ORIGINAL ARTICLE

Metals in urine in relation to the prevalence of pre-diabetes, diabetes and atherosclerosis in rural India

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ABSTRACT

Objective Diabetes and cardiovascular diseases are growing burdens in rural communities worldwide. We have observed a high prevalence of diabetes among rural farming communities in India and sought to evaluate the association of non-traditional risk factors, such as metals, with diabetes and other cardiometabolic risk factors in this community.

Methods Anthropometric measurements, chemistries and carotid intima-media thickness were determined in 865 participants of the Kovai Medical Center and Hospital-Nallampatti Non-Communicable Disease Study-I (KMCH-NNCD-I, 2015), a cross-sectional study conducted in a farming village in South India. Urinary metal levels were determined by inductively couped plasma-mass spectrometry analysis and corrected to urinary creatinine level. Statistical analyses were performed to study the association between urinary metal levels and clinical parameters.

Results 82.5% of the study population were involved in farming and high levels of toxic metals were detected in the synthetic fertilisers used in the study village. The prevalence of pre-diabetes, diabetes and atherosclerosis was 43.4%, 16.2% and 10.3%, respectively. On logistic regression analysis, no association of traditional risk factors such as body mass index, blood pressure and total cholesterol with disease conditions was observed, but urinary levels of metals such as arsenic, chromium, aluminium and zinc showed an association with diabetes, while arsenic and zinc showed an association with pre-diabetes and atherosclerosis.

Conclusions Our data suggest a probable role of metals in the aetiology of diabetes and cardiovascular diseases in rural communities. Identifying and eliminating the causes of increased levels of these environmental chemicals could have a beneficial impact on the burden of non-communicable diseases in rural population.

Key messages

What is already known about this subject?
► There has been a worrying increase in the prevalence of diabetes and cardiovascular diseases in rural India.
► Previous studies indicate the probable role of environmental chemicals such as toxic metals in the epidemic of metabolic diseases.
► But the association of metals and metabolic diseases in the rural world has been largely overlooked.

What are the new findings?
► Our cross-sectional study of 865 subjects from a village in South India indicated a high prevalence of pre-diabetes, diabetes and atherosclerosis with no association with traditional risk factors.
► Urine metals showed an association with pre-diabetes, diabetes and atherosclerosis in this rural population.

How might this impact on policy or clinical practice in the foreseeable future?
► This study adds to the growing body of evidence that the use of metals in anthropogenic activities contributes to increasing burden of metabolic diseases in the rural world.
► This study implicates the necessity for inclusion of metals in clinical diagnostics and therapies could be developed on targeting metal chelation.

INTRODUCTION

With successes in the treatment and eradication of many communicable diseases worldwide, non-communicable diseases (NCDs) such as obesity, type 2 diabetes mellitus (T2D) and cardiovascular diseases (CVDs) have become major threats to population health, particularly in South Asia.[1] The WHO projects diabetes prevalence to expand from 422 million (8.5%) in 2014 to 592 million (12%) in 2035.[2] Recently, either pre-diabetes or T2D has been found in six out of ten adults in large South Asian cities such as Chennai, Delhi and Karachi.[3]

Globally, a high prevalence of T2D, dyslipidaemia and hypertension has been observed in educated and more affluent groups in urban areas.[4] Such studies reinforce the idea that urbanisation, westernisation and affluence have significant roles in the explosion of NCDs. Intuitively, one would expect a low prevalence of NCDs in a rural population, where adherence to traditional lifestyles includes more physical activity and access to a more nutrient-rich (less processed foods) diet. However, our recent studies have demonstrated increased burden of T2D and CVDs in rural India.[5–9] Anecdotally, in recent years we have also observed increasing numbers of farmers in rural India seeking medical management for diabetes and hypertension who do not have the traditional risk factors for these

conditions, such as older age, physical inactivity, high-fat diet and obesity. This provided us with the rationale to investigate the role of other, non-traditional risk factors for NCDs in rural Indian communities.

As an example of a non-traditional risk factor, evidence is accumulating that environmental chemicals may be linked with increased risk for T2D and CVDs. Much of this evidence is summarised in two position statements released by the Endocrine Society on the role of environmental endocrine-disrupting chemicals in population health and disease.[10][11] Epidemiological studies from urban areas have shown an association of metals such as cadmium, arsenic, lead, mercury and uranium with T2D[12][13] and CVDs.[14] These toxic metals are ubiquitous environmental contaminants and have been implicated in abnormal glucose metabolism, beta cell dysfunction and atherosclerosis.[12–15] Synthetic fertilisers and pesticides are rich sources of toxic metals in rural farming regions. However, the role of metals in the aetiology of NCDs in rural farming populations, where metal-rich agrochemicals are used,[16] is poorly understood. Therefore, the aim of this study is to examine the associations of urinary metals with the prevalence of T2D, other cardiovascular risk factors and atherosclerosis in Nallampatti, a typical Indian farming village dominated by modern synthetic chemical-based agricultural practices.

MATERIALS AND METHODS
Kovai Medical Center and Hospital-Nallampatti Non-Communicable Disease Study-I

Nallampatti is a typical farming village in Tamil Nadu, South India (latitude: 11°21'2.39' N; longitude: 77°32'4.79' E) (online supplementary figure 1) with a population of around 3000. All participants older than 20 years of age and native of that village were invited to participate in this study through pamphlets and word of mouth. This study, named the 'Nallampatti non-communicable disease study-I – 2015 (NNCD-I, 2015)[7]', was conducted every Sunday during a period of 4 weeks (15 March–5 April 2015). Informed written consent was obtained from all participants prior to participation and followed the principles of the Declaration of Helsinki.

Data collection

Demographics, anthropometric data, non-fasting blood and spot urine samples were collected from all consented participants as previously described.[7] Body weight was measured using an electronic weighing scale (SECA 813), height was measured by a stadiometer (SECA 208), and waist circumference was measured in centimetres using a non-stretchable measuring tape between the costal margins and the iliac crest at the end of of expiration. Blood pressure was recorded using the electronic Omron machine in sitting position in the right arm (Model HEM-7130, Omron Healthcare, Singapore) on two occasions 15 min apart. The average value was used to determine the hypertension status.

Serum and plasma samples were prepared from whole blood collected appropriately by standard protocols. Glycated haemoglobin (HbA$_{1c}$) was measured using an automated high-performance liquid chromatography method (D-10-Bio-Rad), cystatin-c was determined by nephelometric method (BN ProSpec-Siemens), glucose was measured using a glucose oxidase – peroxidase method and lipid levels were measured using an automated analyser (Abbott Architect ci8200), and uric acid and creatinine levels were measured using the endpoint method (Abbott Architect ci8200). Urine protein was determined using commercially available kits as per manufacturers' instructions

and haemoglobin was determined by sodium lauryl sulfate method (Sysmex XN).

Carotid intima-media thickness (CIMT) was measured using two high-resolution B-mode ultrasound machines (GE Healthcare, Venue 40, USA), in supine position on a scan bed with the head of the patient resting comfortably, neck slightly hyperextended and the head tilted towards the opposite of the examined side. Both left and right common carotid arteries were depicted. The imaging was performed on field by two trained, final-year radiology residents under the supervision of a senior radiologist.

Definition of outcomes

Diabetes was defined as either having a history of diabetes on medications or diagnosed with HbA$_{1c}$ of ≥6.5%. People with self-reported diabetes were confirmed by reviewing their medical records and their medications. For those reported as non-diabetic, HbA$_{1c}$ ≥6.5% was considered diabetic, and HbA$_{1c}$ ranging between 5.7% and 6.4% was defined as pre-diabetic, as per the American Diabetes Association guidelines (2017). Obesity and central obesity were defined using the criteria specific for Indian populations as body mass index (BMI) ≥25 kg/m^2 and waist circumference ≥90 cm for men and ≥80 cm for women, respectively.[17] Hypertension was defined as either having a history of hypertension on medications or a systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg. Abnormal non-fasting lipid levels were defined as total cholesterol ≥200 mg/dL, low-density lipoprotein-cholesterol (LDL-C) ≥130 mg/dL, high-density lipoprotein-cholesterol (HDL-C) <40 mg/dL in men and <50 mg/dL in women, and non-HDL-C ≥160 mg/dL.[18] Participants with self-reported diseases were confirmed by review of their medical records and medications. Carotid atherosclerosis was defined as a CIMT ≥1 mm in either left or right or in both measurements.[19]

Metal analysis in fertilisers

Fertiliser samples frequently used in the study village were purchased from agrochemical shops in the village and the surrounding area of 10 km. All the fertiliser products were government-subsidised products. Briefly, 1 g of the fertiliser sample was digested in a microwave oven in nitric acid:perchloric acid (2:1) mixture as per standard protocols. Finally, the level of metals was studied by inductively coupled plasma-mass spectrometry (ICP-MS (NexION 300X; PerkinElmer, USA)) and expressed as mg/kg of fertiliser.

Assessment of exposure to metals

Among the 865 participants, 18 cases missing urine samples or other information were excluded from the study. Urine samples were immediately frozen and shipped for storage in a −80° C deep freezer. The level of metals such as cadmium, arsenic, lead, chromium, aluminium, zinc, copper and nickel in urine was determined using ICP-MS (NexION 300X; PerkinElmer) as per the standard protocol.[20] Briefly, 200 μL of urine sample was diluted with 1.8 mL diluent (5% nitric acid + 1.5% ethanol) and filtered through a 20 μm filter. The filtrate was subjected to ICP-MS analysis. The concentrations of metals were normalised to urinary creatinine and expressed as μg/mg creatinine.

Statistical methods

All statistical analyses were performed using the statistical software SPSS V.20.0. Urine metal concentrations were categorised in quartiles based on the weighted sample distribution. The association between risk factors and disease outcomes was studied
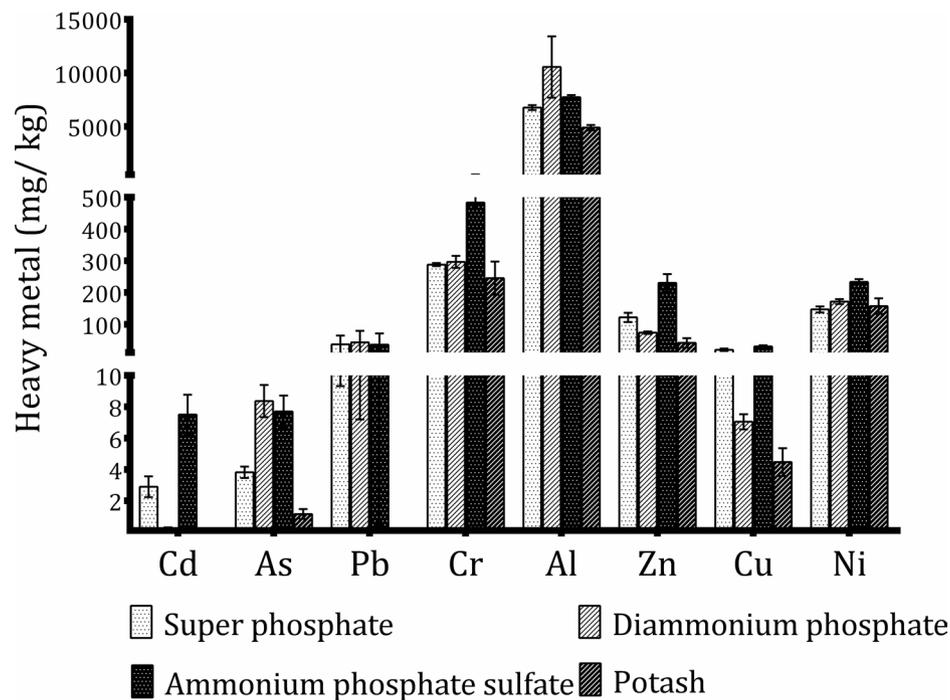
Figure 1 Concentration of toxic metals in the synthetic fertilisers being used in the study village.

by multiple logistic regression analysis, with age, sex, familial diabetic history, BMI, systolic and diastolic blood pressure, LDL-C, smoking, and alcohol and tobacco usage as confounders for adjustment in diabetes and pre-diabetes. In the case of atherosclerosis, along with above-mentioned confounders, diabetes and familial ischaemic heart disease history were used for adjustment. For each metal, we used logistic regression to estimate ORs and CI levels for diabetes, pre-diabetes and carotid atherosclerosis by comparing each quartile with the lowest quartile. Our logistic regression models were fitted with appropriate degrees of adjustment. In each analysis, we also evaluated the significance of the differences of the average proportion of disorder across the four quartiles of the model by a generalised maximum likelihood Wald χ^2 test. Subsequently, we tested for linear trends across quartiles of urine metals by including the median of each quartile as a continuous variable in logistic regression models. Spearman's correlation coefficient was calculated between metals and cardiometabolic risk factors. Statistical significance was determined on the basis of two-sided p values of <0.05.

RESULTS

Out of 865 participants in our study, 82.7% (n=715) were involved in chemical-based farming practices. Rice is the staple food of the people, and largely grown from their own land is used for day-to-day consumption. All the characteristics of the study population are shown in online supplementary table 1. The prevalence of pre-diabetes and diabetes in this population based on HbA_{1c} levels was 43.4% and 16.2%, respectively. Out of the total diabetes population (n=141), 79 (56%) were self-reported and the rest (44%) were newly diagnosed based on HbA_{1c} analysis. Pre-diabetes and diabetes were almost equally prevalent among both sexes, while atherosclerosis prevalence was relatively higher among men. Among people with pre-diabetes and diabetes, more than 50% were middle-aged (41–60 years). A very low prevalence of diabetes and atherosclerosis was noted among young-aged

people (20–40 years). On multivariate regression analysis after adjustment for confounders, among the traditional risk factors, only age and BMI showed an association with diabetes and pre-diabetes, while in the case of atherosclerosis only age showed a significant association (online supplementary table 2).

On our survey of agricultural practices in the study village, we found that superphosphate, diammonium phosphate, ammonium phosphate sulfate and potash were the major synthetic fertilisers being used. The level of toxic metals was extremely high in all types of phosphate fertilisers but not in potash fertiliser (figure 1). No significant difference in the level of aluminium, zinc, copper and nickel was found between the types of fertilisers. An increased level of these urinary metals was noted among the people with pre-diabetes, diabetes and atherosclerosis in comparison with non-diabetic and non-atherosclerotic people, respectively (online supplementary table 3). On correlation and regression analyses of the urinary metals with cardiometabolic risk factors (HbA_{1c}, systolic and diastolic blood pressure, BMI, total cholesterol, CIMT-left, CIMT-right and cystatin-c), only HbA_{1c} and CIMT showed significant correlation with the metals (table 1). Hence subsequent analyses focused only on diabetes, pre-diabetes and atherosclerosis.

Based on metal accumulation, the population was divided into quartiles (online supplementary table 4). Significant trends for pre-diabetes associated with the highest quartile of metal compared with the lowest quartile of metal (table 2) were found for arsenic and zinc. Significant trends for T2D associated with the highest quartile of metal compared with the lowest quartile of metal (table 3) were found for arsenic, chromium, aluminium and zinc. Similar to pre-diabetes, significant trends for carotid atherosclerosis associated with the highest quartile of metal compared with the lowest quartile of metal (table 4) were found for arsenic and zinc. Among the metals studied, arsenic and zinc showed association with pre-diabetes, diabetes and atherosclerosis.

Table 1 Correlation analysis between the cardiometabolic risk factors and urinary metals

| | Correlation coefficient | | | | | | | | |
	HbA$_{1c}$	Systolic BP	Diastolic BP	BMI	Total cholesterol	LDL-cholesterol	CIMT-left	CIMT-right	Cystatin-c
Cadmium	0.08*	0.01	−0.03	−0.11	−0.00	−0.03	0.02	−0.01	0.01
Arsenic	0.16***	0.37	0.02	0.01	0.10**	0.07*	0.18†	0.16†	0.09*
Lead	0.13***	−0.01	−0.03	−0.12	−0.03	−0.04	0.14†	0.13***	0.06
Chromium	0.12**	−0.01	−0.02	−0.14	−0.08	−0.07	0.07*	0.07*	0.03
Aluminium	0.09**	0.01	−0.02	−0.14	−0.04	−0.05	0.08*	0.08*	0.02
Zinc	0.17***	0.02	0.01	−0.05	0.02	−0.01	0.13***	0.12***	0.12***
Copper	0.11**	0.03	0.01	−0.08	0.01	−0.01	0.02	0.02	0.03
Nickel	0.92**	0.01	−0.03	−0.12	−0.07	−0.06	−0.02	0.01	0.03

Spearman's correlation, two-tailed: *p<0.05, **p<0.01, ***p<0.001, †p<0.0001 .
BMI, body mass index; BP, blood pressure; CIMT, carotid intima-media thickness; HbA$_{1c}$, glycated haemoglobin; LDL, low-density lipoprotein.

DISCUSSION

Although the prevalence of NCDs is commonly reported in urban populations, the WHO (2014) has estimated that 47% of the global and 67% of the Indian population live in rural villages, where NCDs are often underestimated or overlooked. Our study highlights the burden of diabetes and pre-diabetes in a rural population of India, which exceeds that estimated in the urban population centres, and raises vital questions on the association between traditional cardiometabolic risk factors and prevalent hyperglycaemic continuum in rural India. In terms of pre-diabetes, it is possible that the criteria for pre-diabetes (HbA$_{1c}$ 5.7–6.4) determined by the American Diabetes Association may over-represent pre-diabetes in an Indian population. We are particularly concerned that a high proportion of rural population in our study has either diabetes or pre-diabetes, far higher than a similar, larger study done in rural India.[6] This led us to question whether traditional risk factors alone are enough to explain the huge prevalence of NCD in this population.

Because other studies have linked toxic metals in fertilisers and other agricultural products with islet cell impairment and diabetes risk, we studied the relationships between excretion rates of several metals and prevalence of pre-diabetes, T2D

and carotid atherosclerosis. The association and possible role of two metals, arsenic and zinc, with diabetes, pre-diabetes and atherosclerosis in our study are especially intriguing. Possible links between exposure to arsenic and incident of diabetes have been reported in urban studies from various parts of the world.[13 14] This element has been proposed to increase the risk of diabetes by multiple mechanisms, including altered gene expression, increased oxidative stress, upregulation of inflammatory markers such as interleukin-6 and tumour necrosis factor alpha, disruption of glucose uptake and transport, increased gluconeogenesis, pancreatic beta cell dysfunction due to amyloid deposition, adipocyte differentiation and altered gut microbiota.[21 22] Oxidised LDL-C and several inflammatory molecules have also been implicated in arsenic-associated atherosclerosis risk.[23]

Synthetic phosphate fertilisers are important sources of metals, particularly arsenic, which can accumulate in soil with repeated application.[16] Arsenic accumulated in the soil easily enters into the groundwater or is readily adsorbed by plants, entering the animal kingdom, including humans, through the food chain. This is especially a concerning issue in the Indian context, where often poor monitoring, lack of

Table 2 ORs (95% CI) of pre-diabetes associated with quartiles of urinary metals (n=847)

| | | OR (95% CI) | | | | |
	Model	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P$_{trend}$
Cadmium	Unadjusted	1.00	1.23 (0.81 to 1.86)	1.19 (0.78 to 1.80)	**1.80 (1.18 to 2.75)**	0.110
	Adjusted*	1.00	1.09 (0.69 to 1.71)	1.02 (0.65 to 1.60)	**1.67 (1.06 to 2.64)**	0.352
Arsenic	Unadjusted	1.00	1.46 (0.96 to 2.22)	**1.82 (1.20 to 2.77)**	**2.14 (1.40 to 3.26)**	**<0.001**
	Adjusted*	1.00	**1.41 (1.01 to 2.21)**	**1.75 (1.12 to 2.73)**	**1.93 (1.23 to 3.04)**	**<0.001**
Lead	Unadjusted	1.00	1.02 (0.68 to 4.54)	1.24 (0.82 to 1.89)	1.48 (0.97 to 2.24)	**0.044**
	Adjusted*	1.00	0.91 (0.59 to 1.43)	1.29 (0.82 to 2.03)	1.36 (0.86 to 2.15)	0.065
Chromium	Unadjusted	1.00	1.10 (0.73 to 1.66)	1.17 (0.77 to 1.77)	**1.54 (1.01 to 2.35)**	**0.048**
	Adjusted*	1.00	1.08 (0.69 to 1.69)	1.16 (0.74 to 1.83)	1.48 (0.93 to 2.36)	0.069
Aluminium	Unadjusted	1.00	0.98 (0.65 to 1.48)	1.16 (0.76 to 1.77)	1.40 (0.92 to 2.13)	0.082
	Adjusted*	1.00	1.03 (0.66 to 159)	1.14 (0.72 to 1.80)	1.53 (0.97 to 2.41)	0.102
Zinc	Unadjusted	1.00	1.12 (0.75 to 1.69)	**2.16 (1.42 to 3.28)**	**1.49 (1.02 to 2.27)**	**0.005**
	Adjusted*	1.00	1.10 (0.71 to 1.72)	**2.18 (1.39 to 3.42)**	**1.24 (1.09 to 1.96)**	**0.006**
Copper	Unadjusted	1.00	1.12 (0.75 to 1.70)	1.48 (0.98 to 2.25)	1.47 (0.97 to 2.24)	**0.032**
	Adjusted*	1.00	1.26 (0.81 to 1.95)	1.45 (0.93 to 2.28)	**1.57 (1.00 to 2.46)**	0.085
Nickel	Unadjusted	1.00	1.07 (0.71 to 1.62)	1.39 (0.92 to 2.12)	1.46 (0.96 to 2.23)	**0.038**
	Adjusted*	1.00	1.03 (0.66 to 1.61)	1.23 (0.78 to 1.94)	1.36 (0.86 to 2.16)	0.077

*Adjusted: multivariate adjustment included age, sex, education, occupation, waist circumference, body mass index, diastolic and systolic blood pressure, low-density lipoprotein-cholesterol, familial diabetic history, smoking, and alcohol and tobacco usage. The OR or p trend value shown in bold indicates statistical significance.

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Table 3 ORs (95% CI) of diabetes associated with quartiles of urinary metals (n=847)

| | | OR (95% CI) | | | | |
	Model	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P$_{trend}$
Cadmium	Unadjusted	1.00	1.25 (0.72 to 2.17)	1.07 (0.61 to 1.87)	1.60 (0.91 to 2.79)	0.142
	Adjusted*	1.00	0.99 (0.54 to 1.84)	0.92 (0.49 to 1.72)	1.46 (0.79 to 2.75)	0.352
Arsenic	Unadjusted	1.00	**2.05 (1.14 to 3.71)**	**2.44 (1.35 to 4.42)**	**2.94 (1.63 to 5.30)**	**0.001**
	Adjusted*	1.00	1.87 (0.98 to 3.57)	**2.44 (1.27 to 4.69)**	**2.68 (1.40 to 3.80)**	**0.005**
Lead	Unadjusted	1.00	**1.34 (0.75 to 2.38)**	**2.09 (1.22 to 3.60)**	**1.90 (1.10 to 3.31)**	0.091
	Adjusted*	1.00	**1.35 (0.56 to 3.25)**	**2.46 (1.10 to 5.50)**	**2.70 (1.16 to 6.32)**	0.134
Chromium	Unadjusted	1.00	1.04 (0.58 to 1.89)	1.69 (0.96 to 2.97)	**2.15 (1.22 to 3.77)**	0.034
	Adjusted*	1.00	1.05 (0.58 to 2.02)	1.87 (0.99 to 3.51)	**2.40 (1.26 to 4.56)**	0.012
Aluminium	Unadjusted	1.00	0.79 (0.43 to 1.46)	**1.93 (1.11 to 3.35)**	**1.80 (1.02 to 3.16)**	0.019
	Adjusted*	1.00	0.86 (0.44 to 1.67)	**2.03 (1.10 to 3.74)**	**2.19 (1.16 to 4.12)**	0.025
Zinc	Unadjusted	1.00	**2.15 (1.14 to 4.04)**	**3.54 (1.87 to 6.71)**	**4.12 (2.23 to 7.60)**	**<0.001**
	Adjusted*	1.00	**2.10 (1.10 to 4.38)**	**3.93 (1.96 to 7.87)**	**3.32 (1.70 to 6.49)**	**<0.001**
Copper	Unadjusted	1.00	1.51 (0.85 to 2.67)	**1.98 (1.13 to 3.46)**	**2.16 (1.25 to 3.74)**	**0.048**
	Adjusted*	1.00	1.32 (0.57 to 3.09)	1.66 (0.76 to 3.67)	**2.27 (1.02 to 5.02)**	0.272
Nickel	Unadjusted	1.00	0.85 (0.48 to 1.51)	1.34 (0.76 to 2.33)	1.47 (0.85 to 2.54)	0.187
	Adjusted*	1.00	0.75 (0.40 to 1.41)	1.12 (0.61 to 2.08)	1.36 (0.74 to 2.57)	0.256

*Adjusted: multivariate adjustment included age, sex, education, occupation, waist circumference, body mass index, diastolic and systolic blood pressure, low-density lipoprotein-cholesterol, familial diabetic history, smoking, and alcohol and tobacco usage. The OR or p trend value shown in bold indicates statistical significance.

quality control mechanisms, ignorance of the danger and poor training are prevalent with farmers opting for more toxic chemicals to expedite and enhance crop yields. A World Bank report (2010) revealed that South Asia is the leader in synthetic fertiliser consumption, which is primarily applied for cultivation of rice, the staple food of this subcontinent.

Our recent study showcased the accumulation of different metals including arsenic in different varieties of rice.[24] The rice grown in the fields in the study village applied with the metal-rich fertilisers is largely used for their consumption. South Indian people will consume 200–400 g of rice per day, and hence it is logical to link the metals in agrochemicals with rice consumption and urinary metals. There have been studies linking higher white rice intake to elevated risk of T2D, especially in Asian populations. This has led to coining of a new terminology called 'Riceabetes'.[25] Over the last few years, there has been a heightened concern on the levels of arsenic in rice and vegetables.[24–26] It is therefore reasonable to speculate that arsenic may be one 'cog in the wheel' contributing to an increase in diabetes and CVDs in rural India. Apart from arsenic, zinc was the only other metal associated with pre-diabetes, diabetes and carotid atherosclerosis in our study. Phosphate fertilisers were shown to be rich in both arsenic and zinc.[27] An unexpected finding in our study was the association of urinary zinc with diabetes, pre-diabetes and atherosclerosis. Numerous studies, both in vivo and in vitro, had demonstrated the beneficial effects of zinc in both type 1 and type 2 diabetes.[28 29] Zinc has antioxidant

Table 4 ORs (95% CI) of atherosclerosis associated with quartiles of urinary metals (n=847)

| | | ORs (95% CI) | | | | |
	Model	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P$_{trend}$
Cadmium	Unadjusted	1.00	0.99 (0.52 to 1.90)	1.15 (0.61 to 2.16)	1.33 (0.72 to 2.46)	0.309
	Adjusted*	1.00	0.76 (0.38 to 1.52)	0.96 (0.48 to 1.90)	1.12 (0.57 to 2.18)	0.486
Arsenic	Unadjusted	1.00	**2.94 (1.28 to 6.76)**	**2.63 (1.13 to 6.11)**	**5.69 (2.59 to12.5)**	**<0.001**
	Adjusted*	1.00	**2.65 (1.11 to 6.31)**	**2.45 (1.02 to 5.90)**	**5.56 (2.42 to 12.7)**	**<0.001**
Lead	Unadjusted	1.00	1.39 (0.68 to 2.86)	**2.05 (1.05 to 4.04)**	**2.23 (1.14 to 4.35)**	**0.008**
	Adjusted*	1.00	1.29 (0.61 to 2.75)	1.95 (0.94 to 4.04)	**2.29 (1.14 to 4.75)**	**0.012**
Chromium	Unadjusted	1.00	1.13 (0.56 to 2.28)	1.86 (0.97 to 3.54)	1.78 (0.93 to 3.41)	0.032
	Adjusted*	1.00	1.08 (0.52 to 2.27)	1.84 (0.92 to 3.72)	1.77 (0.87 to 3.61)	0.065
Aluminium	Unadjusted	1.00	1.06 (0.52 to 2.16)	1.56 (0.80 to 3.02)	**2.17 (1.15 to 4.08)**	0.036
	Adjusted*	1.00	1.20 (0.57 to 2.55)	1.50 (0.74–3.05)	**2.54 (1.28 to 5.06)**	0.056
Zinc	Unadjusted	1.00	1.89 (0.88 to 4.04)	**2.90 (1.41 to 5.97)**	**2.88 (1.40 to 5.93)**	**0.002**
	Adjusted*	1.00	1.92 (0.86 to 4.29)	**2.86 (1.33 to 6.14)**	**2.13 (1.06 to 4.59)**	**0.001**
Copper	Unadjusted	1.00	0.88 (0.45 to 1.71)	1.51 (0.82 to 2.76)	1.10 (0.58 to 2.08)	0.409
	Adjusted*	1.00	0.87 (0.43 to 1.76)	1.30 (0.68 to 2.52)	0.99 (0.50 to 2.00)	0.658
Nickel	Unadjusted	1.00	0.90 (0.47 to 1.70)	1.10 (0.59 to 2.02)	1.05 (0.56 to 1.94)	0.738
	Adjusted*	1.00	0.82 (0.41 to 1.63)	0.85 (0.44 to 1.66)	0.75 (0.45 to 1.77)	1.056

*Adjusted: multivariate adjustment included age, sex, education, occupation, waist circumference, body mass index, glycated haemoglobin, diastolic and systolic blood pressure, low-density lipoprotein-cholesterol, familial ischaemic heart disease history, smoking, and alcohol and tobacco usage. The OR or p trend value shown in bold indicates statistical significance.

properties, stimulates glycolysis, inhibits gluconeogenesis, reversibly inhibits alpha glucosidase activity in intestines, enhances glucose transport in adipocytes, increases the expression of peroxisome proliferator-activated receptor gamma and thereby promoting adipogenesis, increases free insulin concentrations and inhibits amyloid fibrillogenesis that is implicated in beta cell destruction.[28] Therefore, it was surprising to see a positive association between increasing urinary zinc concentrations and diabetes in our study. The answer may lie in the observations from as early as 1970s of 'hyperzincuria' in subjects with diabetes compared with the control population.[30] Studies have shown that subjects with type 1 diabetes excrete four times more zinc in the urine compared with non-diabetic controls.[31] The exact molecular mechanisms to explain a link between hyperzincuria and diabetes are not clear at this point but may relate to reduced renal tubular absorption of zinc due to hyperglycaemia and insulin deficiency. While it is a speculation to link hyperzincuria to CVDs at this point, there are studies linking hyperzincuria to incipient nephropathy and 'Near Sudden Unexplained Death Syndrome'.[32] Further research is needed to evaluate the mechanisms of hyperzincuria and its association with diabetes and CVDs in a rural population.

The role of chromium and aluminium, the other two metals showing an association with T2D in our study, is less clear. Besides synthetic fertiliser use, the other possible sources of these elements might be leaching from cooking utensils since chromium, which is associated with stainless steel and aluminium utensils, is commonly used for cooking purposes in rural India. This metal is linked to hyperinsulinaemia and insulin resistance.[33] However, the biological roles or plausible mechanisms of these two metals in relation to diabetes or CVDs need to be investigated.

Our study has several limitations. The sampling was convenient as it was difficult to do randomised cluster sampling as it may be perceived as an offence by the local population. Anyone >20 years of age was invited for study through door to door and speaker announcements. A total of 865 participants turned up for the study. As a cross-sectional study it cannot establish causality of any of the significant relationships we find. We cannot rule out the possibility that changes in metabolism, lifestyle or medication use after the development of diabetes affected exposure, absorption or excretion of some metals. Another limitation is the use of a single urine metal measurement, which may not reflect cumulative exposure and does not address the route of exposure or different forms of the metals. A 24-hour urine sample may have been preferable to a spot urine sample, but we adjusted for creatinine to control for concentration dilution of urine. Also, the use of only HbA$_{1c}$ to determine undiagnosed diabetes may have missed some people who would have been considered to have diabetes based on fasting glucose or 2-hour glucose after a glucose challenge. Due to logistics and manpower issues, we were not able to have an independent review of carotid intima thickness nor could we do quality assurance on measurement readings. Finally, exposure to these metals may involve coexposure to other potentially harmful substances, and we cannot rule out confounding by substances not measured.

In conclusion, our study provides new evidence that suggests a probable role of metals in the prevalence of diabetes and CVDs in rural India. If verified, these data should simultaneously lead to implementation of large-scale epidemiological and mechanistic studies on the role of metals in the aetiopathogenesis of the hyperglycaemic spectrum and CVDs in rural populations

and prompt governmental action to closely monitor fertilisation practices or promote safer alternative farming practices.

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