant impact on cardiovascular events in people with CKD stages 3–5.

Conclusions: Serum phosphate is associated with cardiovascular events in people with and without CKD. Further research is required to determine the mechanisms underlying these associations.

Introduction

Observational data suggest that an elevated serum phosphate increases the risk of cardiovascular events and mortality in patients with chronic kidney disease (CKD) [1–4]. Furthermore, hypophosphatemia is associated with reduced cardiovascular events in people with CKD [1,3]. Hyperphosphatemia is also associated with increased vascular and valvular calcification in end-stage renal disease [5,6]. Secondary hyperparathyroidism is common in people with CKD and subsequently altered calcium and vitamin D metabolism may be responsible for this increased arterial calcification [7]. However, repeated investigation has not been able to identify a relationship between serum calcium and cardiovascular events, and there is inconclusive evidence for an association with parathyroid hormone levels [3].

Given the diverse biochemical roles of phosphate, it is possible that high serum phosphate is more directly responsible for cardiovascular events than previously suggested. Indeed, elevated phosphate also correlates with increased vascular and valvular calcification in people with normal renal function [8,9] and an association with cardiovascular events has also been reported in people with pre-existing coronary artery disease [10] and in the general population [11–13].

A number of potential mechanisms by which phosphate leads to increased cardiovascular risk have been proposed [14]. Elevated phosphate levels induce degradation of the extracellular matrix and causes osteochondrogenic change in vascular smooth muscle.
Methods

The data analysed were from the Quality Improvement in Chronic Kidney Disease (QICKD) cluster randomised trial (clinical trials registration: ISRCTN56023731) [25]. These data consist of the primary care records of the population of 127 primary care practices across England; a nationally representative sample of urban, sub-urban and rural practices in London, Surrey, Sussex, Leicester, Birmingham and Cambridge. The complete protocol used for sampling and data collection from these practices for the QICKD trial have been previously described [26]. A sub-population of individuals in whom phosphate and renal indices were available was selected for inclusion in this study. Routine data from electronic records were collected over a five year period; between January 2006 and December 2010. Data recorded between January 2006 and June 2008 was used to determine the baseline characteristics of the people included in the study, with the most recent values of creatinine, phosphate and PTH used for comparison. A second data collection was undertaken at 30 months to obtain follow-up data on outcomes.

The study population was subdivided by renal function into three groups; people with normal renal function, people with CKD stage 1–2, and people with CKD stage 3–5. CKD stage was identified using estimated glomerular filtration rate (eGFR) measurements calculated using the modified diet in renal disease equation from serum creatinine measurements [27,28] and measurements of proteinuria. Normal renal function was defined as an eGFR ≥90 ml/min and absence of significant proteinuria. CKD stage 1–2 was defined as an eGFR of 60–89 ml/min or the presence of significant proteinuria with an eGFR ≥90 ml/min. CKD stages 3–5 were defined as an eGFR <60 ml/min. Proteinuria was analysed using the diagnostic criteria described by the National Institute for Health and Clinical Excellence with lower significance thresholds in people with diabetes (NICE) [28]. People without creatinine measurements were excluded from the analysis. People were only included in the normal renal function group if no proteinuria was present; those with no proteinuria measurements were excluded from this group.

Outcomes

We used a combined outcome measure of all-cause mortality and incident stroke, transient ischaemic attack (TIA), myocardial infarction (MI), advanced coronary artery disease, new cardiac failure and death, during the follow up period. Advanced coronary artery disease was defined as at least one of; coronary artery revascularisation procedures, progressive angina, angina at rest, and acute coronary syndrome not otherwise diagnosed as MI.

Predictors

Traditional cardiovascular risk factors were identified from analysis of the electronic patient records at baseline. These were the Framingham risk factors; age, ethnicity, smoking status, alcohol use, and body mass index (BMI), diabetes, hypertension, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, and total cholesterol (TC).

BMI was calculated from routinely gathered measurements of weight and height performed by physicians and practice nurses. The presence of diabetes was defined from clinical coding records and serum glucose values using a method we have validated previously [29]. The presence of significant hypertension was defined by prescription of antihypertensive medications (angiotensin converting enzyme inhibitors and angiotensin II receptor blockers) using electronic prescription records kept by every practice included in the study.

Serum phosphate levels were analysed as a categorical variable grouped in 0.25 mmol/l bands (≤0.75, 0.75–1.00, 1.01–1.25, 1.26–1.50, and >1.50 mmol/l). Serum PTH was categorised as high (>60 ng/l), normal (10–60 ng/l), low (<10 ng/l) or not measured.

The presence of proteinuria was determined by examining a hierarchy of clinical tests; albumin creatinine ratio (ACR), then if not available; protein creatinine ratio (PCR), 24 hour urinary protein, or urine dipstick testing in turn. Threshold values are shown in Table 1. The lower significance thresholds for people with diabetes are also shown [28].

Statistical analysis

Numerical data was refined, to adjust for inputting errors, by removing values outside of physiological limits. Where the variable of interest had not been measured or recorded in the patient record individuals were categorised as being not monitored for that given parameter and included separately for analysis.

Multilevel binary logistic regression models were built to account for outcome variation between primary care practices. Patients were nested within practices using a random intercept. Model selection was performed using the approach described by Maindonald and Braun [30] by minimising the Bayesian information criterion (BIC) using backward stepwise elimination. Models were validated using receiver operating characteristic (ROC) curves and Hosmer-Lemeshow testing. People who left their practice during the follow up period were excluded from the logistic regression analysis. The analysis was performed using the statistical package R and the multilevel R package lme4 [31].

Cox proportional hazards multivariate models were also constructed using the statistical package R to provide a time-event
Results

Ethical considerations

Events of cardiovascular death and non-fatal events were ascertained from primary care records. This data was available for all outcome events.

Analysis

Individuals who left their practice during the follow up period were included as censored data. The date of each outcome event was taken as the date it was recorded as occurring in the primary care records. This data was available for all outcome events.

Ethical considerations

No patient identifiable data was used in the analysis described here. Other ethical considerations of the QICKD study are described elsewhere [26,32].

Discussion

Principal findings

In this large community-based population, serum phosphate levels were associated with cardiovascular events and mortality in both people with normal renal function and those with CKD. In people with normal renal function the relationship appears to be linear with no minimum threshold for improved cardiovascular outcomes. In those with CKD stages 3–5, hyperphosphatemia (which we have defined as phosphate >1.5 mmol/l) was found to be associated with increased cardiovascular risk. No protective effect of low phosphate (which we have defined as <0.75 mmol/l) is suggested in people with CKD stages 3–5 (figure 1).

Implications

These findings add to the growing weight of evidence that phosphate is an independent predictor of cardiovascular disease, in both people with and without CKD. Furthermore, these findings call into question the definition of a ‘normal range’ for phosphate, as low phosphate is associated with better outcomes in people with normal renal function. If phosphate is demonstrated to be an effective cardiovascular target, perception of serum levels should be similar to that of serum cholesterol or blood pressure, where the focus is on achieving values below a threshold. We propose that an increased research focus on the relationship between serum phosphate and cardiovascular events in the general population is needed.

Comparison with the literature

We found the prevalence of CKD stages 3–5 to be 7.6% in the adult population; this is in good agreement with the UK based population estimate of 6%, which includes under 18s [33].

Table 1. Protienuria threshold values by clinical test. Threshold values are adapted from the 2008 NICE guidelines [28] and Lamb et al. [45].

| Clinical test                  | Non diabetes threshold value | Diabetes threshold value |
|-------------------------------|------------------------------|----------------------------|
| Albumin creatinin ratio (mg/mmol) | ≥30                          | ≥2.5 males, ≥3.5 females |
| Protein creatinine ratio (mg/mmol) | ≥50                          | ≥15                        |
| 24 h urinary protein (mg/24h)     | ≥300                         | ≥150                       |
| Urine dipstick testing            | ‘trace’ or above             | ‘trace’ or above           |

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Correlations with known cardiovascular risk factors were also consistent with those derived from the Framingham studies [34]. Previous data suggests increased phosphate is associated with increased mortality and cardiovascular risk in the general population and that hypophosphatemia is associated with reduced risk [10,11]. This study confirms these findings. It also is in agreement with previous findings that hyperphosphatemia is associated with increased cardiovascular risk in people with CKD [1–3]. In people with CKD, phosphate below the normal range has been associated with both improved [1,35] and worsened [36] cardiovascular outcomes. We found no effect of low phosphate on cardiovascular outcomes in the CKD population. Low phosphate is a marker of malnutrition [37], which is common in people with advanced renal disease [38]. This correlation may mask any potential benefits of low phosphate in this group and account for discrepancies between previous studies.

Limitations of the method

The limitations of this study include those of working with routinely collected data (34). A particular consideration here is that only a small proportion of the population had serum phosphate measurements. There is a probable bias for increased co-morbidities amongst this group, however, this is likely to be a systematic effect and is therefore unlikely to explain the relationship between phosphate and cardiovascular events observed.

The number of people with phosphate levels at the extremes of the range was small. There is therefore greater uncertainty in our calculated odds of a cardiovascular event at these extremes.

Table 2. Demographics of people included for analysis in each study group; those with normal renal function (N = 24,184), those with CKD stages 1–2 (N = 20,356), and those with CKD stages 3–5 (N = 13,292) where phosphate measurements were available.

|                        | Total population | Normal renal function | CKD stages 1–2 | CKD stages 3–5 |
|------------------------|------------------|-----------------------|----------------|---------------|
| n (%)                  | n (%)            | n (%)                 | n (%)          | n (%)         |
| Female                 | 271,503 (49.6)   | 14,082 (58.2)         | 12,330 (60.6)  | 9,057 (68.1)  |
| Age: mean (SD)         | 49.0 years (±17.8) | 52.8 years (±17.0) | 56.0 years (±16.2) | 72.8 years (±12.9) |
| Never smoked           | 181,662 (33.2)   | 9,552 (39.5)          | 9,266 (45.5)   | 5,950 (44.8)  |
| Current Smoker         | 94,042 (17.2)    | 5,074 (21.0)          | 3,813 (18.7)   | 1,720 (12.9)  |
| Ex-smoker              | 49,584 (9.1)     | 2,833 (11.7)          | 2,643 (13.0)   | 2,539 (19.1)  |
| Diabetes               | 33,811 (6.2)     | 1,953 (8.1)           | 3,163 (15.5)   | 2,536 (19.1)  |
| Antihypertensive drug   | 77,056 (14.1)    | 4,503 (18.6)          | 5,354 (26.3)   | 7,388 (55.6)  |
| HDL cholesterol: measured | 167,607 (30.6)  | 14,502 (60.0)         | 14,281 (70.2)  | 10,321 (77.6) |
| HDL cholesterol: mean (SD) | 1.44 mmol/l (±0.43) | 1.45 mmol/l (±0.42) | 1.41 mmol/l (±0.41) | 1.46 mmol/l (±0.43) |
| Creatinine: measured   | 234,800 (42.9)   | 24,184 (100.0)        | 20,356 (100.0) | 13,292 (100.0) |
| Protienuria: measured  | 231,925 (42.3)   | 24,184 (100.0)        | 14,290 (70.2)  | 8,995 (67.7)  |
| Phosphate: measured    | 57,832 (10.6)    | 24,184 (100.0)        | 20,356 (100.0) | 13,292 (100.0) |
| Phosphate: <0.75 mmol/l | 1,340 (0.2)      | 627 (2.6)             | 522 (2.6)      | 273 (2.1)     |
| Phosphate: 0.75–1.00 mmol/l | 14,601 (2.7)   | 6,726 (27.8)          | 5,506 (27.0)   | 3,270 (24.6)  |
| Phosphate: 1.00–1.25 mmol/l | 27,562 (5.0)   | 12,201 (50.5)         | 10,405 (51.5)  | 6,750 (50.8)  |
| Phosphate: 1.25–1.50 mmol/l | 9,943 (1.8)    | 4,295 (17.8)          | 3,644 (17.5)   | 2,687 (20.2)  |
| Phosphate: >1.50 mmol/l | 861 (0.2)       | 335 (1.4)             | 279 (1.4)      | 312 (2.3)     |
| PTH measured           | 1,633 (0.3)      | 225 (0.9)             | 250 (1.2)      | 764 (5.7)     |

For comparison, the demographics of the total adult population (N = 547,494) from which these groups are extracted is also included. SD = standard deviation. doi:10.1371/journal.pone.0074996.t002

Table 3. Number of outcome events in each study group by phosphate range in; the total population (N = 57,832), those with normal renal function (N = 24,184), those with CKD stages 1–2 (N = 20,356), and those with CKD stages 3–5 (N = 13,292) where phosphate measurements were available.

|                      | Total population | Normal renal function | CKD stages 1–2 | CKD stages 3–5 |
|----------------------|------------------|-----------------------|----------------|---------------|
| n (%)                | n (%)            | n (%)                 | n (%)          | n (%)         |
| Phosphate: <0.75 mmol/l | 68 (4.8)       | 15 (2.4)              | 19 (3.6)       | 34 (12.5)     |
| Phosphate: 0.75–1.00 mmol/l | 872 (5.6)   | 264 (3.9)             | 223 (4.1)      | 385 (11.8)    |
| Phosphate: 1.00–1.25 mmol/l | 1,721 (5.9)  | 517 (4.2)             | 435 (4.2)      | 769 (11.4)    |
| Phosphate: 1.25–1.50 mmol/l | 706 (6.6)    | 192 (4.5)             | 172 (4.7)      | 342 (12.7)    |
| Phosphate: >1.50 mmol/l | 99 (10.7)     | 17 (5.1)              | 12 (4.3)       | 70 (22.4)     |

The percentages shown are the proportion of people within category who had a cardiovascular event (not of the group population). doi:10.1371/journal.pone.0074996.t003
Table 4. Logistic regression analysis: The clinical characteristics of people with normal renal function, CKD stages 1–2, and people with CKD stages 3–5 and odds ratio of cardiovascular events and mortality during 30 months of follow up.

| Model performance: | Complete population | Normal renal function | CKD stages 1–2 | CKD stages 3–5 |
|--------------------|---------------------|-----------------------|----------------|---------------|
| Bayesian information criteria | 21,510 | 4,991 | 4,803 | 7,090 |
| -log-likelihood | 10,673 | 2,428 | 2,335 | 3,480 |
| ROC curve statistic | 0.833 | 0.822 | 0.811 | 0.7465 |

**Random effects:**
Random intercepts for primary care practice:

|   | Variance | Standard deviation |
|---|----------|-------------------|
|   | 0.199    | 0.446             |

**Fixed effects:**

|   | Odds Ratio (Confidence interval) | P value |
|---|---------------------------------|---------|
| Female | 1.00 [reference] | 1.00 [reference] | 1.00 [reference] | 1.00 [reference] | 1.00 [reference] |
| Male | 1.70 (1.57–1.84) | <0.001 | 2.01 (1.68–2.40) | <0.001 | 1.76 (1.47–2.11) | <0.001 | 1.44 (1.25–1.66) | <0.001 |
| Age (years) | 1.08 (1.08–1.09) | <0.001 | 1.08 (1.07–1.09) | <0.001 | 1.08 (1.07–1.09) | <0.001 | 1.07 (1.06–1.08) | <0.001 |
| Never smoked | 1.00 [reference] | 1.00 [reference] | 1.00 [reference] | 1.00 [reference] | 1.00 [reference] |
| Current Smoker | 1.60 (1.44–1.78) | <0.001 | 1.49 (1.21–1.84) | <0.001 | 1.62 (1.30–2.02) | <0.001 | 1.46 (1.20–1.77) | <0.001 |
| Ex-smoker | 1.19 (1.08–1.32) | <0.001 | 0.97 (0.77–1.22) | 0.755 | 1.21 (0.97–1.52) | 0.091 | 1.32 (1.12–1.55) | <0.001 |
| Diabetes | 1.43 (1.30–1.57) | <0.001 | 1.43 (1.16–1.76) | <0.001 | 1.27 (1.05–1.54) | 0.014 | 1.44 (1.24–1.66) | <0.001 |
| Antihypertensive medication | 1.25 (1.16–1.36) | <0.001 | 1.20 (1.01–1.43) | 0.036 | 1.32 (1.11–1.56) | 0.001 | 1.16 (1.01–1.33) | 0.040 |
| HDL Cholesterol | 1.25 (1.16–1.36) | <0.001 | 0.73 (0.60–0.90) | 0.004 | 0.87 (0.70–1.08) | 0.203 | 0.77 (0.65–0.91) | 0.002 |
| Phosphate: <0.75 mmol/l | 0.83 (0.64–1.09) | 0.175 | 0.59 (0.36–0.97) | 0.049 | 0.60 (0.32–1.14) | 0.117 | 1.11 (0.71–1.73) | 0.647 |
| Phosphate: 0.75–1.00 mmol/l | 1.00 [reference] | 1.00 [reference] | 1.00 [reference] | 1.00 [reference] | 1.00 [reference] |
| Phosphate: 1.00–1.25 mmol/l | 1.09 (0.99–1.19) | 0.069 | 1.19 (0.98–1.43) | 0.077 | 1.12 (0.92–1.36) | 0.270 | 1.07 (0.91–1.25) | 0.420 |
| Phosphate: 1.25–1.50 mmol/l | 1.27 (1.13–1.42) | <0.001 | 1.36 (1.06–1.74) | 0.016 | 1.40 (1.09–1.81) | 0.010 | 1.21 (1.00–1.46) | 0.054 |
| Phosphate: >1.50 mmol/l | 2.19 (1.72–2.80) | <0.001 | 1.80 (0.89–3.63) | 0.100 | 1.51 (0.72–3.13) | 0.272 | 2.34 (1.64–3.32) | <0.001 |

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Table 5. Cox regression analysis: The clinical characteristics of people with normal renal function and people with CKD stages 3–5, CKD stages 1–2, and odds ratio of cardiovascular events and mortality during 30 months of follow up.

| Clinical characteristic | Complete population | Normal renal function | CKD stages 1–2 | CKD stages 3–5 |
|-------------------------|---------------------|-----------------------|----------------|----------------|
|                         | Hazard Ratio        | P value               | Hazard Ratio   | P value        |
|                         | (Confidence interval)|                      | (Confidence interval)|                      |
| Female                  | 1.00 [reference]    |                      | 1.00 [reference]    |                      |
| Male                    | 1.58 (1.45–1.72)    | <0.001                | 1.08 (1.07–1.08)    | <0.001                |
| Age (years)             | 1.07 (1.07–1.08)    | <0.001                | 1.89 (1.60–2.24)    | <0.001                |
| Never smoked            | 1.00 [reference]    |                      | 1.00 [reference]    |                      |
| Current Smoker          | 1.50 (1.35–1.66)    | <0.001                | 1.43 (1.18–1.73)    | <0.001                |
| Ex-smoker               | 1.22 (1.10–1.35)    | <0.001                | 0.99 (0.80–1.22)    | 0.909                |
| Diabetes                | 1.32 (1.20–1.44)    | <0.001                | 1.39 (1.15–1.68)    | <0.001                |
| Antihypertensive medication | 1.23 (1.13–1.33) | <0.001                | 1.14 (0.97–1.34)    | 0.108                |
| HDL Cholesterol         | 0.78 (0.70–0.86)    | <0.001                | 0.68 (0.56–0.83)    | <0.001                |
| Phosphate: <0.75 mmol/l | 0.80 (0.60–1.07)    | 0.127                 | 0.58 (0.36–0.97)    | 0.049                |
| Phosphate: 0.75–1.00 mmol/l | 1.00 [reference] |                        | 1.00 [reference]    |                        |
| Phosphate: 1.00–1.25 mmol/l | 1.11 (1.01–1.22) | 0.031                 | 1.19 (1.00–1.42)    | 0.054                |
| Phosphate: 1.25–1.50 mmol/l | 1.29 (1.15–1.45) | <0.001                | 1.38 (1.09–1.73)    | 0.007                |
| Phosphate: >1.50 mmol/l | 2.20 (1.75–2.78)    | <0.001                | 1.62 (0.86–3.07)    | 0.138                |
|                         |                      |                      | Hazard Ratio (Confidence interval) | P value |
|                         |                      |                      | 1.06 (1.05–1.07)    | <0.001                |
|                         |                      |                      | 1.31 (1.03–1.66)    | 0.026                |
|                         |                      |                      | 1.44 (0.73–2.83)    | 0.288                |
|                         |                      |                      | 2.40 (1.82–3.16)    | <0.001                |

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Additionally, very few people had serum PTH measurements. It is therefore not possible to conclude from these data that there is no relationship between serum PTH and cardiovascular events.

Serum phosphate levels are subject to diurnal variation with a minimum level in the morning with a subsequent rise throughout the day [39,40]. As we did not have data on the times at which individual serum samples were taken, we were unable to adjust for this diurnal variation. It is likely that this will have weakened the association with cardiovascular outcomes. Fixed time sampling (e.g. 9am phosphate levels) may demonstrate improved correlation with cardiovascular outcomes. However, our data suggests that a random day-time phosphate measurement can be correlated with cardiovascular outcome.

**Further research**

Whilst the mechanisms underlying this association remain to be fully elucidated the possibility that phosphate represents a potential therapeutic target should be considered. Trials looking at the impact of phosphate binders and dietary phosphate restriction have focused on end-stage renal disease and have mostly been underpowered for detection of reduced cardiovascular events [41–43], although sevelamer (a phosphate binder) has been demonstrated to reduce all-cause mortality in CKD stages 3–4.

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**Figure 1.** The adjusted odd ratio of a cardiovascular event during a 30 month follow up period for the complete population and sub populations; 24,184 people with normal renal function, 20,356 people with CKD stages 1–2, and 13,292 people with CKD stages 3–5 by serum phosphate category.

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Author Contributions
Conceived and designed the experiments: APM SdL. Performed the experiments: APM. Analyzed the data: APM MR. Contributed reagents/materials/analysis tools: APM SJ. Wrote the paper: APM SdL JvV HL CT SJ HG MR.

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