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A Cross-Sectional Study Measuring Vanadium and Chromium Levels in Paediatric CKD Patients

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Abbreviations: Vanadium (V), Chromium (Cr), International Agency for Research on Cancer (IARC), chronic kidney disease (CKD), High Resolution Sector Field Inductively Coupled Mass Spectrometry (HR-SF-ICP-MS), Centers for Disease Control (CDC).

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Abstract

Objectives: Although many of the secondary effects of high levels of vanadium (V) and chromium (Cr) overlap with symptoms commonly seen in paediatric patients with chronic kidney disease (CKD), plasma V and Cr levels in paediatric CKD patients are understudied.

Design: Ancillary cross-sectional study to a prospective longitudinal randomized controlled trial.

Setting: Children's Hospital of Western Ontario, London Health Sciences Centre, London, Ontario, Canada.

Participants: 36 children and adolescents 4-18 years of age with CKD.

Interventions: 1-6 trace element measurements per patient. Cystatin C estimated glomerular filtration rate (eGFR) was calculated using the Filler formula. Plasma V and Cr levels were measured using HR-SF-ICP-MS. Anthropomorphic data and other blood parameters were collected from our electronic chart program. Data for water V and Cr levels were obtained from the Ontario Water (Stream) Quality Monitoring Network.

Primary and Secondary Outcome Measures: Primary: plasma zinc levels; Secondary: cystatin C eGFR, plasma chromium, copper, molybdenum, vanadium.

Results: Median (IQR) eGFR was 51 mL/min/1.73 m^2 (35, 75). V levels were significantly greater than the 97.5th percentile of the reference interval of 0.088 µg/L and 32 patients had at least one set of V levels above the published reference interval. The median Cr level was 0.43 µg/L (0.36, 0.54), which was also significantly greater than the established reference interval. V and Cr levels were moderately correlated. Only some of our patients had high environmental exposure.

Conclusions: Our study suggests that paediatric patients with CKD have elevated plasma levels of V and Cr. This may be the result of both environmental exposure and a low eGFR. It may be necessary to monitor V and Cr levels in patients with an eGFR <30 mL/min/1.73m^2.

Trial Registration: NCT02126293; HC #172241

Strengths and Limitations of the Study

- This is the first study to examine V and Cr in children with CKD.
- Our study has a strong cross-sectional design in combination with longitudinal data and a reasonable number of patients for a paediatric study. The instruments used to measure the trace elements are highly precise.
- Our study links publicly-available data measuring trace elements in drinking water to detailed descriptions of patients to differentiate environmental and CKD-related accumulation of V and Cr.
- More subjects would have lead to more precise data, and our patient cohort has a bias towards milder CKD stages. We did not control for fluid or food intake.
- There are no detailed studies on the age dependency of paediatric V and Cr concentrations.

Introduction

Vanadium (V, atomic number 23) and chromium (Cr, atomic number 24) are located beside each other on the periodic table and share many points of similarity. Both are predominantly excreted by the kidneys,[1 2] are associated with normal human health and with the pathogenesis of several diseases (i.e. essential and toxic),[3-6] and occur naturally in our surrounding environment.[7 8] Metallic V and Cr do not occur in nature and are instead are found as compounds in different valence states.[8-10] Humans can be exposed to V and Cr through the air, but the majority of contact stems from food and water [7 8 11] They are usually present in low, harmless concentrations in various foods (particularly seafood for V).[1] Some nutritional supplements and vitamins also contain V, whose levels can exceed healthy levels.[4 11]

The principle pentavalent form of V can easily enter a cell via phosphate and sulphate ion channels, rendering this version of V very toxic.[12] V also interferes with phosphate-containing enzymes[3 4] and can activate several genes and participate in the inflammatory response. The highest initial concentrations of V are found in the kidneys, liver, and lungs, and in the long-term, it is stored in the bones and muscles.[13 14] The effects of V on human health are largely dependent on the type of compound,[11] dose, duration and route of exposure.[3 4 11] The common hexavalent form of Cr easily enters cells through facilitated uptake, which is more efficient than the simple diffusion used to take up the trivalent form. Free radicals are created in cells when Cr (VI) is reduced to Cr (III).[8] It is distributed to and accumulated by the erythrocyte and the highest concentrations of Cr are found in the kidneys and liver. The absorption fraction of ingested Cr is higher when dietary intakes are lower. The major route of elimination of absorbed Cr is through the urine, with unabsorbed Cr recovered in the faeces,[2] while V is also mostly excreted through the urine, with some excretion in the faeces.[15]

V has many systemic effects, including gastrointestinal, respiratory, haematological, immunological, and cardiovascular effects.[1 7 16 17] The International Agency for Research on Cancer (IARC) has classified V pentoxide as "possibly carcinogenic." Other data also suggest the potential of V to induce developmental effects in humans. It is unknown whether children are affected by V-containing compounds in the same ways as adults.[11]

The IARC has classified Cr as carcinogenic. Inhalation has been shown to cause lung cancer in humans, and exposure has been shown to cause tumours in the stomach, intestinal tract, and lungs in animals.[8] Depending on the route of exposure, trivalent and hexavalent Cr are also associated with gastrointestinal, immunological, haematological (including anaemia), reproductive, developmental, and other serious effects. Hexavalent Cr is more toxic than its trivalent form, but trace element measurements rarely differentiate between the two.

Several studies show that the serum concentrations of V and Cr are higher in adult haemodialysis patients and chronic kidney disease (CKD) patients; this suggests that they accumulate in the body.[5 18-20] Another study confirmed this association by measuring trace elements in the hair of adult haemodialysis patients.[6] Although data in paediatric CKD patients are elusive, we hypothesized that similar to dialysis patients, we would find elevated V and Cr levels in paediatric CKD patients.[21] We also hypothesized that levels would increase with worsening glomerular filtration rate (GFR).

Patients and Methods

STUDY DESIGN

The study adhered to the Declaration of Helsinki. The Research Ethics Board of the University of Western Ontario approved the study as part of an intervention study on zinc supplementation in CKD patients centred at McMaster University (NCT02126293; HC #172241; REB #104976). Patients were recruited from April 2014 to April 2016. Given that almost all patients enrolled within the first 3 months had elevated V and Cr levels, we created this ancillary cross-sectional study to specifically analyze the elevated trace elements in this population. The primary outcome of the original study was patient plasma zinc levels, while the secondary outcomes were the patient plasma trace element levels.

STUDY POPULATION

See Figure 1 for inclusion and exclusion criteria. We performed an interim analysis on 36 study patients (16 female, 44%; average age 11.85 ± 4.5 years, age range 4.42-18.98 years) with various renal pathologies and diagnosed CKD (as per the KDIGO guidelines[22]) using the modified Schwartz formula[23] at the London Health Sciences Centre, a tertiary paediatric nephrology centre. Since this was an ancillary study, the study did not specifically select for the stage of CKD, the patient's age, or the location of the patient's residence, which could introduce potential bias. Patients also had different numbers of repeated samples, depending on their clinic visits. Patients did not record their fluid or food intake, which could influence the results. To address potential bias due to contaminated drinking water, we matched the postal code of the patient's home with provincial 2014 water quality data.

EXPERIMENTAL METHODS

eGFR was calculated using the Filler formula,[24] using the new international reference materials.[25] Plasma samples were collected in BD K_2-EDTA Royal Blue Vacutainer tubes (Reference #368381). V and Cr levels were measured using High Resolution Sector Field Inductively Coupled Mass Spectrometry (HR-SF-ICP-MS; https://ltig.lhsc.on.ca/?action=view_rec&test=Vanadium%2C%20Plasma; https://ltig.lhsc.on.ca/?action=view_rec&test=Chromium%2CPlasma; last accessed 27-Jun-2016). Total imprecision (CV) of the V measurements was 10% at low concentration (0.100 µg/L), 4% at medium concentration (0.257 µg/L), and 8% at high concentration (0.356 µg/L). For Cr, the total imprecision (CV) was 3% (0.82 µg/L), 4% (5.40 µg/L) and 5% (43.60 µg/L), respectively. Anthropomorphic data, the first three digits of patients' postal codes, and other blood parameters were collected from our electronic chart program, PowerChart (Cerner). Data were entered into an Excel spread sheet (Excel for Mac 2011, version 14.4.4.).

Data analysis was performed using GraphPad Prism 5 for Mac OS X, version 5.0f, and HLM 7.01, Scientific Software International, Inc., Skokie, IL, USA. Data were analysed for normal distribution using the D'Agostino & Pearson omnibus normality test. As most data were normally distributed, parametric methods were used for all statistical tests, with the exception of the V and Cr levels and estimated glomerular filtration rate (eGFR), which were expressed as median and interquartile range (25^{th}, 75^{th} percentile). Spearman's rank correlation analysis was used to analyse the correlation analysis of V levels that were not normally distributed. The Wilcoxon Signed Rank test was used to compare the V and Cr levels with the 97.5^{th} percentile as neither V nor Cr levels were normally distributed. A repeated measures analysis was conducted to determine whether the results of repeated measurements affected the original results.

Heat maps depicting V and Cr levels in drinking water were generated using data collected by the Government of Ontario for their Provincial (Stream) Water Quality Monitoring Network, which can found at https://www.ontario.ca/data/provincial-stream-water-quality-monitoring-network. Only the most recent data were used (2014, https://files.ontario.ca/moe_mapping/downloads/2Water/PWQMN_by_year/pwqmn_rawdata_2014.xlsx). The station coordinate data (found at https://files.ontario.ca/moe_mapping/downloads/2Water/PWQMN1.xlsx) was used to determine the longitude and latitude of each testing station in order to generate the map. Since each station had a varying number of results, only the most recent measurement was used, unless that measurement was a negative number, in which case the second most recent measurement was used. Measurements used in the maps were taken between spring and winter of 2014. The V or Cr measurement at each station and the longitude and latitude of each station were then uploaded in two separate files to open source mapping software created by Pete Warden, which can be found at www.openheatmap.com. Patients' locations in Southern Ontario were mapped using the first three digits of their postal codes (data on file).

Results

Thirty-six children and adolescents with CKD and at least one set of trace element data were included in the study (Figure 1, Table 1). Median eGFR was 51 mL/min/1.73 m^2 (35, 75). V levels were not normally distributed (D'Agostino & Pearson omnibus test p-value <0.0001). The median V level was 0.12 µg/L (0.09, 0.18) and the maximum V level was 3.350 µg/L. Thirty-two patients had at least one set of V levels above the published reference interval of 0.088 µg/L (Table 2) in either unit, and the results of 75 of the 94 total tests (80%) were above the interval. The V levels were significantly greater than the 97.5th percentile of the reference interval of

0.088 µg/L (Wilcoxon Signed Rank test p<0.0001). There was a weak negative correlation between the V levels and the eGFR (Spearman r=-0.1209, Figure 2). In patients with repeated V levels, there was no statistically significant change between the first and the last measured level (Figure 3).

Cr levels were not normally distributed (D'Agostini & Pearson omnibus test p-value <0.0001). The median Cr level was 0.43 µg/L (0.36, 0.54), which was significantly greater than the 97.5th percentile of the reference interval of 0.31µg/L (Wilcoxon Signed Rank test p<0.0001). Thirty-four patients had at least one Cr level that was above the reference interval for healthy children and adults (77 of 94 tests [82%]). There was a very weak non-significant positive correlation between Cr and eGFR (Spearman r=0.09111, p=0.3851, Figure 4). The median level did not change from first to last measurement, and the perceived rise in the values was not statistically significant (p=0.3381, Wilcoxon matched-pairs signed rank test, Figure 5). There was a moderate but significant correlation between V and Cr levels (Spearman r=0.5973, p=<0.0001). Similar results were found with a repeated measures analysis (HLM 7.01, Scientific Software International, Inc., Skokie, IL, USA) that accounted for all measurement points.

The V levels measured in water ranged from 0.00257 µg/L to 5.87 µg/L, and the measured Cr levels in water ranged from 0.0099 µg/L to 4.32 µg/L. The mapped V data shows higher concentrations grouped along the Detroit and Saint Clair Rivers as well as the shores around Lake Erie and Lake Huron (Figure 6). The mapped Cr levels show the same general distribution pattern but with slightly larger values overall (Figure 7).

Comparing the two maps to a map of the patients' locations from the study, we found that although some groupings of patients corresponded to the areas of high V and Cr (such as

Windsor, St. Thomas, Hanover, and Owen Sound, n=8), others were located in areas of low concentrations. Comparing these distributions leads us to believe that our findings are the result of both environmental factors and impaired kidney function.

Discussion

Our study demonstrates a high prevalence of elevated V and Cr levels in paediatric CKD patients. With V, this trend is strongest in patients with an eGFR of less than 30 mL/min/1.73 m^2. In 28 of the 36 patients, V and Cr exposure in drinking water did not seem to be the major contributing factor.

The results are concerning as chronic V and Cr toxicities have many serious systemic effects. For V this includes neurocognitive deficits and neurobehavioral abilities, which are regularly seen in paediatric CKD patients.[26] Cr appears even more toxic; with oral exposure, it has been linked to (i) death, and at lethal doses respiratory effects such as pleural effusion, pulmonary oedema, bronchitis, and bronchopneumonia, and cardiovascular effects such as cardiopulmonary arrest, and a drop in cardiac output, heart rate and blood pressure; (ii) gastrointestinal effects from Cr in drinking water such as oral ulcer, diarrhoea, abdominal pain, indigestion, and vomiting; (iii) haematological effects from Cr in drinking water including leucocytosis and immature neutrophils; (iv) musculoskeletal effects such as rhabdomyolysis; (v) hepatic effects such as the development of jaundice, and increased bilirubin, serum lactic dehydrogenase, alanine and aspartate aminotransferase, and γ-glutamyl transferase; (vi) renal effects such as renal failure characterized by proteinuria, haematuria and anuria, necrosis of renal tubules, oliguria, and destruction of the tubular epithelium of the kidneys, highly elevated serum creatinine and blood urea nitrogen; (vii) Cr supplementation has also been associated with weight loss.

If ingested orally, V can cause mild gastrointestinal irritation such as stomach cramps, mild diarrhoea, and nausea.[7] It can also induce haematological effects such as microcytic erythrocytosis.[7] Immunological effects such as significant decreases in lymphocyte stimulation and an increase in the incidence of viral and bacterial respiratory infections have been noted in children, a vulnerable population.[16] Another study found a significant association between V and systolic blood pressure and pulse pressure in an elderly population.[17] Of course, haematopoiesis is impaired in the advanced stages of CKD and nephrotoxicity should be avoided.

Homeostasis of both trace elements depends on uptake and elimination. Uptake occurs through different types of environmental exposures, namely through the air, water, food, and soil. More than 70% of Cr in the environment comes from anthropogenic sources, such as non-ferrous base metal smelters, refineries, leather tanning industries, urban storm water runoff, effluent streams from pulp and paper mills and discharges from thermal generating stations.[27] In Canada, it is largely released into the atmosphere through different means of pollution, namely industrial processes (1/3), stationary fuel consumption (1/2), and transportation (3/20).[28] Ground water and other fresh water contain V (in the form of $H_2VO_4^-$) at an approximate concentration of 1.17 µg/L, fats, fruits, and vegetables at 1-5 µg/kg, and meat, seafood, whole grains, and dairy products at 5-30 µg/kg.[29] The daily intake via food is usually between 10-200 µg.[30] Cr (III), the less toxic isoform of Cr, is also naturally found in many foods.[8] Both hexavalent and trivalent Cr are found in water.[27]

Although previous studies have shown elevated V and Cr levels in adult dialysis patients,[19] we are unaware of any published work showing high V and Cr levels in paediatric CKD patients other than in abstract form.[31] Since CKD patients are usually polyuric and develop polydipsia, their exposure to V in drinking water is several-fold greater than the general population.

Combined with the fact that V is mainly eliminated through the kidneys,[1 3] this vulnerable population's environmental exposure to toxic trace elements may pose a danger, especially if they have polyuria and polydipsia and drink contaminated water. We suspect that in addition to our patients' low V and Cr clearance, their significantly greater exposure to V and Cr in drinking water is the reason for their high levels of both elements.

There are few guidelines for maximum V concentrations in the environment, and those that exist either cover a wide range from different sources, are outdated, or were determined using extremely limited and unacceptable methodologies.[32] The Office of Environmental Health Hazard Assessment in California released a statement in August of 2000 in response to the Department of Health Service's proposed level not exceeding 50 µg/L, recommending a lower level of 15 µg/L.[33] Meanwhile, Environment Canada released Federal Environmental Quality Guidelines for V in May of 2016, listing the predicted no-effect concentration for marine water at 5 µg/L and for freshwater at 120 µg/L. The Canadian federal government does not include V in their most recent Guidelines for Canadian Drinking Water Quality (2014),[34] nor is it included in the water quality guidelines used by Ontario.[35] Other Canadian water quality guidelines list standards ranging from 3.9 to 250 µg/L.[36 37] The standard for Cr in Ontario drinking water is 50 µg/L. The Centers for Disease Control (CDC) has established some minimal risk levels (MRLs) for humans, defined as an estimate of daily human exposure to V and Cr that is likely to be without an appreciable risk of adverse effects over a specified duration of exposure.[7] These limits are as follows: oral acute (none), intermediate-duration (10 µg/kg/day for V and 0.5 µg/kg/day), and chronic-duration (none for V and 0.9 µg/kg/day for Cr).

The average concentration of Cr in uncontaminated surface and marine water is generally below 1.0 µg/L,[28] but in Ontario these numbers can be much greater at sites most severely affected by

pollution. This includes the St. Marys River system with concentrations of 31 000 µg/g dry weight (d.w.) in Tannery Bay and concentrations exceeding 5120 µg/g (d.w.) in the Welland River downstream from a steel manufacturing plant compared to 10 µg/g (d.w.) upstream.[28] Up to 1920 µg/g (d.w.) has been found in Detroit River sediments and 564 µg/g (d.w.) in Hamilton Harbour sediments.[6] Elevated concentrations of two- to four-fold above local background levels have also been reported in sediments from Lake Simcoe, the Detroit River, Lake Ontario off the Niagara River and the St. Lawrence River.[30] Our catchment area overlaps with several of these areas.

Twenty-eight of our patients were not from the highly polluted areas. However, some of the highest V and Cr levels were observed in patients with a low GFR, despite living in areas with low V and Cr exposure. We believe that the high water intake with CKD and the low GFR are two additional factors that result in the high prevalence of elevated V and Cr in these patients.

Clearly, the scientific evidence indicating both the safe levels and the resultant toxicity of V in our air, water, and food is lacking, of poor quality, and conflicting, especially considering how little, if any, of this research has been done on i) vulnerable populations such as children and ii) children with impaired renal or hepatic function. The guidelines for Cr are slightly clearer considering its increased toxicity, but there are still little data on chronic safe levels and toxicity. Since high V and Cr levels are seen in such a large proportion of our study population, and since the environmental levels of V and Cr and the resultant health implications are mostly unknown, the question becomes: how can we limit the exposure of children with poor renal function to these potentially toxic trace elements?

Several studies have shown the potential of chelating agents such as tiron in reducing V body burden and toxicity.[7] The safety and efficacy of this strategy has not been established in children. Therefore, preventing high exposure may be the only feasible strategy, but using bottled water with low trace element levels may be very costly.

A strength of our study is its cross-sectional design in combination with longitudinal data and a reasonable number of patients for a paediatric pilot study. Another strength is the high precision of the instruments used to measure the trace elements. There are still several limitations. More subjects would have lead to more precise data, and our patient cohort has a bias towards milder CKD stages, which could have potentially minimized the trace element levels seen in this population. We did not assess whether the patients drink municipal water, well water or bottled water (with presumably lower concentrations) nor did we assess their total fluid intake. We also did not control for food intake. Certain foods such as fish, shellfish[38] and grains and cereals[39] may contain dangerous V concentrations. These potential confounders could have minimized the environmental impact of the high levels in this population. Finally, detailed studies on the age dependency of paediatric V and Cr concentrations are elusive.

Despite these limitations, our data robustly demonstrate a high prevalence of elevated V and Cr levels in children with CKD. There was a trend toward higher V levels with worsening kidney function. Our data would favour monitoring V and Cr in paediatric CKD patients, especially in areas with high exposures or in very polyuric patients.

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Contributors' Statement

Contributors GF and VB articulated the conceptual framework for both the original RCT study and this ancillary study. GF developed the analytical approach and GF and MK analysed the data. GF, MK, and HKS drafted and edited the manuscript. VB, SHSH and LY contributed to the interpretation of data, added intellectual content during manuscript preparation and provided valuable feedback on various aspects of the manuscript. All authors read and approved the final manuscript.

Variable	n (%)
Gender	
Male	22 (58%)
Female	16 (42%)
Age group (years)	
4 to 10	18 (47%)
11 to 15	9 (24%)
16 to 18	11 (29%)
Primary Diagnosis	
Hereditary	
Renal Dysplasia	11 (31%)
Metabolic Disorders	5 (14%)
Nephronophthisis	2 (6%)
Autosomal Recessive Polycystic Kidney Disease	2 (6%)
Autosomal Dominant Polycystic Kidney Disease	1 (3%)
Congenital Nephrotic Syndrome	1 (3%)
Alport Syndrome	1 (3%)
Acquired	
Reflux Nephropathy	4 (11%)
Haemolytic Uremic Syndrome	3 (8%)
Glomerulonephritis/Focal Segmental Glomerulosclerosis	3 (8%)
Tubulopathy	2 (6%)
Ischaemic Renal Injury	1 (3%)
Kidney Transplant	
Yes	13 (36%)
No	23 (64%)

Table 1. Patient Demographics.

Paediatric Reference Intervals				
	µg/L		µmol/L	
	Lower	Upper	Lower	Upper
Vanadium	0.032	0.088	0.6	1.7
Chromium	0.13	0.31	2.5	6

Table 2. Paediatric reference intervals used in our study. Paediatric-specific vanadium and chromium reference intervals were not available. Source: http://www.lhsc.on.ca/lab/memos/Reference_Ranges_for_Trace_Elements_2014_11_03.pdf.

Figure 1. Patient flowchart. Of the 42 subjects assessed for eligibility in the study, we included 36 in our analysis. These 36 patients had 94 trace element panel measurements.

Figure 2. Scatter plot of vanadium levels versus eGFR. Vanadium levels [µg/L] were not normally distributed. The non-linear regression line (one-phase exponential decay) has been included. GFR was measured in mL/min/1.73 m^2. The formula reads: Y=(Y0 - Plateau)*exp(-K*x) + Plateau, where Y0 is the value when x is zero, plateau is Y at a large value, K is the rate constant, and the values were Y0=4.555, Plateau=0.1457, and K=0.1111 (GraphPad Prism).

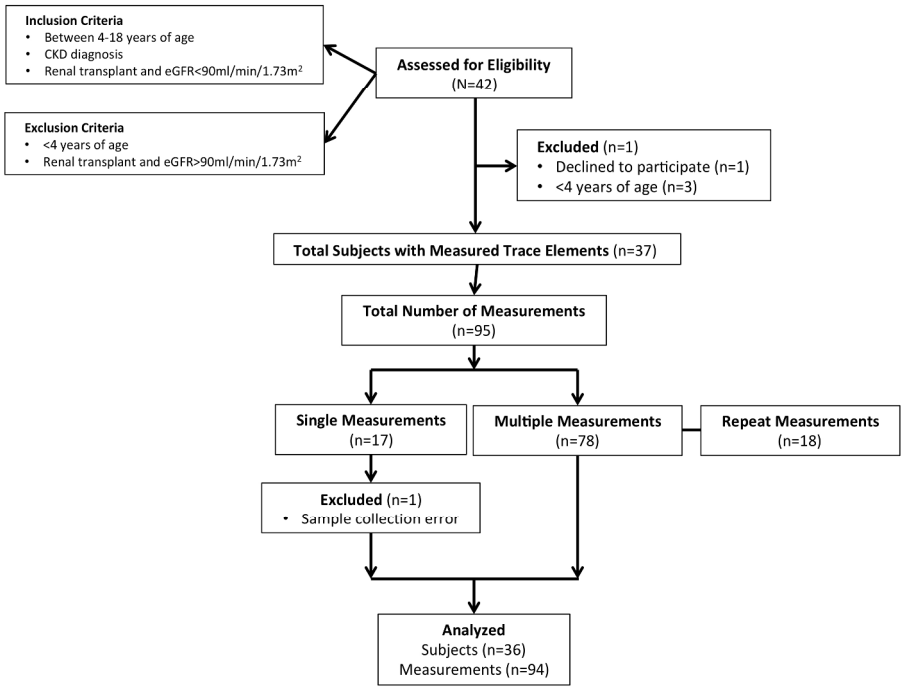
Figure 3. Repeated measures plot of first and last vanadium level in those patients who had repeated levels. Vanadium levels [µg/L] were not normally distributed. While the median vanadium level lowered from 0.1510 to 0.1410 µg/L and many patients demonstrated an increase of their vanadium level with repeated measures, this did not reach statistical significance (p=0.4140, Wilcoxon matched-pairs signed rank test).

Figure 4. Scatter plot of chromium levels versus eGFR. Chromium levels [µg/L] were not normally distributed. The non-linear regression line (one-phase exponential decay) has been included. GFR was measured in mL/min/1.73 m^2. The formula reads: Y=(Y0 - Plateau)*exp(-K*x) + Plateau, where Y0 is the value when x is zero, plateau is Y at a large value, K is the rate constant, and the values were Y0=1.641, Plateau=0.4841, and K=0.09060 (GraphPad Prism).

Figure 5. Repeated measures plot of first and last chromium level in those patients who had repeated levels. Chromium levels [µg/L] were not normally distributed. The median chromium level did not change from 0.44 µg/L and the rise in the values was not statistically significant (p=0.3381, Wilcoxon matched-pairs signed rank test).

Figure 6. Heat map showing the concentration of vanadium in 2014 in various streams around Southwestern Ontario. Map created using 2014 data from the Provincial (Stream) Water Quality Monitoring Network at https://www.ontario.ca/data/provincial-stream-water-quality-monitoring-network in open source software found at www.openheatmap.com

Figure 7. Heat map showing the concentration of chromium in 2014 in various streams around Southwestern Ontario. Map created using 2014 data from the Provincial (Stream) Water Quality Monitoring Network at https://www.ontario.ca/data/provincial-stream-water-quality-monitoring-network in open source software found at www.openheatmap.com



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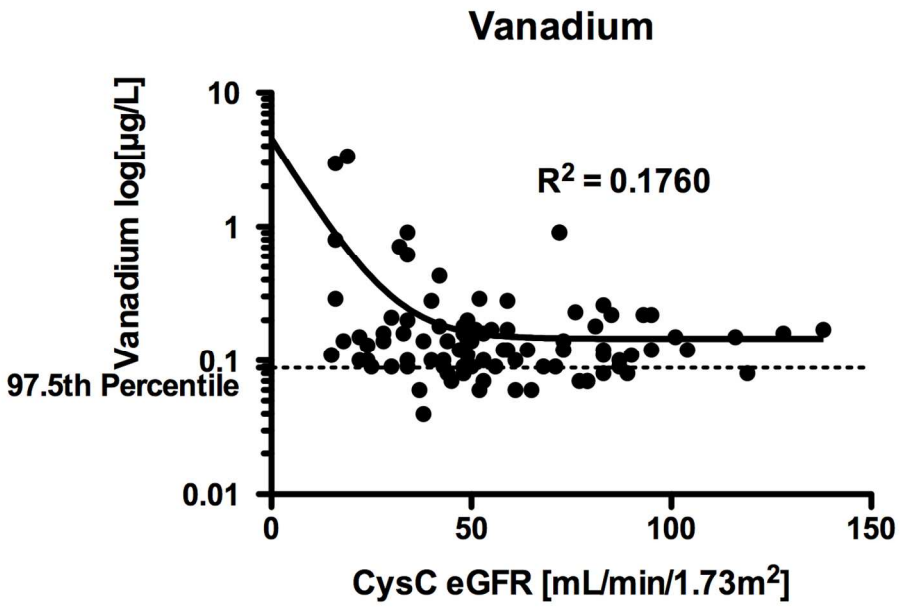

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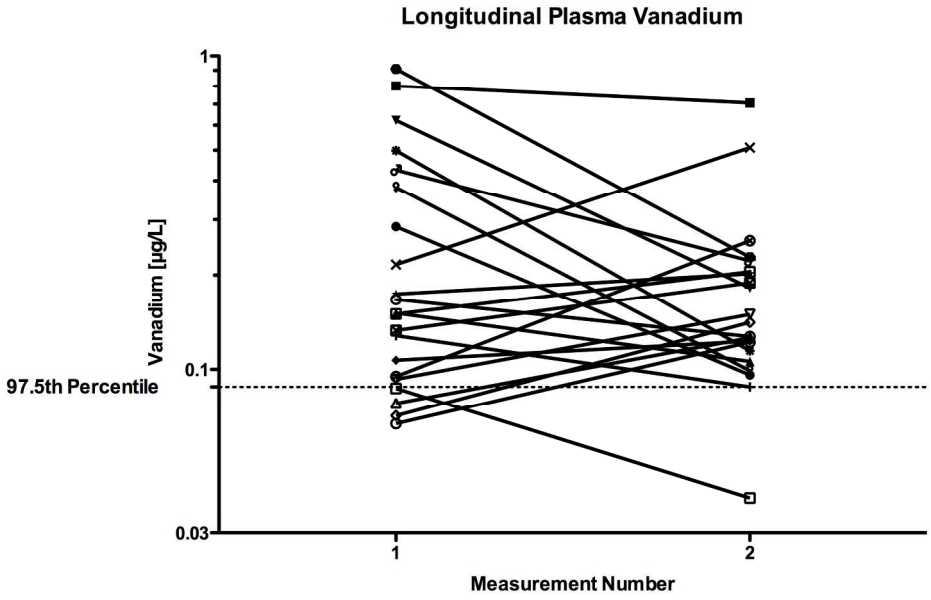
127x85mm (300 x 300 DPI)



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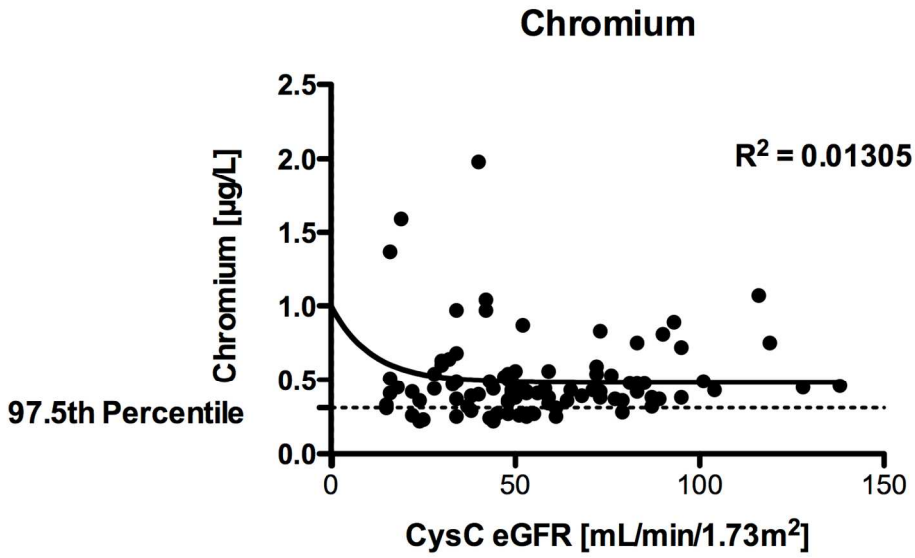

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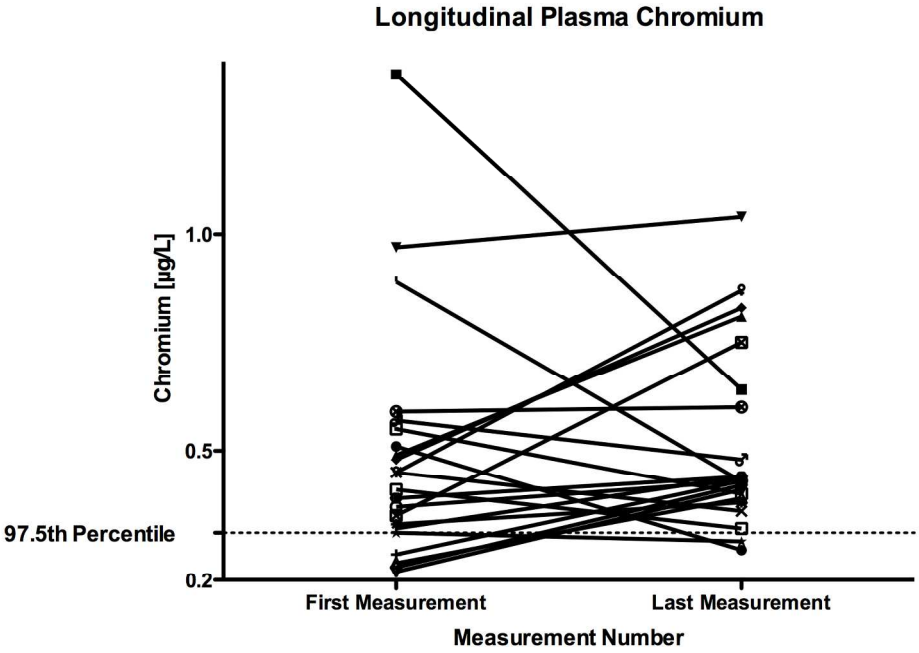
138x85mm (300 x 300 DPI)



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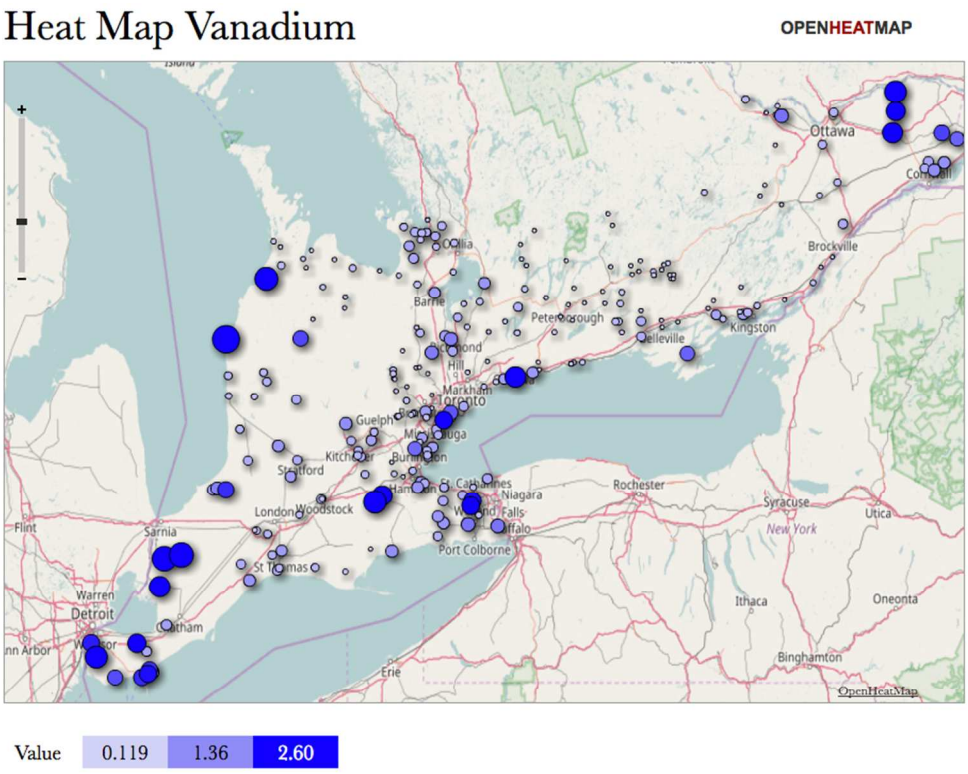

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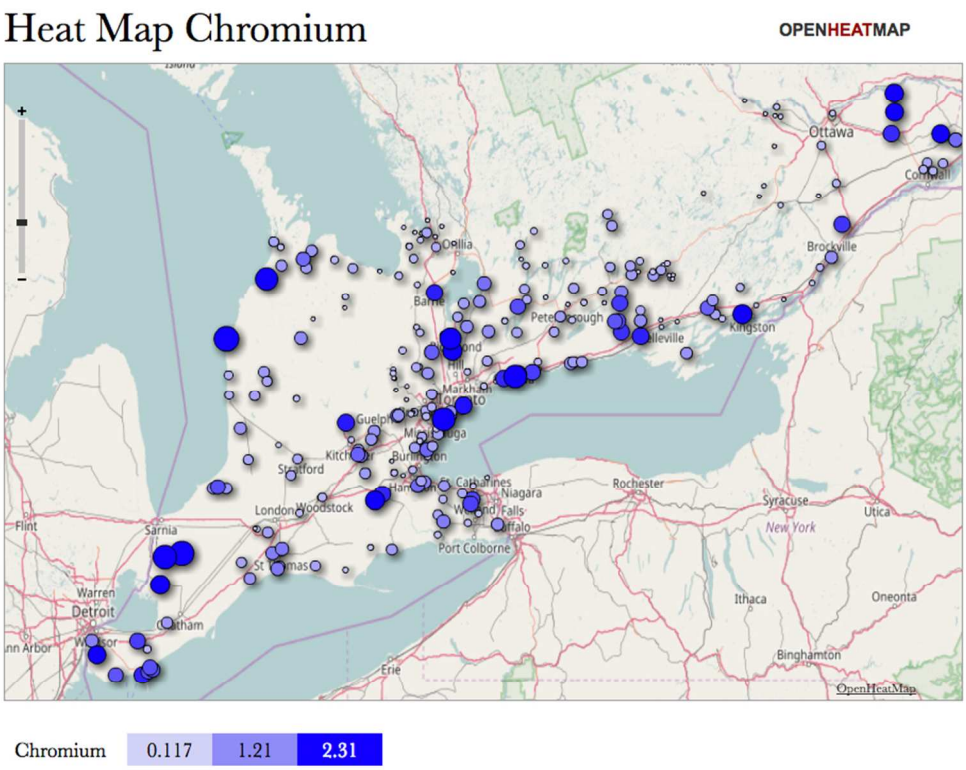
330x258mm (72 x 72 DPI)



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332x257mm (72 x 72 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	6 Figure 1
Variables	7	Clearly define all *outcomes*, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	7-8
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1
		(b) Give reasons for non-participation at each stage	Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	Figure 1
Outcome data	15*	Report numbers of outcome events or summary measures	8-9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included.	8-9
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11, 13-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

A Cross-Sectional Study Measuring Vanadium and Chromium Levels in Paediatric CKD Patients

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A Cross-Sectional Study Measuring Vanadium and Chromium Levels in Paediatric CKD Patients

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Short title: Vanadium in CKD

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Financial Disclosure: The authors have no financial relationships relevant to this article to disclose.

Conflict of Interest: The authors have no conflicts of interest relevant to this article to disclose.

Clinical Trial Registration: clinicaltrials.gov, NCT02126293, https://clinicaltrials.gov/ct2/show/NCT02126293

Abbreviations: Vanadium (V), Chromium (Cr), International Agency for Research on Cancer (IARC), chronic kidney disease (CKD), High Resolution Sector Field Inductively Coupled Mass Spectrometry (HR-SF-ICP-MS), Centers for Disease Control (CDC).

Word Count: Abstract: 281; Manuscript: 3,104

Abstract

Objectives: Although many secondary effects of high levels of vanadium (V) and chromium (Cr) overlap with symptoms seen in paediatric patients with chronic kidney disease (CKD), their plasma V and Cr levels are understudied.

Design: Ancillary cross-sectional study to a prospective longitudinal randomized controlled trial.

Setting: Children's Hospital of Western Ontario, London Health Sciences Centre, London, Ontario, Canada.

Participants: 36 children and adolescents 4-18 years of age with CKD.

Interventions: 1-6 trace element measurements per patient. Cystatin C (CysC) estimated glomerular filtration rate (eGFR) was calculated using the Filler formula. Plasma V and Cr levels were measured using HR-SF-ICP-MS. Anthropomorphic data and blood parameters were collected from our electronic chart program. Water Cr and V data were obtained from the Ontario Water (Stream) Quality Monitoring Network.

Primary and Secondary Outcome Measures: Primary outcomes: Plasma Cr and V. Secondary outcomes: Age, season, CysC, CysC eGFR, and Cr and V levels in environmental water.

Results: Median (IQR) eGFR was 51 mL/min/1.73 m^2 (35, 75). The median V level was 0.12 µg/L (0.09, 0.18), which was significantly greater than the 97.5th percentile of the reference interval of 0.088 µg/L; 32 patients had at least one set of V levels above the published reference interval. The median Cr level was 0.43 µg/L (0.36, 0.54), which was also significantly greater than the established reference interval; 34 had at least one set of Cr levels above the published reference interval. V and Cr levels were moderately correlated. Only some patients had high environmental exposure.

Conclusions: Our study suggests that paediatric patients with CKD have elevated plasma levels of V and Cr. This may be the result of both environmental exposure and a low eGFR. It may be necessary to monitor V and Cr levels in patients with an eGFR<30 mL/min/1.73m^2.

Trial Registration: NCT02126293; HC #172241

Strengths and Limitations of the Study

- This is the first study to examine V and Cr in children with CKD.
- Our study has a strong cross-sectional design in combination with longitudinal data and a reasonable number of patients for a paediatric study. The instruments used to measure the trace elements are highly precise.
- Our study links publicly-available data measuring trace elements in drinking water to detailed descriptions of patients to differentiate environmental and CKD-related accumulation of V and Cr.
- More subjects would have lead to more precise data, and our patient cohort has a bias towards milder CKD stages. We did not control for fluid or food intake.
- There are no detailed studies on the age dependency of paediatric V and Cr concentrations.

Introduction

Vanadium (V, atomic number 23) and chromium (Cr, atomic number 24) are located beside each other on the periodic table and share many points of similarity. Both are predominantly excreted by the kidneys,[1 2] are associated with normal human health and with the pathogenesis of several diseases (i.e. essential and toxic),[3-6] and occur naturally in our surrounding environment.[7 8] Metallic V and Cr do not occur in nature and are instead found as compounds in different valence states.[8-10] Humans can be exposed to V and Cr through the air, but the majority of contact stems from food and water [7 8 11] They are usually present in low, harmless concentrations in various foods (particularly seafood for V).[1] Some nutritional supplements and vitamins also contain V, whose levels can exceed healthy levels.[4 11]

The principle pentavalent form of V can easily enter a cell via phosphate and sulphate ion channels, rendering this version of V very toxic.[12] V also interferes with phosphate-containing enzymes[3 4] and can activate several genes and participate in the inflammatory response. The highest initial concentrations of V are found in the kidneys, liver, and lungs, and in the long-term, it is stored in the bones and muscles.[13 14] The effects of V on human health are largely dependent on the type of compound,[11] dose, duration, and route of exposure.[3 4 11] The common hexavalent form of Cr easily enters cells through facilitated uptake, which is more efficient than the simple diffusion used to take up the trivalent form. Free radicals are created in cells when Cr (VI) is reduced to Cr (III).[8] It is distributed to and accumulated by the erythrocyte and the highest concentrations of Cr are found in the kidneys and liver. The absorption fraction of ingested Cr is higher when dietary intakes are lower. The major route of elimination of absorbed Cr is through the urine, with unabsorbed Cr recovered in the faeces,[2] while V is also mostly excreted through the urine, with some excretion in the faeces.[15]

V has many systemic effects, including gastrointestinal, respiratory, haematological, immunological, and cardiovascular effects.[1 7 16 17] The International Agency for Research on Cancer (IARC) has classified V pentoxide as "possibly carcinogenic." Other data also suggest the potential of V to induce developmental effects in humans. It is unknown whether children are affected by V-containing compounds in the same ways as adults.[11]

The IARC has classified Cr as carcinogenic. Inhalation has been shown to cause lung cancer in humans, and exposure has been shown to cause tumours in the stomach, intestinal tract, and lungs in animals.[8] Depending on the route of exposure, trivalent and hexavalent Cr are also associated with gastrointestinal, immunological, haematological (including anaemia), reproductive, developmental, and other serious effects. Hexavalent Cr is more toxic than its trivalent form, but trace element measurements rarely differentiate between the two.

The danger V and particularly Cr pose to our health are made clear when the serious systemic effects associated with either acute or chronic exposure to either element in humans are explored in greater depth.

Acute oral exposure to V can cause mild gastrointestinal irritation such as stomach cramps, mild diarrhoea, and nausea.[7] Although there are no studies examining the effect of chronic oral exposure to V on human health, chronic exposure to V through inhalation may cause neurocognitive deficits and may impair neurobehavioral abilities, symptoms which are regularly seen in paediatric CKD patients.[18] Chronic exposure to V through inhalation has also been shown to cause immunological effects such as significant decreases in lymphocyte stimulation and an increase in the incidence of viral and bacterial respiratory infections in children, a vulnerable population.[16] Another study examining the health effects of chronically inhaling V

found a significant association between V and systolic blood pressure and pulse pressure in an elderly population.[17]

Cr appears even more toxic; acute oral exposure has been linked to (i) death, and at lethal doses respiratory effects such as pleural effusions, pulmonary oedema, bronchitis, and bronchopneumonia; (ii) cardiovascular effects such as cardiopulmonary arrest, cardiac arrest, and a drop in cardiac output, heart rate and blood pressure; (iii) gastrointestinal haemorrhage and necrosis, abdominal pain and vomiting; (iv) haematological effects such as inhibited coagulation; (v) hepatic effects such as the development of jaundice, fatty degeneration, hepatic necrosis, and increased bilirubin, serum lactic dehydrogenase, alanine and aspartate aminotransferase, and γ-glutamyl transferase; and (vi) renal effects such as renal failure characterized by proteinuria, haematuria and anuria, renal necrosis, necrosis and swelling of renal tubules, oliguria, and destruction of the tubular epithelium of the kidneys, highly elevated serum creatinine and blood urea nitrogen; and (vii) metabolic acidosis.[8] Two studies in particular have shown the effects of chronic oral exposure to Cr(VI). Both studies are exceptionally relevant to our study and consisted of populations who 1had Cr(VI)-contaminated drinking water. The first study, from an area near a ferrochromium production plant in the Liaoning Province in China, found an association between contaminated water and gastrointestinal effects such as oral ulcers, diarrhoea, abdominal pain, indigestion, and an increased incidence of lung and stomach cancer.[8] [19] The second study, from an area of Greece with elevated Cr(VI) in the public drinking water, found significantly higher standardized mortality ratios for primary liver, lung, kidney, and genitourinary cancer.[8] [20]

Several studies show that the serum concentrations of V and Cr are higher in adult haemodialysis patients and chronic kidney disease (CKD) patients; this suggests that they accumulate in the

body.[5 21-23] Another study confirmed this association by measuring trace elements in the hair of adult haemodialysis patients.[6] Although data in paediatric CKD patients are elusive, we hypothesized that similar to dialysis patients, we would find elevated V and Cr levels in paediatric CKD patients who were not receiving dialysis treatments.[24] We also hypothesized that levels would increase with worsening glomerular filtration rate (GFR).

Patients and Methods

STUDY DESIGN

The study adhered to the Declaration of Helsinki. The Research Ethics Board of the University of Western Ontario approved the study as part of an intervention study on zinc supplementation in CKD patients centred at McMaster University (NCT02126293; HC #172241; REB #104976). Patients were recruited from April 2014 to April 2016. Given that almost all patients enrolled within the first 3 months had elevated V and Cr levels, we created this ancillary cross-sectional study to specifically analyze the elevated trace elements in this population. The primary outcome of the original study was patient plasma zinc levels, while the secondary outcomes were the patient plasma trace element levels.

STUDY POPULATION

See Figure 1 for inclusion and exclusion criteria. We performed an interim analysis on 36 study patients (16 female, 44%; average age 11.85 ± 4.5 years, age range 4.42-18.98 years) with various renal pathologies and diagnosed CKD (as per the KDIGO guidelines[25]) using the modified Schwartz formula[26] at the London Health Sciences Centre, a tertiary paediatric nephrology centre. Patients with Stage 1 (eGFR> 90 mL/min/1.73 m^2) to Stage 5 (<15 mL/min/1.73 m^2) CKD were included in the study. Patients receiving dialysis treatments were

excluded from the study because the dialysis water could further affect the patients' V and Cr levels. Since this was an ancillary study, the study did not specifically select for the stage of CKD, the patient's age, or the location of the patient's residence, which could introduce potential bias. Patients also had different numbers of repeated samples, depending on their clinic visits. Patients did not record their fluid or food intake, which could influence the results. To address potential bias due to contaminated drinking water, we matched the postal code of the patient's home with provincial 2014 water quality data.

EXPERIMENTAL METHODS

eGFR was calculated using the Filler formula,[27] using the new international reference materials.[28] Plasma samples were collected in BD K_2-EDTA Royal Blue Vacutainer tubes (Reference #368381). V and Cr levels were measured using High Resolution Sector Field Inductively Coupled Mass Spectrometry (HR-SF-ICP-MS; https://ltig.lhsc.on.ca/?action=view_rec&test=Vanadium%2C%20Plasma; https://ltig.lhsc.on.ca/?action=view_rec&test=Chromium%2CPlasma; last accessed 27-Jun-2016). Total imprecision (CV) of the V measurements was 10% at low concentration (0.100 µg/L), 4% at medium concentration (0.257 µg/L), and 8% at high concentration (0.356 µg/L). For Cr, the total imprecision (CV) was 3% (0.82 µg/L), 4% (5.40 µg/L) and 5% (43.60 µg/L), respectively. Anthropomorphic data (patient height measured by stadiometer, necessary to calculate the Schwartz eGFR), the first three digits of patients' postal codes, and creatinine and cystatin C were collected from our electronic chart program, PowerChart (Cerner). Data were entered into an Excel spread sheet (Excel for Mac 2011, version 14.4.4.).

Data analysis was performed using GraphPad Prism 5 for Mac OS X, version 5.0f, and HLM 7.01, Scientific Software International, Inc., Skokie, IL, USA. Data were analysed for normal

distribution using the D'Agostino & Pearson omnibus normality test. As most data were normally distributed, parametric methods were used for all statistical tests, with the exception of the V and Cr levels and estimated glomerular filtration rate (eGFR), which were expressed as median and interquartile range (25^{th}, 75^{th} percentile). Spearman's rank correlation analysis was used to analyse the correlation analysis of V levels that were not normally distributed. The Wilcoxon Signed Rank test was used to compare the V and Cr levels with the 97.5^{th} percentile as neither V nor Cr levels were normally distributed. A repeated measures analysis was conducted to determine whether the results of repeated measurements (from the same day) affected the original results.

Heat maps depicting V and Cr levels in drinking water were generated using data collected by the Government of Ontario for their Provincial (Stream) Water Quality Monitoring Network, which can found at https://www.ontario.ca/data/provincial-stream-water-quality-monitoring-network. Only the most recent data were used (2014, https://files.ontario.ca/moe_mapping/downloads/2Water/PWQMN_by_year/pwqmn_rawdata_2014.xlsx). The station coordinate data (found at https://files.ontario.ca/moe_mapping/downloads/2Water/PWQMN1.xlsx) was used to determine the longitude and latitude of each testing station in order to generate the map. Since each station had a varying number of results, only the most recent measurement was used, unless that measurement was a negative number, in which case the second most recent measurement was used. Measurements used in the maps were taken between spring and winter of 2014. The V or Cr measurement at each station and the longitude and latitude of each station were then uploaded in two separate files to open source mapping software created by Pete Warden, which can be

found at www.openheatmap.com. Patients' locations in Southern Ontario were mapped using the first three digits of their postal codes (data on file).

Results

Thirty-six children and adolescents with CKD and at least one set of trace element data were included in the study (Figure 1, Table 1). Median eGFR was 51 mL/min/1.73 m^2 (35, 75). V levels were not normally distributed (D'Agostino & Pearson omnibus test p-value <0.0001). The median V level was 0.12 µg/L (0.09, 0.18) and the maximum V level was 3.350 µg/L. Thirty-two patients had at least one set of V levels above the published reference interval of 0.088 µg/L (Table 2) in either unit, and the results of 75 of the 94 total tests (80%) were above the interval. The V levels were significantly greater than the 97.5th percentile of the reference interval of 0.088 µg/L (Wilcoxon Signed Rank test p<0.0001). There was a weak negative correlation between the V levels and the eGFR (Spearman r=-0.1209, Figure 2). In patients with repeated V levels, there was no statistically significant change between the first and the last measured level (Figure 3).

Cr levels were not normally distributed (D'Agostini & Pearson omnibus test p-value <0.0001). The median Cr level was 0.43 µg/L (0.36, 0.54), which was significantly greater than the 97.5th percentile of the reference interval of 0.31µg/L (Wilcoxon Signed Rank test p<0.0001). Thirty-four patients had at least one Cr level that was above the reference interval for healthy adults (77 of 94 tests [82%]). There was a very weak non-significant positive correlation between Cr and eGFR (Spearman r=0.09111, p=0.3851, Figure 4). The median level did not change from first to last measurement, and the perceived rise in the values was not statistically significant (p=0.3381, Wilcoxon matched-pairs signed rank test, Figure 5). There was a moderate but significant

correlation between V and Cr levels (Spearman r=0.5973, p=<0.0001). Similar results were found with a repeated measures analysis (HLM 7.01, Scientific Software International, Inc., Skokie, IL, USA) that accounted for all measurement points. Neither V nor Cr levels were associated with age or season.

The V levels measured in water ranged from 0.00257 µg/L to 5.87 µg/L, and the measured Cr levels in water ranged from 0.0099 µg/L to 4.32 µg/L. The mapped V data shows higher concentrations grouped along the Detroit and Saint Clair Rivers as well as the shores around Lake Erie and Lake Huron (Figure 6). The mapped Cr levels show the same general distribution pattern but with slightly larger values overall (Figure 7).

Comparing the two maps to a map of the patients' locations from the study, we found that although some groupings of patients corresponded to the areas of high V and Cr (such as Windsor, St. Thomas, Hanover, and Owen Sound, n=8), others were located in areas of low concentrations.

Discussion

Our study demonstrates a high prevalence of elevated V and Cr levels in paediatric CKD patients. With V, this trend is strongest in patients with an eGFR of less than 30 mL/min/1.73 m^2. In 28 of the 36 patients, V and Cr exposure in drinking water did not seem to be the major contributing factor.

Homeostasis of both trace elements depends on uptake and elimination. Uptake occurs through different types of environmental exposures, namely through the air, water, food, and soil. More than 70% of Cr in the environment comes from anthropogenic sources, such as non-ferrous base metal smelters, refineries, leather tanning industries, urban storm water runoff, effluent streams

from pulp and paper mills and discharges from thermal generating stations.[29] In Canada, it is largely released into the atmosphere through different means of pollution, namely industrial processes (1/3), stationary fuel consumption (1/2), and transportation (3/20).[30] Ground water and other fresh water contain V (in the form of $H_2VO_4^-$) at an approximate concentration of 1.17 µg/L, fats, fruits, and vegetables at 1-5 µg/kg, and meat, seafood, whole grains, and dairy products at 5-30 µg/kg.[31] The daily intake via food is usually between 10-200 µg.[32] Cr (III), the less toxic isoform of Cr, is also naturally found in many foods.[8] Both hexavalent and trivalent Cr are found in water.[29]

Although previous studies have shown elevated V and Cr levels in adult dialysis patients,[22] we are unaware of any published work showing high V and Cr levels in paediatric CKD patients other than in abstract form.[33] Since CKD patients are usually polyuric and develop polydipsia, their exposure to V in drinking water is several-fold greater than the general population. Combined with the fact that V is mainly eliminated through the kidneys,[1 3] this vulnerable population's environmental exposure to toxic trace elements may pose a danger, especially if they have polyuria and polydipsia and drink contaminated water. We suspect that in addition to our patients' low V and Cr clearance, their significantly greater exposure to V and Cr in drinking water is the reason for their high levels of both elements. Since some groupings of patients corresponded to the areas of high V and Cr and others did not, this leads us to believe that our findings are the result of both environmental factors and impaired kidney function.

There are few guidelines for maximum V concentrations in the environment, and those that exist either cover a wide range from different sources, are outdated, or were determined using extremely limited and unacceptable methodologies.[34] The Office of Environmental Health Hazard Assessment in California released a statement in August of 2000 in response to the

Department of Health Service's proposed level not exceeding 50 µg/L, recommending a lower level of 15 µg/L.[35] Meanwhile, Environment Canada released Federal Environmental Quality Guidelines for V in May of 2016, listing the predicted no-effect concentration for marine water at 5 µg/L and for freshwater at 120 µg/L. The Canadian federal government does not include V in their most recent Guidelines for Canadian Drinking Water Quality (2014),[36] nor is it included in the water quality guidelines used by Ontario.[37] Other Canadian water quality guidelines list standards ranging from 3.9 to 250 µg/L.[38 39] The standard for Cr in Ontario drinking water is 50 µg/L. The Centers for Disease Control (CDC) has established some minimal risk levels (MRLs) for humans, defined as an estimate of daily human exposure to V and Cr that is likely to be without an appreciable risk of adverse effects over a specified duration of exposure.[7] These limits are as follows: oral acute (none), intermediate-duration (10 µg/kg/day for V and 0.5 µg/kg/day), and chronic-duration (none for V and 0.9 µg/kg/day for Cr).

The average concentration of Cr in uncontaminated surface and marine water is generally below 1.0 µg/L,[30] but in Ontario these numbers can be much greater at sites most severely affected by pollution. This includes the St. Marys River system with concentrations of 31 000 µg/g dry weight (d.w.) in Tannery Bay and concentrations exceeding 5120 µg/g (d.w.) in the Welland River downstream from a steel manufacturing plant compared to 10 µg/g (d.w.) upstream.[30] Up to 1920 µg/g (d.w.) has been found in Detroit River sediments and 564 µg/g (d.w.) in Hamilton Harbour sediments.[6] Elevated concentrations of two- to four-fold above local background levels have also been reported in sediments from Lake Simcoe, the Detroit River, Lake Ontario off the Niagara River and the St. Lawrence River.[32] Our catchment area overlaps with several of these areas.

Twenty-eight of our patients were not from the highly polluted areas. However, some of the highest V and Cr levels were observed in patients with a low GFR, despite living in areas with low V and Cr exposure. We believe that the high water intake with CKD and the low GFR are two additional factors that result in the high prevalence of elevated V and Cr in these patients.

Clearly, the scientific evidence indicating both the safe levels and the resultant toxicity of V in our air, water, and food is lacking, of poor quality, and conflicting, especially considering how little, if any, of this research has been done on i) vulnerable populations such as children and ii) children with impaired renal or hepatic function. The guidelines for Cr are slightly clearer considering its increased toxicity, but there are still little data on chronic safe levels and toxicity. Since high V and Cr levels are seen in such a large proportion of our study population, and since the environmental levels of V and Cr and the resultant health implications are mostly unknown, the question becomes: how can we limit the exposure of children with poor renal function to these potentially toxic trace elements?

Several studies have shown the potential of chelating agents such as tiron in reducing V body burden and toxicity.[7] The safety and efficacy of this strategy has not been established in children. Therefore, preventing high exposure may be the only feasible strategy, but using bottled water with low trace element levels may be very costly.

A strength of our study is its cross-sectional design in combination with longitudinal data and a reasonable number of patients for a paediatric pilot study. Another strength is the high precision of the instruments used to measure the trace elements. There are still several limitations. More subjects would have lead to more precise data, and our patient cohort has a bias towards milder CKD stages, which could have potentially minimized the trace element levels seen in this

population. Additionally, although the reference intervals that we used were specific to our catchment area and to the equipment used to measure plasma trace elements at our site, they are based on a cohort of adults and have not been published. We did not assess whether the patients drink municipal water, well water or bottled water (with presumably lower concentrations) nor did we assess their total fluid intake. We also did not control for food intake. Certain foods such as fish, shellfish[40] and grains and cereals[41] may contain dangerous V concentrations. These potential confounders could have minimized the environmental impact of the high levels in this population. Finally, detailed studies on the age dependency of paediatric V and Cr concentrations are elusive. Additionally, the tubulotoxicity of Cr, in particular, may influence the progression of CKD. Although we did not examine the tubulotoxicity found in our patient cohort, there is often mixed tubular and glomerular proteinuria in CKD patients and proximal tubular dysfunction in CKD.[42] Some recent research in animal models has suggested that selenium supplementation could reverse some of the tubular damage.[43] Of course, haematopoiesis is impaired in the advanced stages of CKD and nephrotoxicity should be avoided.

Despite these limitations, our data robustly demonstrate a high prevalence of elevated V and Cr levels in children with CKD. There was a trend toward higher V levels with worsening kidney function. Our data would favour monitoring V and Cr in paediatric CKD patients, especially in areas with high exposures or in very polyuric patients.

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Contributors' Statement

Contributors GF and VB articulated the conceptual framework for both the original RCT study and this ancillary study. GF developed the analytical approach and GF and MK analysed the data. GF, MK, and HKS drafted and edited the manuscript. VB, SHSH and LY contributed to the interpretation of data, added intellectual content during manuscript preparation and provided valuable feedback on various aspects of the manuscript. All authors read and approved the final manuscript.

Variable	n (%)
Gender	
Male	22 (58%)
Female	16 (42%)
Age group (years)	
4 to 10	18 (47%)
11 to 15	9 (24%)
16 to 18	11 (29%)
Primary Diagnosis	
Hereditary	
Renal Dysplasia	11 (31%)
Metabolic Disorders	5 (14%)
Nephronophthisis	2 (6%)
Autosomal Recessive Polycystic Kidney Disease	2 (6%)
Autosomal Dominant Polycystic Kidney Disease	1 (3%)
Congenital Nephrotic Syndrome	1 (3%)
Alport Syndrome	1 (3%)
Acquired	
Reflux Nephropathy	4 (11%)
Haemolytic Uremic Syndrome	3 (8%)
Glomerulonephritis/Focal Segmental Glomerulosclerosis	3 (8%)
Tubulopathy	2 (6%)
Ischaemic Renal Injury	1 (3%)
Kidney Transplant	
Yes	13 (36%)
No	23 (64%)

Table 1. Patient Demographics.

Paediatric Reference Intervals				
	µg/L		µmol/L	
	Lower	Upper	Lower	Upper
Vanadium	0.032	0.088	0.6	1.7
Chromium	0.13	0.31	2.5	6

Table 2. Paediatric reference intervals used in our study. Paediatric-specific vanadium and chromium reference intervals were not available. Source: http://www.lhsc.on.ca/lab/memos/Reference_Ranges_for_Trace_Elements_2014_11_03.pdf.

Figure 1. Patient flowchart. Of the 38 subjects assessed for eligibility in the study, we included 36 in our analysis. These 36 patients had 94 trace element panel measurements.

Figure 2. Scatter plot of vanadium levels versus eGFR. Vanadium levels [µg/L] were not normally distributed. The non-linear regression line (one-phase exponential decay) has been included. GFR was measured in mL/min/1.73 m^2. The formula reads: Y=(Y0 - Plateau)*exp(-K*x) + Plateau, where Y0 is the value when x is zero, plateau is Y at a large value, K is the rate constant, and the values were Y0=4.555, Plateau=0.1457, and K=0.1111 (GraphPad Prism).

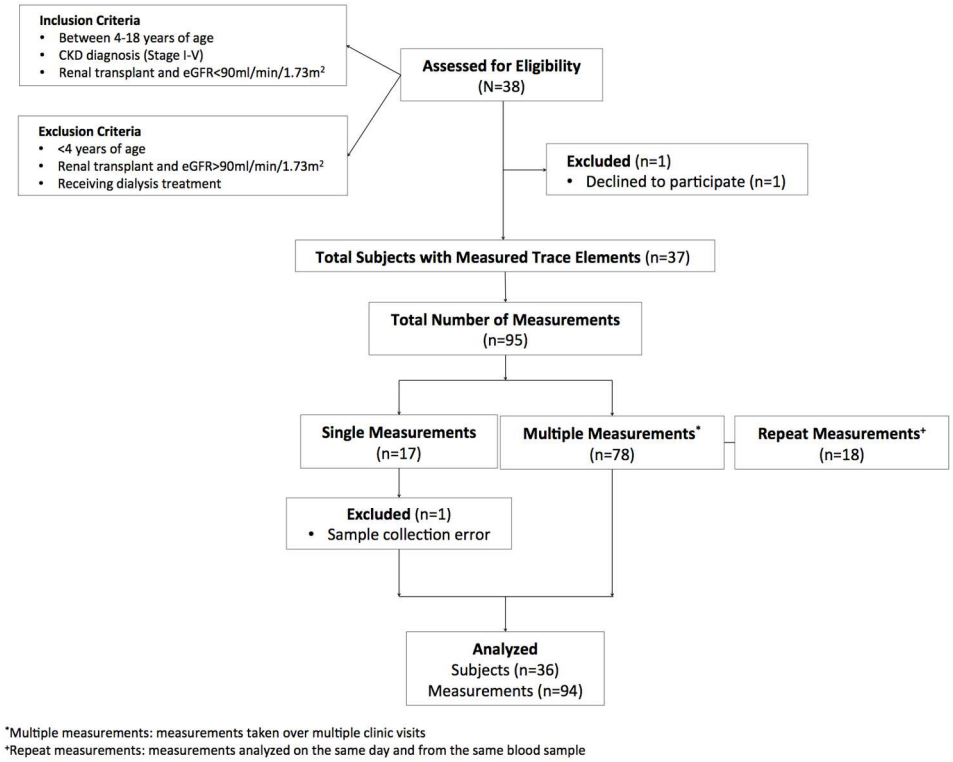
Figure 3. Repeated measures plot of first and last vanadium level in those patients who had repeated levels. Vanadium levels [µg/L] were not normally distributed. While the median vanadium level lowered from 0.1510 to 0.1410 µg/L and many patients demonstrated an increase of their vanadium level with repeated measures, this did not reach statistical significance (p=0.4140, Wilcoxon matched-pairs signed rank test).

Figure 4. Scatter plot of chromium levels versus eGFR. Chromium levels [µg/L] were not normally distributed. The non-linear regression line (one-phase exponential decay) has been included. GFR was measured in mL/min/1.73 m^2. The formula reads: Y=(Y0 - Plateau)*exp(-K*x) + Plateau, where Y0 is the value when x is zero, plateau is Y at a large value, K is the rate constant, and the values were Y0=1.641, Plateau=0.4841, and K=0.09060 (GraphPad Prism).

Figure 5. Repeated measures plot of first and last chromium level in those patients who had repeated levels. Chromium levels [µg/L] were not normally distributed. The median chromium level did not change from 0.44 µg/L and the rise in the values was not statistically significant (p=0.3381, Wilcoxon matched-pairs signed rank test).

Figure 6. Heat map showing the concentration of vanadium in 2014 in various streams around Southwestern Ontario. Map created using 2014 data from the Provincial (Stream) Water Quality Monitoring Network at https://www.ontario.ca/data/provincial-stream-water-quality-monitoring-network in open source software found at www.openheatmap.com

Figure 7. Heat map showing the concentration of chromium in 2014 in various streams around Southwestern Ontario. Map created using 2014 data from the Provincial (Stream) Water Quality Monitoring Network at https://www.ontario.ca/data/provincial-stream-water-quality-monitoring-network in open source software found at www.openheatmap.com



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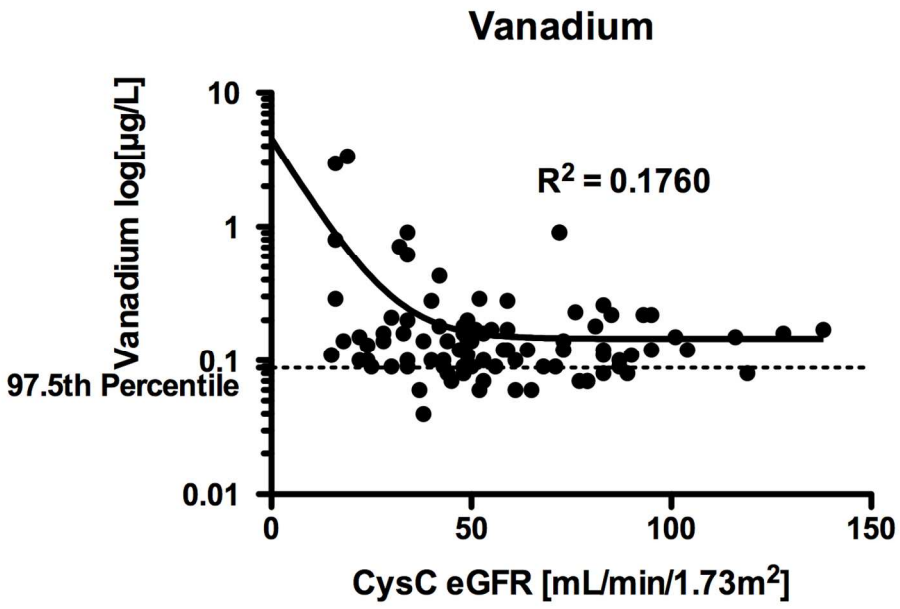

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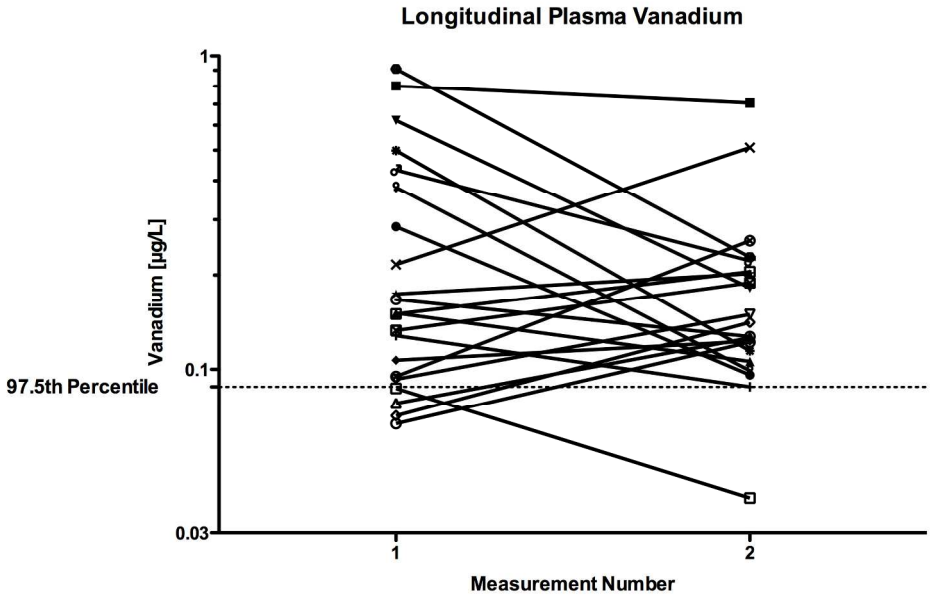

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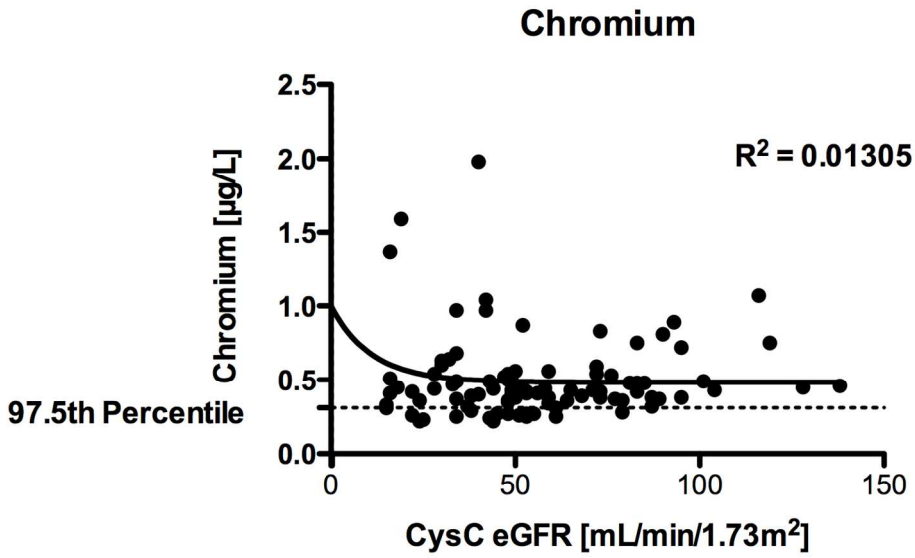

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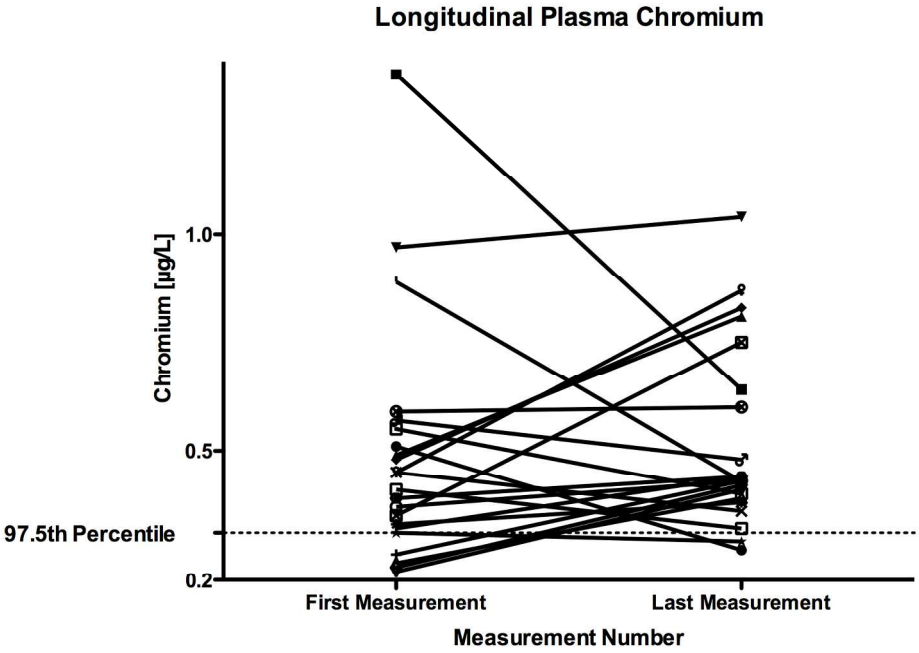

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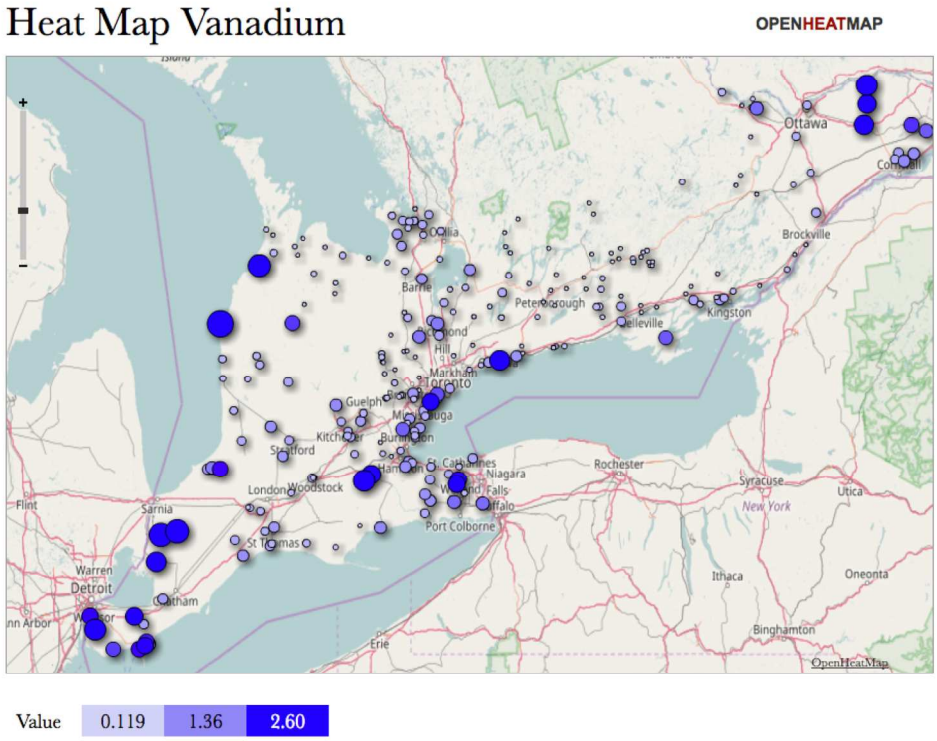

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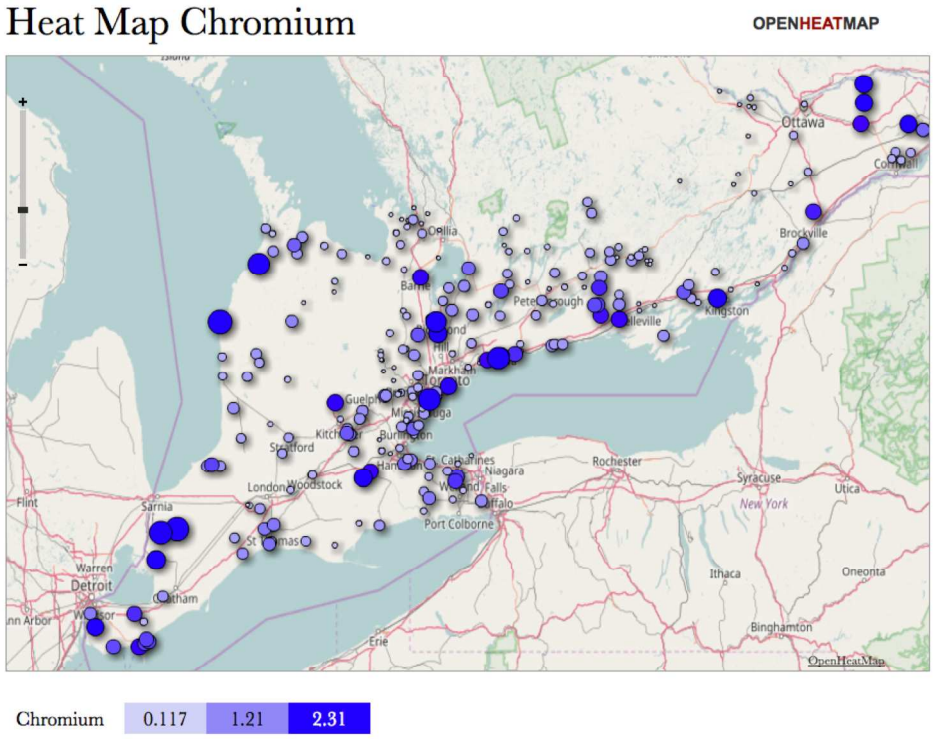

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190x149mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	6 Figure 1
Variables	7	Clearly define all *outcomes*, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	7-8
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	7
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1
		(b) Give reasons for non-participation at each stage	Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	Figure 1
Outcome data	15*	Report numbers of outcome events or summary measures	8-9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included.	8-9
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13-14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11, 13-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.