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ORIGINAL ARTICLE

Association of co-accumulation of arsenic and organophosphate insecticides with diabetes and atherosclerosis in a rural agricultural community: KMCH-NNCD-I study

Ganesan Velmurugan[1,2] · Krishnan Swaminathan[1,2] · Sundaresan Mohanraj[2] · Mani Dhivakar[1] · Ganesh Veerasekar[2] · Thomas Alexander[2] · Mathew Cherian[2] · Nalla G. Palaniswami[2] · Thalappil Pradeep[1]

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Abstract

Aims In last few decades, the prevalence of diabetes and vascular diseases has intensified concurrently with increased use of synthetic chemicals in agriculture. This study is aimed to evaluate the association of co-accumulation of arsenic and organophosphate (OP) insecticides with diabetes and atherosclerosis prevalence in a rural Indian population.

Methods This study included observations from KMCH-NNCD-I (2015) cross-sectional study ($n = 865$) from an Indian farming village. The participants had assessment of clinical parameters including HbA_{1c} and carotid intima–media thickness and urinary heavy metals. Serum OP residues were extracted and quantified by GC–MS. Statistical analyses were performed to unravel the co-association of arsenic and OPs on prevalence of diabetes and atherosclerosis.

Results On multivariate regression analyses, total organophosphate level and arsenic accumulation showed association with diabetes and atherosclerosis. Higher odds ratio with significant trends were observed for the sub-quartiles formed by the combination of higher quartiles of arsenic and total organophosphates in association with diabetes and atherosclerosis.

Conclusions We observed evidence of possible synergism between arsenic and OPs in association with prevalence of diabetes, pre-diabetes and atherosclerosis in the study population. Our findings highlight the importance of understanding health effects of mixed exposures and raises vital questions on the role of these agrochemicals in the etiology of diabetes and vascular diseases.

Keywords Rural health · Organophosphates · Arsenic · Endocrine-disrupting chemicals · Diabetes · Atherosclerosis

Abbreviations

As	Arsenic
AsQ	Quartiles based on arsenic values
CIMT	Carotid intima–media thickness
EDC	Endocrine disrupting chemicals
HbA_{1c}	Glycated hemoglobulin
LOD	Limit of detection
LOQ	Limit of quantification
OP	Organophosphates
OPQ	Quartiles based on total organophosphate levels

Introduction

The prevalence of diabetes has increased alarmingly and today represents one of the largest global health emergencies of the twenty-first century. Diabetes confers a twofold increased risk of a wide range of vascular diseases including myocardial infarction and stroke. As per WHO estimation

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Ganesan Velmurugan and Krishnan Swaminathan have contributed equally to this work.

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✉ Ganesan Velmurugan
vel@kmchrf.org

✉ Thalappil Pradeep
pradeep@iitm.ac.in

[1] DST Unit of Nanoscience and Thematic Unit of Excellence in Water Research, Department of Chemistry, Indian Institute of Technology, Chennai, Tamil Nadu 600 036, India

[2] Chemomicrobiomics Laboratory, KMCH Research Foundation, Kovai Medical Centre and Hospital, Coimbatore, Tamil Nadu 641 014, India

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[1], the prevalence of diabetes has increased from 4.7% (108 million adults) in 1980 to 8.5% (422 million adults) in 2014. This sudden increase in diabetes rate was higher particularly in middle- and low-income countries and the rural world [2]. South Asia, specifically India houses 15.3% of global diabetic population and frequently referred as the diabetic capital of the world. It is an interesting corollary that though India witnessed adoubling in diabetes rate during 1980–2014, there was no significant increase in the incidence oftraditional risk factors like obesity, hypertension, hypercholesterolemia and smoking during the same period [2]. On the other hand, this same period witnessed a massive production and release of toxic chemicals into environment, which are potent endocrine-disrupting chemicals (EDC) [2, 3]. There is now a growing body of evidence for the role of environmental chemicals in the etiology of diabetes [2, 3] and vascular diseases [4, 5].

We noticed increasing number of farmers with minimal traditional risk factors around our catchment areas seeking medical management for diabetes and vascular diseases. In our KMCH-NNCD-I (2015) cross-sectional study, executed in Nallampatti, a farming village in South India, we observed 42%, 16.2% and 10.3% prevalence of pre-diabetes (as per ADA criteria), diabetes and atherosclerosis [6]. There was no association of traditional risk factors with diabetes in this rural population, Velmurugan et al. [7] indicating the probable role of non-traditional risk factors particularly environmental chemicals. The major source of environmental chemicals in a farming village are chemicals (synthetic fertilizers and pesticides) used for agriculture. The phosphate fertilizers are a rich source of heavy metals including arsenic. Urine heavy metal analyses in this population suggested a significant association between arsenic and prevalence of pre-diabetes, diabetes and atherosclerosis [7].

The other important EDC in rural environment are insecticides used for pest control. Organophosphates (OPs) are the largely used group of insecticides in the world, accounting for more than 40% of insecticide usage. Our interest in the role of OPs and diabetes was sparked by a case of insecticide poisoning in a 15-year-old girl masquerading as diabetic ketoacidosis [8]. Studies done by our study group raised vital questions on this intriguing link between OPs and human diabetes in rural India [8–10]. Except for a few, all studies focused on a single environmental chemical that does not reflect the real-world scenario where humans are exposed to multiple chemicals. Therefore, the aim of this study is to understand the combined effect of co-accumulation of arsenic and OP insecticides on prevalence of pre-diabetes, diabetes and atherosclerosis among the participants of KMCH-NNCD-I study performed in rural India.

Materials and methods

Chemicals

All the organophosphate insecticides used are of analytical pure grade purchased from Sigma-Aldrich Inc., (PESTNAL). The solvents used were of chromatographic grade. Glassware was cleaned thoroughly with aqua regia (HCl/HNO_3, 3:1 vol%), rinsed with distilled water, and dried in oven prior to use. Deionized water was used throughout the experiments.

Study population

KMCH-NNCD-I is a cross-sectional study designed to be representative of farming rural population is South India. The study was performed in Nallampatti, an agricultural village in South India (latitude: 11°21′2.39″ N; longitude: 77°32′4.79″ E; Figure S1) during March–April 2015. The details of this study have been described previously [6]. Inclusion criteria included all those native to the village and ≥ 20 and ≤ 85 years of age. Pregnant ladies, people outside of this age criteria and those not native to Nallampatti were excluded. The study design and protocol were approved by KMCH Ethics Committee, Kovai Medical Center and Hospital Limited, Coimbatore (Approval no EC/AP/02/2015 dated 16 February 2015).

Data retrieval

All the demographic, clinical and heavy metal data of the 865 participants of KMCH—Nallampatti non-communicable disease—I (NNCD-I) study were retrieved from our database and used for the study. The variables includes basic demographic details, life style, disease history, medications, body weight, height, waist circumference, blood pressure and carotid intima–media thickness (CIMT). CIMT was measured using two high resolution B-mode ultrasound machines (GE Healthcare, Venue 40, USA). Blood investigations included a random glucose (Hexokinase/GOD-POD/endpoint method), HbA_{1c} (Automated HPLC method), cystatin-c (Nephlometric method-BN Prospec), non-fasting lipid profile, uric acid (Uricase Endpoint method) and hemoglobin (SLS method). Fasting and post-meal glucose were not considered due to logistical issues. Urinary arsenic was determined by ICP-MS (NHANES, 2011–2012) and normalized to urinary creatinine level and expressed as µg/mg creatinine [7].

Diabetes and atherosclerosis

People with glycated hemoglobin (HbA_{1c}) of ≥ 6.5% or under diabetic medications were considered as diabetic. People with no history of diabetes and HbA_{1c} ranging between 5.7 and 6.4% were defined as pre-diabetic as per American Diabetic Association guidelines (2017). People with CIMT ≥ 1 mm in either left or right arteries were considered atherosclerosis [7, 11].

Survey on use of insecticides

A survey on name, type and frequency of insecticides being used was conducted among the insecticide sellers, insecticide applicators and land owners in Nallampatti and other villages within five kilometers circumference of study village. Based on this survey, the insecticides were selected for the study.

Standards calibration

Primary stock solutions of each insecticide (1 mg/l) were prepared in methanol. Working standard solutions of the compounds were prepared by combining the aliquots of each primary solution and diluting with hexane. The stock solutions were stored at −20 °C in the dark when not in use.

Sample preparation

Serum samples were extracted by dispersive liquid–liquid microextraction technique [10] for insecticide assay. The extraction method was optimized for extraction solvent, dispersive solvent, its volume and pH. Briefly, 200 µl of serum sample was spiked with 1 ppm of azobenzene as internal standard. 20 µl of 5 N HCl was added and made up to 1 ml with deionized water. Subsequently, the sample was incubated at 70 min for 30 min to avoid protein interaction with insecticides. 150 µl of acetonitrile (dispersive solvent) and 50 µl of chloroform (extraction solvent) mixture was forcibly added to the sample using syringe and sonicated for 3 min followed by centrifugation at 10,000 rpm for 5 min. The organic phase at the bottom of the tube was carefully collected and dried under a gentle stream of nitrogen gas and dissolved in 20 µl of hexane.

GC–MS conditions

The GC injector temperature was set at 200° C. The oven temperature program was optimized to hold at 120° C for 1 min and then to increase by 10° C min^{-1} up to 290° C [12]. Helium gas was used as carrier gas. The transfer line temperature was adjusted to 280° C. Mass spectrometry conditions were as follows: electron ionization source set to 70 eV, emission current 500 lA, MS Quad 150 C, MS Source 200° C. The mass spectrometer was run in full-scan mode and in single ion monitoring mode for the fragments specific to each insecticide [10, 12].

Calibration, LOD, LOQ and recoveries

The standards were run at different concentrations, and peak area was calculated and subsequently, linearity was established. Limit of detection (LOD) and limit of quantification (LOQ) were determined by standard methods. The recovery efficiencies for each individual insecticide were determined by spiking known concentration of insecticide and measurement by GC–MS.

Statistical analysis

All statistical analyses were performed using the statistical software SPSS version 20.0. We calculated mean and percentage of participant characteristics by diabetic and pre-diabetic status. The cumulative sum of the level of all organophosphates studied was expressed as total organophosphates. Linear regression was performed to study the strength of association of serum organophosphate residues with HbA_{1c} and CIMT. Based on detection level, the participants were categorized as below and above detectable limit, and the odds ratio was calculated by logistic regression. We included likely or suspected confounders in models based on previously published data. Our logistic regression models were fitted with appropriate degrees of adjustment. We adjusted for age, sex, family diabetic history, education, occupation, smoking, smoking frequency, alcohol consumption, frequency of alcohol usage, tobacco usage and frequency of tobacco usage, waist circumference,obesity, hypertension and hypercholesterolemia. For total organophosphates and arsenic, we used logistic regression to estimate odds ratios and confidence interval (CI) levels for diabetes, prediabetes and atherosclerosis by comparing each quartile with the lowest quartile. Subsequently, we tested for linear trends across quartiles of insecticide by including the median of each quartile as a continuous variable in logistic regression models.The Spearman correlation coefficient was calculated between insecticides with HbA_{1c} and CIMT. Statistical significance was determined on the basis of two-sided tests at a 5% significance level. In addition, linear regression analysis was performed to understand the relation between arsenic accumulation and corresponding serum insecticide residues. To understand the relationship between arsenic and total organophosphates, 16 sub-quartiles were created by combining the quartiles of arsenic and total organophosphates. Subsequently, logistic regression with adjustment for above-mentioned confounding factors was performed for pre-diabetic, diabetic and atherosclerosis populations.

Table 1 Characteristics of the study population (KMCH-NNCD-I Study) [$n = 865$]

KMCH-NNCD, 2015		Whole population ($n = 865$)	Pre-diabetes ($n = 371$)	Diabetes ($n = 142$)	Carotid Atherosclerosis ($n = 90$)
		Percent	Percent	Percent	Percent
Sex	Male	48.0	43.1	59.6	67.5
	Female	52.0	56.9	40.4	32.5
Age (years)	20–40	32.9	25.3	5.7	2.3
	41–60	46.6	53.7	57.4	46.1
	Above 60	20.5	21.0	36.9	51.7
Alcohol intake (only males)	Daily	2.7	2.0	3.8	1.8
	Occasionally	50.4	47.3	58.2	64.9
	Never	47.0	50.7	38.0	33.3
Smoking (only males)	Daily	31.2	20.5	32.8	35.7
	Occasionally	25.0	14.0	30.3	34.0
	Never	43.8	55.5	36.8	30.3
Tobacco use	Daily	14.2	17.5	16.2	12.2
	Occasionally	11.5	11.0	8.5	20.8
	Never	74.3	71.5	75.4	67.1
BMI (kg/m^2)	Obese (≥ 25)	31.6	34.2	36.2	32.6
	Underweight (≤ 18.5)	13.2	11.2	6.4	18
HbA1c (%)	Diabetes (≥ 6.5)	16.2	–	–	32.6
	Pre-diabetes (5.7–6.4)	43.4	–	–	48.4
Blood pressure (mm Hg)	Hypertension ($\geq 140/90$)	37.8	33.0	49.6	54.0
Total cholesterol (mg/dL)	Hypercholesterolemia (≥ 200)	33.4	40.4	34.6	39.4
CIMT (mm)	Atherosclerosis (≥ 1)	10.3	11.6	20.5	–

Results

The characteristics of the study population are summarized in Table 1. 82.5% of the participants were involved in farming occupation. Based on our survey with farmers, pesticide applicators and pesticide vendors, we identified no organochlorine or any other persistent insecticides are being used in the study community and surrounding regions. The insecticides being used are largely dominated by organophosphates followed by carbamates, pyrethroids and neonicotinoids. We noticed 12 insecticides as commonly used that includes ten organophosphates (dichlorvos, acephate, monocrotophos, phorate, dimethoate, methyl parathion, malathion, chlorpyrifos, quinalphos and profenofos) a carbamate (carbofuran) and a pyrethriod (λ-cyhalothrin) (Table S1). Among them, methyl parathion is classified as extremely hazardous (Class Ia), malathion as slightly hazardous (Class III) and others fall within highly (Class Ib) and moderately (Class II) hazardous classes. As organophosphates occupies the 70% of insecticide usage in the study community, we focused on these group of insecticides.

The mass fragments scanned for each organophosphate under single ion monitoring mode in GC–MS are provided in Table S2. The LOD and LOQ for each organophosphate were calculated (Table S3), and the recovery efficiencies ranges from 57.8 to 102.7% (Table S4). Monocrotophos, methyl parathion, malathion and chlorpyrifos were detected in more than 70% of the samples analyzed, while detection rate for other organophosphates ranges between 38 and 65% (Table S5). We observed a strong correlation between the insecticides irrespective of the disease status of the participants (data not shown). Relatively high levels of acephate, monocrotophos, methyl parathion, malathion and chlorpyrifos residues were detected in among diabetes population (Table S6). We observed a significant linear trend between serum organophosphate residues level and of urinary arsenic accumulation by regression analysis (Table S7).

The prevalence of diabetes and pre-diabetes were determined by HbA_{1c} levels. We noticed a significant positive correlation between all the organophosphate residue levels and HbA_{1c} (Table S8) indicating the role of insecticides in glucose homeostasis. Based on detection of insecticide residue level, the population was categorized as "detected below LOD" and "detected above LOD". On multivariate regression analysis between these two groups, significant odds ratio was obtained for monocrotophos, methyl parathion, malathion, chlorpyrifos and profenofos for pre-diabetes

Table 2 Association of organophosphate insecticide residues with prevalence of pre-diabetes

	Total no. of samples above LOD (%)	Samples above LOD		Samples below LOD		Odds ratio (95% CI)
		No. of pre-diabetes	Percentage of pre-diabetes	No. of pre-diabetes	Percentage of pre-diabetes	
Dichlorvos	274 (38%)	81	29.6	290	49.1	0.44 (0.32–0.59)
Acephate	339 (47%)	152	44.8	219	41.6	1.14 (0.87–1.50)
Monocrotophos	563 (78%)	298	43.7	73	39.7	**1.18***** (**1.08–1.65**)
Phorate	312 (41%)	152	48.7	219	39.6	1.45 (1.10–1.92)
Dimethoate	469 (65%)	161	28.5	210	70	0.17 (0.12–0.23)
Methyl parathion	491 (68%)	320	54.7	51	18.2	**1.42***** (**1.12–1.65**)
Malathion	548 (76%)	333	50.4	38	18.6	**1.44**** (**1.02–1.81**)
Chlorpyrifos	527 (73%)	351	55.9	20	8.4	**1.75**** (**1.47–2.31**)
Quinalphos	259 (36%)	122	34.7	249	48.5	0.562 (0.42–0.74)
Profenofos	296 (41%)	185	62.5	186	32.6	**1.43*** (**1.26–2.60**)

The odds ratio values showed in boldness indicates the statistical significance (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). Multivariate adjustment included age, sex, familial diabetic history, education, occupation, smoking, smoking frequency, alcohol consumption, frequency of alcohol usage, tobacco usage, frequency of tobacco usage, waist circumference, obesity, hypertension and hypercholesterolemia

(Table 2), while for diabetes, all the above except profenofos showed significant association (Table 3).

The level of all the ten organophosphates residues detected was added and expressed as total organophosphates. On multivariate regression analysis with the total organophosphates level, significance was observed for only diabetes and not for pre-diabetes (ADA) (Fig. 1a), but urinary arsenic levels showed significant trends with higher odds ratio for both pre-diabetes and diabetes (Fig. 1b). For every unit increase in the level of serum organophosphates residues, a corresponding increase in HbA_{1c} value was found by linear regression analysis. For all the organophosphates analyzed, the regression coefficient for highest arsenic quartiles was relatively higher on comparison with lower quartiles of arsenic (Table S9). By combining the quartiles of arsenic (AsQ) and total organophosphates (OPQ), 16 sub-quartiles were formed, and inter-quartile multiple regression analysis was performed. Higher odds ratio with significant trends was observed for the sub-quartiles formed by the combination of higher quartiles of arsenic and total organophosphates [AsQ3 × OPQ3, AsQ3 × OPQ4, AsQ4 × OPQ3, AsQ4 × OPQ4] for both pre-diabetes and diabetes (Table 5; Fig. S2). The sub-quartiles [AsQ3 × OPQ2, AsQ4 × OPQ2] formed by highest quartile of arsenic and relatively lower quartile of organophosphates showed association for only diabetes and not for pre-diabetes.

On multivariate regression analysis in atherosclerotic population, significant odds ratio was observed for monocrotophos, methyl parathion, chlorpyrifos and (Table 4) total organophosphates (Fig. 1a). The urinary arsenic levels also exhibited higher odds ratio and significant trend in atherosclerotic population (Fig. 1b). Higher regression coefficient for CIMT on analysis with serum insecticide residues was observed for all organophosphates (Table S10). Higher odds ratio with significant trendswas observed for the sub-quartiles formed by the combination of higher quartiles of arsenic and total organophosphates [AsQ3 × OPQ3, AsQ3 × OPQ4, AsQ4 × OPQ3, AsQ4 × OPQ4] for atherosclerosis (Table 5, Fig. S2). In addition, the sub-quartile [AsQ3 × OPQ2] formed by highest quartile of arsenic and relatively lower quartile of organophosphates also showed association for atherosclerosis (Table 5, Fig. S2). Thus, this study revealed the association of agrochemicals with diabetes and cardiovascular diseases in rural communities.

Table 3 Association of organophosphate insecticide residues with prevalence of diabetes

	Total no. of samples above LOD (%)	Samples above LOD		Samples below LOD		Odds ratio (95% CI)
		No. of diabetes	Percentage of diabetes	No. of diabetes	Percentage of diabetes	
Dichlorvos	274 (38%)	25	9.1	116	19.6	0.41 (0.26–0.65)
Acephate	339 (47%)	62	18.3	79	15	0.66 (0.42–1.01)
Monocrotophos	563 (78%)	125	18.4	16	8.7	**2.36*** (**1.37–4.09**)
Phorate	312 (41%)	58	18.5	83	15	1.29 (0.90–1.87)
Dimethoate	469 (65%)	88	15.6	53	17.7	0.860 (0.59–1.25)
Methyl parathion	491 (68%)	112	19.1	29	10.4	**1.25***** (**1.18–1.52**)
Malathion	548 (76%)	135	20.4	6	2.9	**1.47**** (**1.18–2.55**)
Chlorpyrifos	527 (73%)	124	19.7	17	7.2	**1.18***** (**1.07–1.42**)
Quinalphos	259 (36%)	45	12.8	96	18.7	0.64 (0.43–0.93)
Profenofos	296 (41%)	45	15.2	96	16.8	0.88 (0.60–1.30)

The odds ratio values showed inboldness indicates the statistical significance(***$p<0.001$; **$p<0.01$; *$p<0.05$). Multivariate adjustment included age, sex, familial diabetic history, education, occupation, smoking, smoking frequency, alcohol consumption, frequency of alcohol usage, tobacco usage, frequency of tobacco usage, waist circumference, obesity, hypertension and hypercholesterolemia

Table 4 Association of organophosphate insecticide residues with prevalence of atherosclerosis

	Total no. of samples above LOD (%)	Samples above LOD		Samples below LOD		Odds ratio (95% CI)
		No. of atherosclerosis	Percentage of atherosclerosis	No. of atherosclerosis	Percentage of atherosclerosis	
Dichlorvos	274 (38%)	12	4.4	77	13.0	0.31 (0.16–0.57)
Acephate	339 (47%)	22	6.5	67	12.7	0.48 (0.29–0.79)
Monocrotophos	563 (78%)	80	11.7	9	4.9	**2.59***** (**1.27–3.26**)
Phorate	312 (41%)	21	6.7	68	12.3	0.51 (0.31–0.86)
Dimethoate	469 (65%)	18	3.2	71	23.7	0.11 (0.06–0.18)
Methyl parathion	491 (68%)	65	11.1	24	8.6	**1.33*** (**1.02–3.22**)
Malathion	548 (76%)	56	8.5	33	16.2	0.48 (0.30–0.76)
Chlorpyrifos	527 (73%)	78	12.4	11	4.6	**1.91*** (**1.52–2.58**)
Quinalophos	259 (36%)	33	9.4	56	10.9	0.84 (0.54–1.32)
Profenofos	296 (41%)	40	13.5	49	8.6	**1.66*** (**1.07–2.58**)

The odds ratio values showed in boldness indicates the statistical significance (***$p<0.001$; **$p<0.01$; *$p<0.05$). Multivariate adjustment included age, sex, familial diabetic history, education, occupation, smoking, smoking frequency, alcohol consumption, frequency of alcohol usage, tobacco usage, frequency of tobacco usage, waist circumference, obesity, hypertension and hypercholesterolemia

Discussion

We were particularly concerned that nearly more than 10% of our rural participants in the KMCH-NNCD-I study had diabetes (Table 1), higher than previous studies done in India [13]. The non-association of traditional risk factors with incidence of diabetes and atherosclerosis [7] in this study population led us to question whether traditional risk factors alone were enough to explain the huge prevalence of NCDs in this rural population. Two areas that interested us were insecticides and heavy metals. Our previous study indicated the association of urinary heavy metals particularly arsenic with diabetes and atherosclerosis [7]. The pesticide scene in India is a cause for huge concern with more than 100 pesticides registered for use. There has been a more than 15-fold increase in the production of insecticides, from a mere 5000 tons in 1952 to 85,000 tons in 2004 [14]. Rampant injudicious use of such insecticides may be a one "cog in the wheel" that explains the increase in metabolic disorders, cancers, neurodegenerative disorders and reproductive abnormalities [15]. In this study, we noticed withdrawal of usage of persistent insecticides like endosulfan, DDT in the study area indicating development of awareness on their toxicity by government and non-governmental agencies. Organophosphates are christened as one of the most poisonous chemicals in the world by Carson [16]. They are largely used along with carbamates and pyrethroids due to their short half-life. Irrespective of their non-persistent nature, these insecticides pose a heavy risk on human health. Though these organophosphate groups of insecticide possess short half-life, they are detected in large populations in this study and in other previous studies [10, 17]. The probable explanation is these insecticides are being exposed to the farming people day-to-day continuously, and hence, they are under detectable limits.

Organophosphate insecticides seem to affect multiple glucose homeostatic pathways that can lead to hyperglycemia. Physiological stress, oxidative stress, inhibition of paraoxonase, nitrosative stress, pancreatitis, inhibition of cholinesterase, stimulation of adrenal gland and disturbed metabolism of liver tryptophan and gut microbiota were enlisted as proposed mechanisms behind OP-induced disruption of glucose homeostasis [10, 17]. We observed farmers and workers mixing and spraying insecticides without using personal protective equipment [6, 9, 10]. It is well recognized that insecticides can not only get avidly absorbed through layers of epidermis and mucosa but can remain in the skin which acts as a reservoir for release in future [18]. The other possibility is the metabolic effects of chronic exposure through food commodities that is being used by both rural and urban populations. Studies have shown that 20% of food commodities in India have insecticides residue levels above the maximal permissible levels on a worldwide basis [14]. Chronic exposure of mice to organophosphates-induced glucose intolerance was mediated by the gut microbial degradation of organophosphates [10]. Our previous studies with rats showed that prolonged exposure of monocrotophos leads to cardiac oxidative stress and myocardial damage [19]. In addition, organophosphate insecticides have been implicated in hypertension, abnormal geometry of left ventricle, peripheral arterial disease, obesity, atherosclerosis and end-stage renal disease [20–22].

Arsenic exposure is a global public health problem and labeled by WHO as the largest mass poisoning of the world.

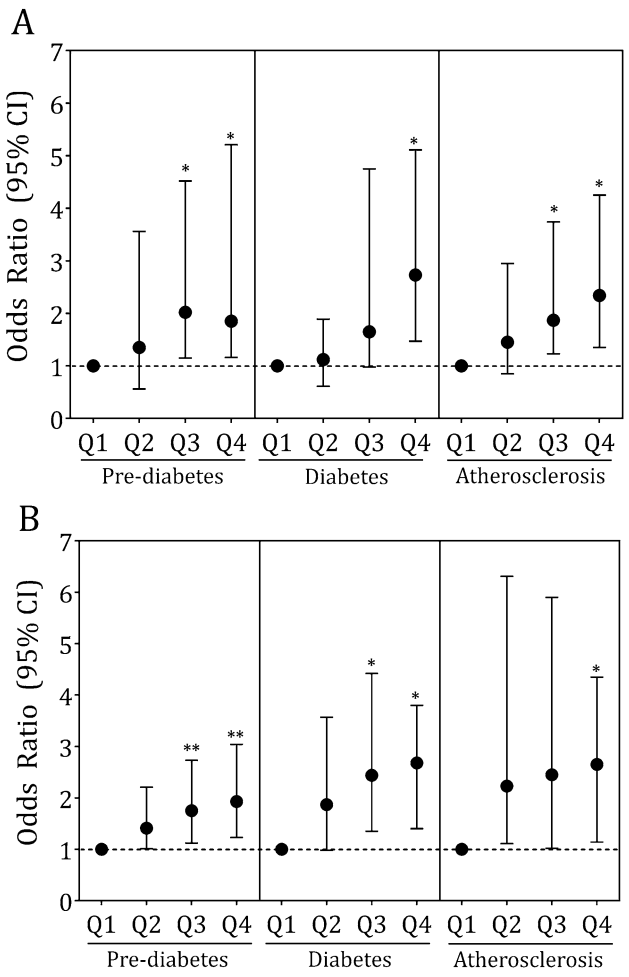

Fig. 1 Odds ratio (95% CI) for pre-diabetes, diabetes and atherosclerosis associated with the quartiles of total organophosphates (**a**) and total arsenic (**b**). *indicates statistical significance (**$p<0.01$; *$p<0.05$). Multivariate adjustment included age, sex, education, occupation, waist circumference, BMI, diastolic and systolic blood pressure, LDL-cholesterol, familial diabetic history, smoking, alcohol and tobacco usage for pre-diabetes and diabetes. Besides the above mentioned, familial ischemic heart disease history and glycated hemoglobulin are also included for atherosclerosis

Table 5 Association of co-accumulation of arsenic and total organophosphate insecticide residues with prevalence of diabetes, pre-diabetes and atherosclerosis

Sub-quartile (SQ) (AsQ × OPQ)	Odds ratio (95% CI)		
	Pre-diabetes	Diabetes	Atherosclerosis
SQ1 (AsQ1 × OPQ1)	1.00	1.00	1.00
SQ2 (AsQ1 × OPQ2)	1.25 (0.72–2.17)	1.60 (0.91–2.79)	1.46 (0.98–1.72)
SQ3 (AsQ1 × OPQ3)	0.91 (0.59–1.43)	1.17 (0.77–1.77)	1.19 (0.78–1.80)
SQ4 (AsQ1 × OPQ4)	0.92 (0.47–1.70)	0.82 (0.41–1.63)	0.87 (0.43–1.76)
SQ5 (AsQ2 × OPQ1)	2.03 (0.85–3.74)	1.80 (0.52–2.25)	0.86 (0.44–1.67)
SQ6 (AsQ2 × OPQ2)	1.10 (0.71–1.72)	1.48 (0.95–2.02)	1.47 (0.97–2.24)
SQ7 (AsQ2 × OPQ3)	1.89 (0.88–4.04)	1.92 (0.86–2.12)	0.87 (0.43–1.76)
SQ8 (AsQ2 × OPQ4)	1.29 (0.35–1.56)	1.95 (0.85–2.86)	0.76 (0.38–1.52)
SQ9 (AsQ3 × OPQ1)	1.08 (0.52–2.27)	1.84 (0.92–3.72)	1.77 (0.87–3.61)
SQ10 (AsQ3 × OPQ2)	0.87 (0.43–1.76)	**2.86 (1.33–4.02)***	0.99 (0.50–1.98)
SQ11 (AsQ3 × OPQ3)	**3.54 (1.87–5.34)***	**2.15 (1.14–3.52)***	**2.19 (1.16–4.12)***
SQ12 (AsQ3 × OPQ4)	**1.48 (1.05–4.82)***	**2.02 (1.82–5.01)***	1.21 (0.84–3.87)
SQ13 (AsQ4 × OPQ1)	1.25 (0.72–2.17)	0.99 (0.55–1.84)	1.46 (0.76–2.75)
SQ14 (AsQ4 × OPQ2)	1.04 (0.58–1.89)	**2.46 (1.10–3.50)***	**2.70 (1.16–3.77)***
SQ15 (AsQ4 × OPQ3)	**3.20 (2.25–5.20)***	**2.86 (1.25–4.52)***	**2.45 (1.34–3.75)***
SQ16 (AsQ4 × OPQ4)	**1.86 (3.54–1.15)***	**2.10 (1.56–4.56)***	**1.56 (1.03–2.98)***

The odds ratio values showed in boldness indicates the statistical significance (***$p < 0.001$; **$p < 0.01$; *$p < 0.05$). Multivariate adjustment included age, sex, familial diabetic history, education, occupation, smoking, smoking frequency, alcohol consumption, frequency of alcohol usage, tobacco usage, frequency of tobacco usage, waist circumference, obesity, hypertension and hypercholesterolemia

The heavy use of synthetic phosphate fertilizers leads to extension of arsenic problem to the different regions of the world that are not previously identified as natural arsenic-rich regions. Arsenic has been implicated in beta-cell dysfunction and atherogenesis with higher risk of diabetes and vascular diseases [23]. Our interest in the synergistic co-exposure hypothesis of arsenic and insecticides in the development of diabetes and atherosclerosis was stimulated by reports of Sri Lankan Agricultural Nephropathy, a new form of nephropathy in paddy farmers in Sri Lanka attributed to synergistic effect of heavy metals like arsenic and glyphosate, an organophosphate herbicide [24]. In an animal study, combined exposure of arsenic and dichlorvos to rats led to changes in glutathione metabolism, neurological and hepatic damage [25, 26]. Higher odds ratio in the sub-quartiles dominated by higher quartiles of arsenic and lower quartile of total organophosphates [AsQ3 × OPQ2, AsQ4 × OPQ2] indicate that arsenic may be the strongest player on comparison with organophosphates (Table 5). Significant regression coefficient for the insecticides in the highest quartile of arsenic in our study suggests that it may promote the accumulation of insecticide residues in blood (Table S7). Arsenic is known to cause hepatic damage with increase in serum aminotransferases, hepatic fibrosis due to progressive reduction in hepatic glutathione, oxidative stress and fatty infiltration [27]. Both metals and insecticides, in the presence of hepatic impairment, can potentially act in concert to produce reactive oxygen species with depletion of antioxidant systems [28]. We hypothesize that arsenic-induced hepatic damage results in impairment of hepatic detoxification mechanisms that may lead to higher accumulation of organophosphate residues. The role of gut microbiota in this process is not ignorable as both arsenic and organophosphates are proven to be metabolized by gut microbiota [2, 10, 29]. There are also possibilities that interaction of arsenic and organophosphates produce a new arsenic species that may have more detrimental effect on the system leading to metabolic and vascular dysregulation. NMR-based studies [30] showed the interaction of cadmium with chlorpyrifos yields a new complex that confers increased hepatoxicity. Future studies focusing on computational and experimental studies are needed to prove these hypotheses.

Limitations

There are some limitations to our study. Being a cross-sectional study, it is difficult to infer causality of any kind, and this study is mere hypothesis generating at this point. Due to practical issues and ethical guidelines, we had participation from two-third of the village population indicating that the prevalence rates reported will have small margin of error (3.3%). 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. A single arsenic or insecticide measurement at one time point may not represent

cumulative exposure. Finally, we cannot rule out confounding effects by other potentially harmful substances in the environment that we could not measure.

Conclusion

In summary, findings from this study highlight the importance of possible synergistic detrimental metabolic and cardiovascular effects of arsenic and organophosphates in a rural population. The results of this study raise vital research questions on the role of these agrochemicals in the etiology of diabetes and vascular diseases.

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Author contributions GV[1], KS, GV[2], MC, TA, NGP and TP conceived and designed the experiments. GV[1], KS, GV[2], MC and NGP are involved in sample collection. GV[1] and KS performed the experiments. GV[1], KS, SM, MD and TP analyzed the data. KS, MC, TA, NGP and TP have contributed reagents/materials/analysis tools. GV[1] and KS wrote the manuscript. All the authors read and approved the final manuscript.

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Availability of data and materials Complete data, clinical details and samples are available for researchers on reasonable request to corresponding author. But providing of data or samples will depend on the approval of Institute ethical committee.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all participants of this study for publication of this data as per the guidelines of institute's ethical committee.

Ethics approval The study design and protocol were approved by KMCH Ethics Committee, Kovai Medical Center and Hospital Limited, Coimbatore (Approval No. EC/AP/02/2015 dated 16 February 2015).

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