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ORIGINAL ARTICLE

Prevalence of chronic kidney disease and anemia in Hirakud Command Area, Odisha, India: unveiling the role of environmental toxicants

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Abstract

Background The present community-based study assessed the prevalence of chronic kidney disease (CKD)/chronic kidney disease of unknown origin (CKDu) as well as anemia in some intense agricultural zones under Hirakud Command Area and evaluated their association with pesticides and heavy metal exposure.

Methods Random cluster sampling method was used to assess the prevalence of CKD and anemia. Hematological analysis was carried out using autoanalyzer. Pesticide residues in soil, water, rice grains, blood and urine samples were analyzed using LCMSMS and GCMS, while heavy metal levels were assessed using ICP-MS. Risks associated with exposure to pesticides and to heavy metals through dietary and non-dietary sources were assessed using the United States Environmental Protection Agency (USEPA) method.

Results CKDu was predominant among the farming community in "blocks" i.e. administrative units in rural governance, functioning as subdivisions of districts in India with intense agricultural activities. Blocks reporting higher prevalence of CKDu showed greater concentrations of nephrotoxic pesticide residues in the soil, water and rice grain. Heavy metals in water, such as cadmium, chromium, lead and arsenic, were found to be above permissible limits in all the hotspot blocks. Dietary exposure to pesticide residues was presumed to contribute significantly to non-carcinogenic risk among the exposed population. Analysis of blood and urine samples collected from patients with CKD/CKDu indicated the presence of nephrotoxic pesticide residues and heavy metals among the directly exposed group. Anemia was found to be prevalent among CKDu patients.

Conclusion The present study indicated a strong association between environmental toxicants, like pesticides and heavy metals, and the onset and progression of CKD, as well as anemia in a high intensity agricultural zone. Dietary exposure to pesticides and heavy metals may pose high risks for kidney diseases.

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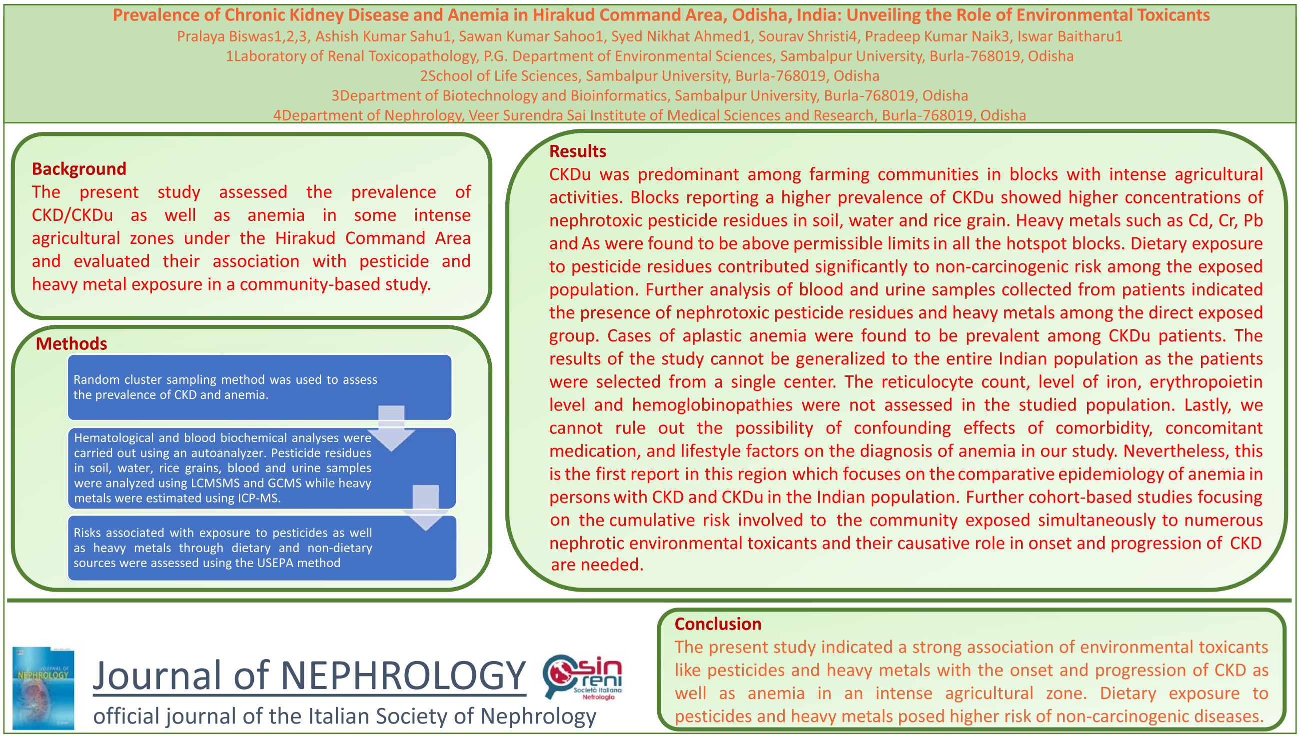
Graphical abstract



Keywords CKD and CKDu · Environmental Toxins · Odisha · Bargarh · Pesticides

Background

Over the years, Chronic Kidney Disease (CKD) has become a significant global public health challenge, contributing notably to the burden of non-communicable diseases. It affects nearly 700 million people worldwide and causes approximately 1.2 million deaths annually (GBD CKD Collaboration 2020). The prevalence of CKD globally ranges between 8 and 16%, equating to over 750 million cases, with 78% of these cases (387.5 million) occurring in lower-middle-income countries [1]. In the United States, more than 8 million people are estimated to have stage 3 CKD, with many more at risk or suffering from milder forms of the disease. According to a systematic analysis of global disease burden, CKD is reported to be a major contributor to global morbidity and is closely linked to cardiovascular diseases. Hypertension and diabetes have traditionally been the primary causes of CKD. However, over the past two decades, there has been a marked increase in cases unrelated to these factors. This rise in CKD of unknown origin, often referred to as "CKDu," has been particularly noteworthy among agricultural workers in regions like the North-Central Province of Sri Lanka [2]. In India, Uddanam nephropathy, especially in the agricultural areas of Andhra Pradesh [3], follows a pattern of chronic tubulointerstitial disease, likely linked to environmental toxins. However, the connection between chronic exposure to pesticide residue and the onset of CKD has not been thoroughly investigated in cohort-based studies worldwide.

Pesticides such as dieldrin, dichlorvos, heptachlor epoxide and many other organochlorines are extensively used in paddy farming. These pesticides are known nephrotoxic agents and are reported to cause CKDu [3, 4]. Traces of these nephrotoxic pesticides have also been detected in the biological samples of CKDu patients [3]. Dichlorvos damages the renal cortex and may cause acute kidney injury (AKI) which can lead to CKD [4]. Exposure to pesticides such as dieldrin and DDT induces oxidative stress and causes structural damage in renal cells that eventually result in a reduction of the kidney function. Heavy metals such as arsenic, cadmium, lead, chromium and zinc are acknowledged nephrotoxic agents. Consumption of water contaminated with these heavy metals may lead to kidney failure. In animal studies, these heavy metals are proven to cause chronic inflammation and fibrosis in the proximal tubules of nephrons, leading to kidney injury. Pesticides and heavy metals, such as arsenic and mercury not only cause CKDu but are also known to cause aplastic anemia [3]. Anemia is one of the consequences of CKD because of the central role played by erythropoietin in the regulation of erythropoiesis [5]. Anemia can manifest early in the course of CKD, and its severity and prevalence gradually increase with the progression of kidney disease especially when the glomerular filtration rate (GFR) decreases to less than 30 mL/min/1.73 m^2 [6]. Anemia also enhances the burden of cardiovascular diseases and decreases patients' quality of life. The use of iron therapies and erythropoiesis-stimulating agents has led to improvement in patients with anemia in CKD. In India, a cross sectional study reported an 82.4% prevalence of anemia in CKD patients [5]. However, no study has focused on the prevalence of anemia among CKD and CKDu patients in the agricultural zones of India and Odisha.

After the commissioning of the Hirakud Dam, Bargarh district and its adjacent area became highly irrigated resulting in a tremendous increase in rice cultivation with a parallel increase in the use of pesticides, agrochemicals, and heavy metals. However, Bargarh district, once known as the rice bowl of the state of Odisha, has been transformed into a disease capital, with a much higher prevalence of cancer as well as CKD within a span of 70 years of commissioning of the dam. In recent years, there have been increasing reports of CKD and CKDu cases among farming communities of the State of Odisha, India. A recent study from our laboratory reported a higher prevalence of CKDu in Bargarh district and identified 16 hotspot villages [7]. Interestingly, all the hotspot blocks (i.e. administrative units in rural governance, functioning as subdivisions of districts in India) were located in intense agricultural pockets and share agroclimatic conditions similar to Andhra Pradesh, where endemic Uddanam nephropathy is localized. Exposure to heat stress, heavy metals and pesticides in agricultural fields is suggested to be a major cause of increased CKDu incidence among agricultural workers worldwide. However, the causative association between agrochemical exposure and the incidence of CKDu in the community is yet to be established. The present work aims to study the prevalence of CKD, its association with environmental factors such as nephrotoxic pesticides and heavy metals, and the occurrence of anemia among CKD and CKDu patients in an intense agricultural zone under Hirakud Command Area. The study further assessed the risks associated with dietary and non-dietary exposure to heavy metals and pesticides using risk modeling.

Materials and methods

Study site

The Bargarh district, once considered the rice bowl of the state, is situated in the western part of Odisha, India. It is geographically located at 21.33° N, 83.62° E, with a total population of 1,481,255 including 749,161 males and 732,094 females, (Census Report, 2011). Of the five blocks in the Bargarh district evaluated for the present study, four blocks fall within intense agricultural zones receiving a continuous supply of irrigation water from the Hirakud reservoir (Fig. 1). Most of the population is involved in agricultural activities and they cultivate paddies as the major crop in the district. Around 60% of the cultivated land in the district is irrigated under the Hirakud command area.

Study population

A community-based survey was conducted in five identified CKD hotspot blocks of the Bargarh district using cluster random sampling and probability proportionate to size methodology followed by a hospital-based survey. A total of 1136 individuals were screened, of whom 656 were males and 480 were females. The present study included participants aged 18 years or above, who voluntarily participated and provided written informed consent for the study. Participants who did not agree to take part in the study or who were diagnosed with disease other than CKD that might influence their hematological profile (hematological disorders, acute or chronic inflammatory conditions, hemorrhagic episodes and cancer), and pregnant or lactating women were excluded.

Diagnostic criteria and definitions

The criteria for diagnosing CKD were based on the Kidney Disease Improving Global Outcomes (KDIGO) guidelines, which define CKD as an estimated glomerular filtration rate (eGFR) below 60 ml/min/1.73m^2 for three or more months, along with the presence of albuminuria or proteinuria. This includes a urine albumin excretion of at least 30 mg/dL or a protein-to-creatinine ratio of at least 30 mg/mmol. Hypertension was diagnosed based on the patient's medical history (if already receiving treatment) or through direct measurement. A diagnosis was confirmed if the measured systolic blood pressure was 140 mmHg or higher and/or the diastolic blood pressure was 90 mmHg or higher on three separate occasions. Diabetes was diagnosed on the basis of either history (with ongoing treatment) or through screening for random plasma glucose, where levels between 70 and 140 mg/dL were considered normal, and levels of 150 mg/dL or higher indicated diabetes. Anemia was defined when hemoglobin levels were below 12 g/dL for women and less than 13.5 g/dL for men, and was further classified as normocytic, microcytic, or macrocytic on the basis of mean corpuscular volume. Normocytic anemia had a mean corpuscular volume of 80–100 fL, microcytic anemia had a mean corpuscular volume less than 80 fL, and macrocytic anemia had a mean corpuscular volume of more than 100 fL [8]. Aplastic anemia was diagnosed on the basis

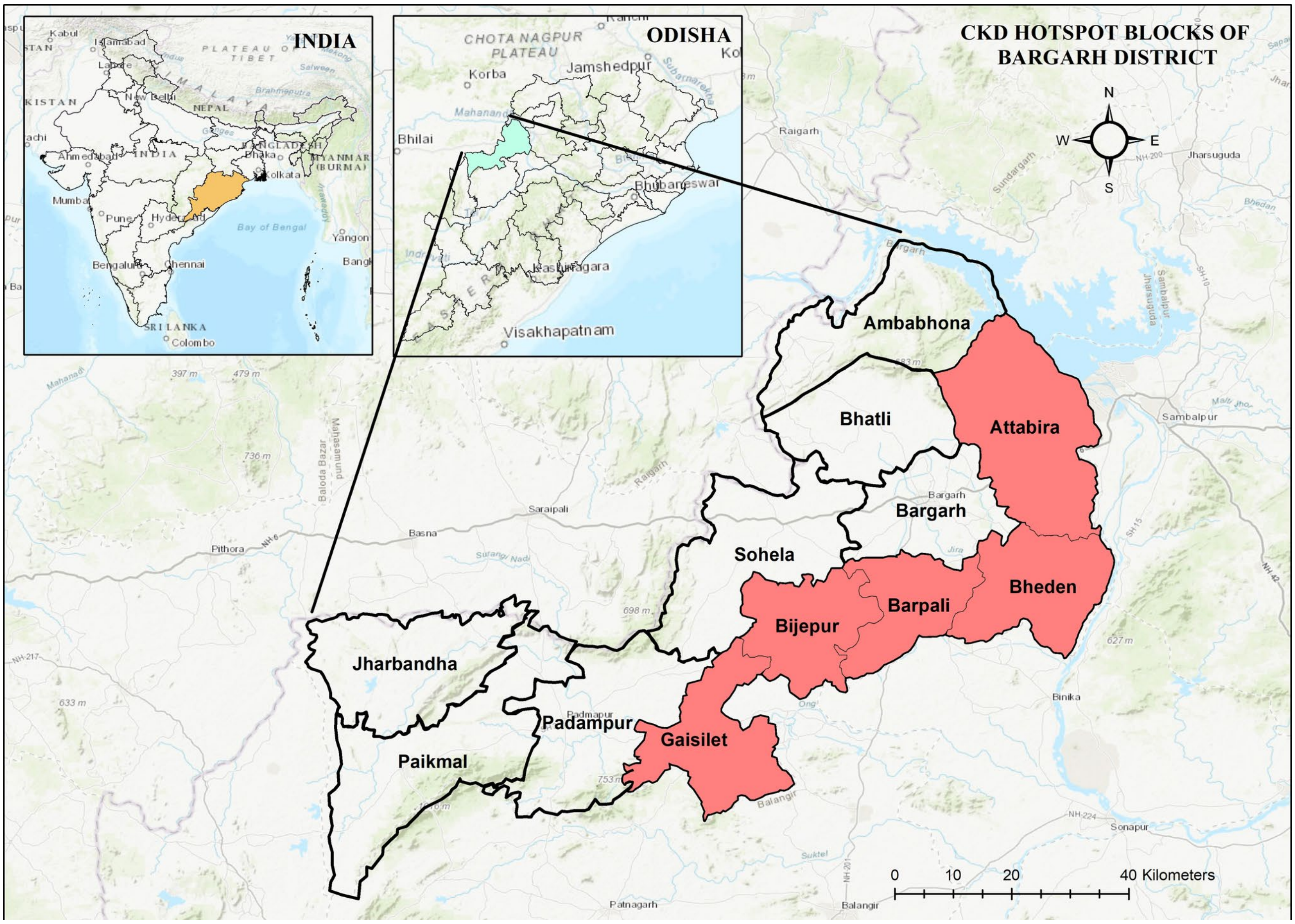


Fig. 1 Map Showing CKD/CKDu hotspot blocks of Bargarh district

of peripheral blood data, requiring at least two of the following criteria: hemoglobin ≤ 100 g/L or hematocrit $\leq 30\%$, platelets $\leq 50 \times 10^9$/L, or leukocytes $\leq 3.5 \times 10^9$/L, or granulocytes $\leq 1.5 \times 10^9$/L [9]. Patients with a history of chronic hypertension, diabetes, or other known causes were classified under the CKD group. In contrast, cases classified as CKDu included individuals with no history of diabetes or hypertension, those with mild hypertension, a protein-creatinine ratio ≤ 1. These cases were predominantly restricted to specific geographical regions, primarily affecting males, younger populations, and individuals with lower income who work in hot environments.

Clinical measurements

An automated, clinically validated digital blood pressure monitor (Dr. Morpen, Model- BP 15, India) was used to measure blood pressure after the participants had rested for 5 min in a seated position. An average of three readings was recorded. The participants' height was measured using a stadiometer (SECA model 213, Hamburg, Germany), and their weight was measured with digital calibrated scales (OMRON Model HN 865) to calculate their Body Mass Index (BMI).

Biological sample collection and analysis

All chemical reagents for the analysis of blood and urine samples were purchased from commercially available, recognized sources. Before the blood samples were collected, the puncture site was sanitized and cleaned. Blood samples were collected from peripheral veins using three different vacutainer tubes, namely plain tube, tube containing EDTA, and fluoride tube, for the estimation of serological parameters, hemoglobin, complete blood count, and random blood sugar of all the participants. The first morning void urine samples (5 ml) were collected from all the participants in polypropylene urine collection bottles. The collected blood and urine samples were transported in ice-carrier boxes to the Regional Diagnostic Center, Veer Surendra Sai Institute of Medical Science and Research, Burla, Odisha, India for analysis. The samples were processed and tested on the same day as sample collection. Serum samples were obtained by centrifugation of blood samples at 3000 rpm for 10 min at 4 °C. Random blood sugar and serological parameters were estimated using Transasia XL 1000 Auto Analyzer (Germany). The photometric method was used to estimate hemoglobin and complete blood count using Mindray BC 1000 Six Part (Mindray, Germany). Urine samples were analyzed for proteinuria using the heat coagulation test and were corrected for creatinine. An additional 2 ml of blood from the EDTA tube and 5 ml of urine were collected for toxicological analysis.

Collection of soil, water, and rice grains

Topsoil samples (n = 144) from 10 to 15 cm depth were collected from agricultural fields of identified hotspot blocks. The soil samples were air dried, powdered and passed through a mesh with a 1 mm pore size. Twenty grams of soil samples were collected in triplicate in 100 ml of polypropylene centrifuge tubes. Surface water samples ($n = 148$) were collected from ponds, canals and rivers for analysis of pesticide residue, while 146 groundwater samples used for direct human consumption in hotspot blocks were collected in glass bottles and acidified on site by the addition of nitric acid for heavy metal analysis. Harvested rice seeds were also collected for analysis.

Quantification of pesticide residue in soil, water, and rice grains

Pesticides were extracted from soil samples using a modified QuEChERS method with ultrasound-assisted microoven techniques [12]. A 10 g soil sample was mixed with acetonitrile and acidified deionized water, and then subjected to ultrasound extraction for 20 min. The mixture was centrifuged at 3000 rpm for 10 min, and the organic phase was separated and evaporated using a vacuum rotary evaporator. The resulting extracts were reconstituted in 6.0 mL of acetonitrile and divided into two 3.0 mL aliquots. Each aliquot was evaporated to near dryness at 40 °C and reconstituted with 1 mL of methanol for UPLC-MS/MS analysis and 1 mL of n-hexane for GC–MS analysis. The surface water samples (20 mL aliquots) were filtered through a 0.45 µm glass-fiber filter, followed by the addition of 20 mL acetonitrile acidified with 120 µL acetic acid. The extraction, purification, concentration, and chromatographic procedures were identical to those used for the soil samples. For rice grain samples, the grains were finely powdered before extraction. The extraction involved the addition of 14 mL of acetonitrile with 1.0% acetic acid and 1.0 mL of sulfamethoxazole to 5 g of powdered sample in a 50 mL PTFE tube. After 30 min, 2.0 g of anhydrous magnesium sulfate and 0.5 g of sodium acetate were added, and the mixture was centrifuged at 4000 rpm for 10 min. A 1.0 mL aliquot of the supernatant was collected for GC–MS chromatographic analysis.

Quantification of heavy metals in soil, water, and rice grain

The soil samples were thoroughly ground using an agate mortar and pestle, then passed through a clean nylon membrane sieve with a pore size of 0.071 mm to obtain a uniform powder. Approximately 100 g of the dried soil samples were added to acid-washed teflon containers containing 5 mL of ultrapure nitric acid and 2 ml of ultrapure concentrated hydrofluoric acid and digested using a microwave digester at 200 °C for 30–40 min. Each batch included at least one reagent blank, a reference standard, and a sample replicate to ensure homogeneity and process efficiency. After cooling for at least 1 h, the containers were opened, and 0.9 g of boric acid was added to dissolve the fluoride precipitates. The containers were then resealed and placed back in the microwave digestion system for an additional 20–30 min. After another hour of cooling, the digested samples were transferred to a graduated plastic test tube with 0.5 ml of hydrofluoric acid, and the volume was brought up to 50 ml with Milli-Q water.

Rice grain samples (0.5 g) were directly weighed into acid-cleaned teflon digestion vessels. Ten mL of ultrapure nitric acid was added to each vessel, which was then heated to 100 °C using an XT-9800 pre-treatment heater until nearly all the nitrogen dioxide was released. A 4 ml aliquot of a concentrated HNO_3: hydrofluoric acid (1:1 v/v) acid mixture was added before microwave digestion. Process efficacy and sample homogeneity were assessed using a reagent blank, a reference standard, and a sample replicate during digestion. The rice samples were digested in the microwave at 0.5 MPa for 1 min, followed by 1.0 MPa for 2 min, and 1.5 MPa for 3 min to ensure complete digestion. The digested samples were then cooled for 1 h, transferred to graduated plastic test tubes, and the volume was adjusted to 100 ml with Milli-Q water.

Heavy metals such as arsenic, cadmium, chromium, and lead in the digested soil and rice samples were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Arsenic and mercury were analyzed using Atomic Fluorescence Spectrometry (AFS). The digested samples were typically diluted 20-fold with 2% nitric acid before analysis. Metals that were present in higher concentrations in soil and rice samples were further diluted up to 10,000-fold to accurately measure their concentrations. Quantification was achieved using an empirical calibration curve created from a multi-element calibration standard, with ultrapure nitric acid (2%) serving as the blank [12].

Quantification of pesticide residue in blood and urine samples of participants

Pesticide extraction from blood samples was performed in duplicate. Two milliliters of blood were mixed with a 10 mL solution of hexane and acetone (1:1 ratio) and agitated for 30 min at room temperature using a mechanical shaker. The mixture was then centrifuged at 2000 rpm for 10 min at 4 °C, after which the clear hexane layers were combined. Clean up was conducted using column chromatography according to the United States Environmental Protection Agency (USEPA) method. The hexane eluate was concentrated through evaporation, and the resulting residue was dissolved in 1 mL of HPLC-grade hexane for analysis. Pesticide residue levels were quantified using GC–MS/MS equipped with Elite-GCDB-5 columns (30 m length and 0.25 mm internal diameter) and a Ni^{63} electron capture detector [12]. A 1 µL sample of the final extract was injected at 170 °C with a 1-min hold time. The temperature was increased from 170 to 225 °C at a rate of 5 °C/min with a 5-min hold, and then from 225 to 275 °C at a rate of 6 °C/min with a 15-min hold, totaling a 40-min run time per sample. The quantitative analysis of all pesticide residue was performed by comparing the peak areas with those from a chromatogram of a mixed pesticide standard with known concentrations (Sigma-Aldrich, USA). The detection limit for all pesticide residue was 4 ng/mL.

For the urine samples, 0.5 mL of urine was placed in a 15 mL polypropylene tube, followed by the addition of 1.5 mL of 2% formic acid (v/v). The samples were purified using Oasis WAX cartridges (50 mg/3 mL), and preconditioned with 3 mL of methanol and HPLC-grade water. After loading, the cartridges were washed with 3 mL of HPLC-grade water and a 50:50 (v/v) mixture of acetonitrile and HPLC-grade water. Analytes were eluted with 3 mL of 5% ammonia in methanol. The extracts were dried under nitrogen and then reconstituted in 250 µL of acetonitrile containing 20 mM ammonium acetate (90:10, v/v), and filtered through a 0.22 µm nylon filter prior to instrumental analysis. Chromatographic separation was achieved with a Waters ACQUITY Class I HPLC system, using a Luna HILIC column (3 µm, 100 × 3 mm, Phenomenex). A 1.5 µL injection volume was used, with a mobile phase flow rate of 400 µL/min. The mobile phase consisted of acetonitrile (A) and 20 mM ammonium acetate in acetonitrile at pH 8.5 (B). The gradient began with 99% B, held for 6 min, then decreased to 50% within 0.1 min and held for 1.4 min, before returning to the initial conditions within 0.1 min and held for 2.4 min. The total run time was 10 min. Mass spectrometry analysis was carried out using GC–MS/MS.

Quantification of heavy metals in blood and urine samples of participants

The blood cadmium, arsenic, chromium, mercury and lead contents of the exposed and unexposed groups were measured using ICP-MS with a Zeeman background correction. Urinary heavy metals (cadmium, chromium, lead and arsenic and mercury) were measured by ICP-MS. The blood and urinary heavy metals are presented as mean ± standard deviation. Values below the detection limit were replaced with the square root of the detection limit divided by two.

Health risk estimations

To evaluate the health risks from exposure to pesticide residue through rice consumption, contaminated drinking water (dietary intake), and contact with contaminated soil (non-dietary intake), the guidelines established by the USEPA were utilized. The EPA provides an acceptable risk range for carcinogens, ranging from 1×10^{-6} to 1×10^{-4}. In contrast, risks exceeding 1×10^{-4}, or one additional case per 10,000 individuals, are typically viewed as unacceptable. For assessing non-carcinogenic risks, the USEPA (2001) guidelines suggest the use of the hazard quotient to gauge potential health impacts. A hazard quotient value of ≤ 1 indicates a low probability of adverse health effects, whereas a hazard quotient > 1 suggests a potential risk of non-carcinogenic health effects (USEPA, 2020).

Human Health Risk Assessment of Pesticide Residue in rice grain (Dietary)

To evaluate the potential health risks posed by pesticide residue in rice, the non-carcinogenic risk was assessed using the USEPA's standard method for determining Estimated Daily Intake and incorporating the slope factor. The target hazard quotient was calculated following methodologies from previous studies [13] and applied to determine non-carcinogenic health risks in adults as per Eqs. 1 and 2:

$$\mathrm{EDIing} = \frac{\mathrm{CF} \times \mathrm{IRCF} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BWCF} \times \mathrm{AT}} \tag{1}$$

$$\mathrm{HQing} = \frac{\mathrm{EDIing}}{\mathrm{RfD}}, \tag{2}$$

where CF represents the residual pesticide concentration (mg/kg), IR represents the food consumption rate (kg/day), EF refers to the exposure frequency (days/year), and ED represents the exposure duration (years). Additionally, BW refers to the average body weight, and AT is the average exposure time for carcinogenic effects (calculated as

EF × ED). The Reference Dose (RfD) of the pesticide and heavy metal was used to calculate the non-carcinogenic risk, as outlined in Eqs. 1 and 2.

Human health risk assessment of pesticides in water samples

To assess the risk posed by pesticides detected in adults through the consumption of contaminated water, the hazard quotient method was utilized [12].

$$\mathrm{HQ} = \frac{\mathrm{ECDI}}{\mathrm{RfD}} \tag{3}$$

$$\mathrm{EDI(ingestion)} = \frac{\mathrm{Cw} \times \mathrm{IR(water)} \times EF \times ED}{\mathrm{BW} \times \mathrm{AT}}, \tag{4}$$

where Cw represents the concentration in the water samples, IR (water) denotes the ingestion rate, EF refers to the exposure frequency, ED is the exposure duration, BW stands for body weight, and AT represents the average lifespan. The specific values for these parameters can be found in Supplementary Table 1. The Reference Dose (RfD) of pesticides and heavy metals is used to calculate the non-carcinogenic risk, as outlined in Eqs. 3 and 4.

Human health risk assessment of pesticides in soil samples (Non-Dietary)

Human exposure to pesticide residue extends beyond dietary intake, encompassing other routes of exposure as well. Non-dietary intake can be assessed using the chronic daily intake model. This model was developed by the USEPA and published in 1992 and 1997, and it estimates the non-carcinogenic risks for both adults and children from non-dietary exposure to contaminants through three primary routes: dermal contact, ingestion, and inhalation [15]. The chronic daily intake calculation was based on the following formulas (Eqs. 5–10).

$$\mathrm{CDIing} = \mathrm{Csoil}\frac{\mathrm{IR} \times \mathrm{CF} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}} \tag{5}$$

$$\mathrm{CRing} = \frac{\mathrm{CDIing}}{\mathrm{RfD}} \tag{6}$$

$$\mathrm{CDIinh} = \mathrm{Csoil}\frac{\mathrm{IAR} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{PEF} \times \mathrm{BW} \times \mathrm{AT}} \tag{7}$$

$$\mathrm{CRinh} = \frac{\mathrm{CDinh}}{\mathrm{RfD}} \tag{8}$$

$$\mathrm{CDIderm} = \mathrm{Csoil} \times \frac{\mathrm{IAR} \times \mathrm{EF} \times \mathrm{ED}}{PEF \times \mathrm{BW} \times \mathrm{AT}} \tag{9}$$

$$\mathrm{CRderm} = \frac{\mathrm{CDIderm}}{\mathrm{RfD}}, \tag{10}$$

where CS represents the concentration of sediment samples, IR (sediment) refers to the sediment ingestion rate, EF represents the exposure frequency, and ED represents the duration of exposure. BW indicates body weight, FE is the dermal exposure ratio, AF refers to the dermal surface factor, ABS is the dermal absorption factor, AT denotes the average lifespan, SA is the surface area, and PEF is the particle emission factor. The Reference Doses (RfD) for pesticides and heavy metals were used to calculate the non-carcinogenic risk as provided in Supplementary Table 1.

Data analysis

Data were analyzed using Microsoft Excel 2016 and IBM SPSS (version 25.0). The results are expressed as mean ± standard deviation.

Results

CKDu dominates among the farming community in blocks with intense agricultural activities

Of the 1136 individuals screened in the hotspot blocks of Bargarh district, 157 were found to be affected with CKDu and 75 were diagnosed with CKD of known etiology. The current study revealed a greater incidence of CKDu (26 cases with a prevalence of 7.3%) cases in Attabira block followed by Bhatli, Bheden, Gaisilate and Bijepur compared to CKD of known etiology. The average age of patients with CKD and CKDu across all the studied blocks was found to be approximately 40 years, with the number of cases among males being greater than that among females. The Attabira block reported the highest number of male CKD patients followed by the Gaisilat block, whereas CKD cases among males were found to be minimal in the Bijepur block. No marked differences in BMI were noted across the hotspot blocks. Compared to other occupational groups such as laborers and others, approximately 65% of the individuals directly engaged in farming and related activities were found to be affected by CKD. Most of the CKD patients in these hotspot blocks had lower socioeconomic backgrounds and less education. The history of tobacco and alcohol abuse was minimal in all the studied areas (Table 1).

Blocks reporting higher prevalence of CKDu showed higher concentrations of nephrotoxic pesticide residue in soil, water and rice grain

Table 2 shows the concentration of nephrotoxic pesticides and heavy metals in soil, water, and rice grains across the studied hotspot blocks. The present study detected

Table 1 Demographic characteristics and prevalence of CKD and CKDu in hotspot blocks

Demographic Characteristic	Attabira Block	Bheden Block	Bijepur Block	Bhatli Block	Gaisilat Block
Total Population Screened	353	173	177	249	184
Number of CKD Cases *n* (% prevalence)	26 (7.3)	16 (9.2)	10 (5.6)	5 (2.0)	18 (9.7)
Number of CKDu Cases *n* (% prevalence)	73 (20.6)	25 (14.4)	11 (6.2)	26 (10.4)	22 (11.9)
Age (Mean ± SD)	46 ± 11.23	49 ± 9.3	51 ± 6.2	48 ± 3.5	41 ± 9.61
No. of Males	71	34	10	23	36
No. of Females	28	7	11	8	4
Hypertension	24	11	8	5	12
Diabetes	21	9	6	4	16
Body Mass Index (Mean ± SD)	28 ± 5.6`	21 ± 3.21	25 ± 9.6	29 ± 8.4	27 ± 5.8
Occupation					
Farmers	68	29	14	21	19
Laborers	27	9	6	8	15
Other	4	3	1	2	6
Level of Education					
Illiterate	38	21	10	18	27
Primary	21	15	8	9	10
Upper Primary	32	4	3	3	4
Degree	8	1	0	1	0
Income					
High	27	7	2	5	4
Average	40	15	9	9	14
Low	32	19	10	17	22
h/o Tobacco use	35	15	5	8	13
h/o Alcohol use	28	20	12	11	18

nephrotoxic pesticide residues such as DDT, dichlorvos, heptachlor epoxide and dieldrin in water, soil and rice grains collected from the study areas. Among the pesticide residues detected in the water samples, the concentration of DDT was found to be greater than the permissible limit (0.01 mg/kg FSSAI MRL) in all the hotspot blocks, with the highest concentration detected in the Attabira (0.09 ± 0.04 µg/l). The concentrations of dichlorvos and heptachlor epoxide in water samples across the hotspot blocks were found to be 0.023–0.046 µg/l and 0.003–0.016 µg/l, respectively which are below the permissible limits. A total of six pesticide residues were detected in the soil, of which dichlorvos residue was found to be the most abundant (0.072–0.076 µg/g) across the hotspot blocks, with marginal differences in concentration. The current study detected variable concentrations of pesticide residues such as dieldrin, heptachlor epoxide, dichlorvos and chlordane in rice grains collected from different hotspot blocks. Rice grains collected from Attabira presented a relatively high concentration (0.027 ± 0.014 µg/g) of dieldrin, whereas dichlorvos residue (0.06 ± 0.00 and 0.04 ± 0.00 µg/g) was found to be high in grains collected from the Bheden and Barpali blocks, respectively. On the other hand, the concentration of heptachlor epoxide was high (0.015 ± 0.01 µg/g) in rice grains collected from the Bijepur block, whereas the concentration of chlordane was high in rice grains collected from the Gaisilat block.

Nephrotoxic heavy metals such as chromium, lead, and arsenic were found to be above permissible limits in all hotspot blocks

The concentration of chromium was greater in the soil, water and grains of the Attabira, Bheden, Barpali and Gaisilat blocks. In the case of Bijepur block, although the concentration of chromium was reported to be high in soil and water, the concentration of arsenic in grain samples was relatively high compared with those of the other heavy metals. The concentration of lead was found to be high in all the samples analyzed across the hotspot blocks, next to chromium. The highest concentration of arsenic was found in the soil samples of Attabira (1.07 µg/g) followed by Barpali (0.53 µg/g) and Bijepur block (0.43 µg/g). However, in the water samples from the Gaisilat block, lead was found to be prominent, exceeding the concentration of chromium.

Table 2 Concentration of nephrotoxicants (pesticides and heavy metals) in environmental and biological samples

Concentrations of Nephrotoxicants (pesticides and heavy metals) in environmental samples (soil (μg/g), water sample (μg/l) and rice grain (μg/g)) of hotspot blocks

Name of the Block	Sample	DDT	Heptachlor epoxide	Dieldrin	Aldrin	Chlordane	Dichlorovos	As	Cd	Pb	Hg	Cr
Attabira	Soil	0.068 ± 0.021	0.073 ± 0.05	0.067 ± 0.021	0.014 ± 0.01	0.017 ± 0.01	0.076 ± 0.027	19.00 ± 3.17	0.7 ± 0.34	34.61 ± 4.46	0.37 ± 0.07	47.43 ± 11.27
	Water	0.09 ± 0.04	0.019 ± 0.011	0.016 ± 0.01	0.014 ± 0.01	0.017 ± 0.01	0.046 ± 0.017	3.41 ± 0.73	0.48 ± 0.04	4.83 ± 0.35	0.35 ± 0.02	38.26 ± 2.01
	Grain	0.016 ± 0.012	0.021 ± 0.01	0.027 ± 0.014	0.007 ± 0.00	0.012 ± 0.01	0.07 ± 0.00	1.07 ± 0.04	0.25 ± 0.01	0.45 ± 0.03	0.18 ± 0.01	1.34 ± 0.25
Bheden	Soil	0.066 ± 0.022	0.071 ± 0.07	0.065 ± 0.020	0.012 ± 0.007	0.016 ± 0.005	0.074 ± 0.025	14.61 ± 3.66	0.66 ± 0.09	27.91 ± 2.34	0.29 ± 0.13	43.70 ± 13.87
	Water	0.08 ± 0.03	0.017 ± 0.010	0.014 ± 0.004	0.013 ± 0.003	0.015 ± 0.002	0.035 ± 0.015	3.26 ± 0.25	0.43 ± 0.02	4.26 ± 0.77	0.25 ± 0.02	33.42 ± 3.02
	Grain	0.014 ± 0.009	0.020 ± 0.01	0.025 ± 0.012	0.007 ± 0.001	0.012 ± 0.01	0.06 ± 0.00	0.94 ± 0.01	0.23 ± 0.01	0.34 ± 0.09	0.16 ± 0.01	1.21 ± 0.22
Barpali	Soil	0.065 ± 0.020	0.070 ± 0.05	0.061 ± 0.021	0.011 ± 0.01	0.014 ± 0.01	0.073 ± 0.024	12.71 ± 2.34	0.43 ± 0.04	25.37 ± 3.78	0.26 ± 0.10	41.59 ± 12.76
	Water	0.07 ± 0.02	ND	0.012 ± 0.01	0.011 ± 0.01	0.014 ± 0.002	0.031 ± 0.013	3.01 ± 0.32	0.37 ± 0.05	3.95 ± 0.22	0.23 ± 0.01	24.32 ± 1.06
	Grain	0.013 ± 0.009	0.017 ± 0.01	0.023 ± 0.012	0.006 ± 0.001	0.01 ± 0.003	0.04 ± 0.001	0.53 ± 0.06	0.20 ± 0.01	0.25 ± 0.03	0.15 ± 0.01	0.38 ± 0.04
Bijepur	Soil	0.060 ± 0.017	0.065 ± 0.02	0.060 ± 0.012	0.010 ± 0.001	0.011 ± 0.01	0.072 ± 0.021	11.40 ± 2.23	0.33 ± 0.10	22.08 ± .11	0.10 ± 0.04	37.60 ± 10.87
	Water	0.07 ± 0.02	ND	0.012 ± 0.01	0.010 ± 0.01	ND	0.023 ± 0.011	2.73 ± 0.73	0.25 ± 0.03	3.46 ± 0.21	0.18 ± 0.03	21.56 ± 1.08
	Grain	0.013 ± 0.003	0.015 ± 0.01	ND	0.005 ± 0.001	0.013 ± 0.007	ND	0.45 ± 0.09	0.17 ± 0.01	0.17 ± 0.09	0.14 ± 0.01	0.37 ± 0.13
Gaisilat	Soil	0.057 ± 0.015	0.061 ± 0.01	0.057 ± 0.010	0.007 ± 0.001	0.009 ± 0.003	0.072 ± 0.021	9.00 ± 3.13	0.9 ± 0.43	30.52 ± 8.33	0.34 ± 0.04	45.21 ± 7.30
	Water	0.05 ± 0.01	0.015 ± 0.01	0.009 ± 0.003	0.009 ± 0.001	0.027 ± 0.012	0.023 ± 0.011	2.73 ± 0.71	0.43 ± 0.03	43.27 ± 7.01	4.93 ± 0.37	0.45 ± 0.03
	Grain	0.011 ± 0.001	0.013 ± 0.01	0.003 ± 0.001	0.003 ± 0.001	0.007 ± 0.002	0.03 ± 0.001	0.43 ± 0.07	0.18 ± 0.01	0.21 ± 0.03	0.16 ± 0.01	0.39 ± 0.09

Concentration of Nephrotoxicants (pesticides/ heavy metals (ng/mL)) in biological samples of the study cohort

Group	Biological Samples	DDT	Heptachlor epoxide	Dieldrin	Aldrin	Chlordane	Dichlorvos	Arsnic	Cadmium	Lead	Mercury	Chromium
Occupationally Exposed (N = 117)	Blood	2.32 ± 0.007	ND	1.93 ± 0.002	2.10 ± 0.003	ND	ND	11.1 ± 2.7	9.8 ± 0.7	22 ± 4.1	ND	ND
	Urine	ND	ND	ND	0.17 ± 0.003	ND	ND	21.11 ± 2.81	1.42 ± 0.32	17.43 ± 3.71	ND	ND
Occupationally Unexposed (N = 40)	Blood	0.72 ± 0.003	ND	0.81 ± 0.001	1.7 ± 0.001	ND	ND	3.7 ± 4.3	2.7 ± 0.6	9 ± 0.16	ND	ND
	Urine	ND	ND	ND	ND	ND	ND	25.18 ± 2.33	1.09 ± 0.16	11.10 ± 2.30	ND	ND

ND not detected

Dietary exposure to pesticide residue and non-carcinogenic risk

The estimation of the non-carcinogenic risk of nephrotoxic heavy metals and pesticides is shown in Table 3. In the Attabira block, the non-carcinogenic risk of heptachlor epoxide through non-dietary sources was noted to be greater than that of other pesticides, followed by dieldrin and DDT. Exposure to heptachlor epoxide through the ingestion route was found to have greater risk followed by the dermal and inhalation routes. In case of dietary non-carcinogenic risk, DDT was found to have the highest risk, followed by heptachlor epoxide and dichlorvos through ingestion of water. However, in grains, heptachlor epoxide posed a greater health risk. Similarly, exposure to arsenic through the ingestion and dermal routes presented a greater risk, whereas chromium presented a potent non-carcinogenic risk through the inhalation route over other heavy metals. In the case of the Bheden block, heptachlor epoxide posed a greater risk for non-carcinogenic diseases when exposed via non-dietary routes such as inhalation. Compared with other pesticides, exposure to DDT through the dermal route resulted in greater health risks. Arsenic represented a potent risk through the ingestion route, followed by chromium via the dermal route. DDT and chromium in water and heptachlor epoxide in grains showed much greater risk when exposed through the dietary route. Similar to the Attabira block, greater health risk of heptachlor epoxide and arsenic in both dietary and non-dietary exposure was observed in Barpali block. In addition to arsenic, lead through ingestion (water) was found to impart a prominent non-carcinogenic risk. In the Bijepur block the non-dietary risk of heptachlor epoxide through the ingestion route was markedly higher, followed by the risk related to aldrin through the same route of exposure. In case of dietary exposure, the risk related to dichlorvos in grain samples was found to be greater compared to other pesticides. Dermal exposure of lead and exposure to arsenic via ingestion route was found to impart a potent non-carcinogenic risk. With regard to the Gaisilat block, health risk due to exposure to heavy metals through dietary as well as non-dietary routes was observed to be very high in comparison to other toxicants.

Direct exposure and nephrotoxic pesticide residues and heavy metals in blood and urine samples

Toxicological analysis of blood and urine samples was performed for 157 CKDu patients, 117 of whom were in the direct exposure group to pesticides and the remaining 40 were in the indirect exposure group. Individuals practicing farming were considered the direct exposure group while those with other occupations, such as construction laborers, teachers, government employees, were grouped as the indirect exposure group. Table 2 shows the concentration of analyzed nephrotoxic heavy metals and pesticide residues in the blood and urine samples of occupationally exposed and unexposed CKDu patients. The present study detected pesticide residues such as DDT and aldrin in the blood and urine samples of CKDu patients. The concentration of all the analyzed pesticides was greater in the blood of the occupationally exposed cohort than that in the unexposed group. The concentration of DDT was found to be the highest (2.32 ± 0.007 ng/mL), followed by aldrin i.e., 2.10 ± 0.003 ng/mL in the blood of the exposed group. Traces of lead, arsenic and cadmium were detected in the blood of the exposed group compared to the unexposed group. Lead was found in the highest concentration i.e., 22 ± 4.1 ng/mL, followed by arsenic in the blood samples of exposed individuals. In the exposed group, the concentration of lead in the urine samples was relatively higher (i.e., 17.43 ± 3.71) than that in the unexposed cohort. Conversely, arsenic was detected in higher concentrations in the urine samples of the unexposed group. None of the pesticide residues were detected in the urine samples of either cohort, except aldrin in the exposed group.

Aplastic anemia predominates among CKDu patients in hotspot blocks

To estimate the prevalence of anemia among CKD-affected individuals, 232 patients of the total screened population were included. Based on the diagnostic criteria, 77 individuals were diagnosed with CKD while the remaining 155 were found to be affected with CKDu. Among the 77 CKD patients, 66 were found to have anemia whereas 140 of the 157 CKDu patients were found to be anemic. In the present study, the number of male CKD and CKDu patients affected with anemia was very high compared to that of female individuals, indicating that males are more vulnerable to CKD and anemia. No significant differences in age or BMI were observed between anemic CKD patients and CKDu patients in the study population. Occupational analysis of the study population revealed that the majority of the affected anemic male CKD and CKDu patients had been actively farming rice and other crops for a long time and had a long history of pesticide use in their crop fields. The prevalence of CKD and CKDu with anemia was found to be greater among the illiterate and lower income groups. The detailed demographic characteristics of the study participants are presented in Supplementary Table 2.

Large numbers of individuals with CKDu in hotspot blocks were found to be affected with anemia, i.e., 89.17%, as compared to those with CKD and normal healthy individuals (25.3%). Prevalence of anemia was significantly greater among CKDu patients (89.42%) than CKD patients carrying out farming activities ($p < 0.05$). Microcytic anemia was

Table 3 Non-Dietary and Dietary Non-Carcinogenic risk of pesticides and heavy metals exposed through soil, water and rice grain in an adult population

Blocks	Route of Exposure		DDT	Hcle	Dieldrin	Aldrin	Chld	Dichl	Arsenic	Cadmium	Lead	Mercury	Chromium
Attabira	NDNCR Soil	Ingestion	0.002329	0.009615	0.002295	0.000799	5.82E–05	0.00026	0.108447	0.0004	0.014816	0.002112	0.027072
		Dermal	0.001208	0.004988	0.00119	0.000414	3.02E–05	0.000135	0.062502	0.020724	0.007685	0.001095	0.014042
		Inhalation	1.71E–06	7.07E–06	1.69E–06	5.88E–07	4.28E–08	1.91E–07	7.97E–05	4.9E–07	1.09E–05	1.55E–06	0.000597
	DNCR	Ingestion (Water)	0.000362	7.65E–05	6.44E–05	5.63E–05	6.84E–05	0.000185	0.274435	0.023178	0.029154	0.028168	0.307914
		Ingestion (Grain)	0.60274	3.042677	1.017123	0.439498	0.045205	0.263699	6.718037	0.47089	0.211901	1.130137	0.841324
Bheden	NDNCR Soil	Ingestion	0.00226	0.009352	0.002226	0.000685	5.48E–05	0.000253	0.08339	0.000377	0.011948	0.001655	0.024943
		Dermal	0.001172	8.51E–07	2.03E–07	0.000355	4.99E–09	2.31E–08	0.048061	0.01954	0.006197	0.000859	0.012938
		Inhalation	1.66E–06	6.88E–06	1.64E–06	5.04E–07	4.03E–08	1.86E–07	6.13E–05	4.62E–07	8.79E–06	1.22E–06	0.00055
	DNCR	Ingestion (Water)	0.000322	6.84E–05	5.63E–05	5.23E–05	6.04E–05	0.000141	0.262363	0.020764	0.025713	0.02012	0.268962
		Ingestion (Grain)	0.527397	2.897787	0.941781	0.439498	0.045205	0.226027	5.901826	0.433219	0.160103	1.004566	0.759703
Barpali	NDNCR Soil	Ingestion	0.002226	0.00922	0.002089	0.000628	4.79E–05	0.00025	0.072546	0.000245	0.01086	0.001484	0.023739
		Dermal	0.001155	0.004783	0.001084	0.000326	2.49E–05	0.00013	0.04181	0.012731	0.005633	0.00077	0.012313
		Inhalation	1.64E–06	6.78E–06	1.54E–06	4.62E–07	3.53E–08	1.84E–07	5.33E–05	3.01E–07	7.99E–06	1.09E–06	0.000524
	DNCR	Ingestion (Water)	0.000282	0.002854	3.42E–05	4.02E–05	0.002854	6.56E–05	0.220031	0.012072	0.020884	0.014486	0.173514
		Ingestion (Grain)	0.489726	2.463119	0.866438	0.376712	0.037671	0.150685	3.327626	0.376712	0.117723	0.941781	0.238584
Bijepur	NDNCR Soil	Ingestion	0.002055	0.008562	0.002055	0.000571	3.42E–05	0.000247	0.065068	0.000188	0.009452	0.000571	0.021461
		Dermal	0.001066	0.004441	0.001066	0.000296	1.78E–05	0.000128	0.037501	0.00977	0.004903	0.000296	0.011132
		Inhalation	1.51E–06	6.3E–06	1.51E–06	4.2E–07	2.52E–08	1.81E–07	4.78E–05	2.31E–07	6.95E–06	4.2E–07	0.000473
	DNCR	Ingestion (Water)	0.000282	0	4.83E–05	4.02E–05	0	9.26E–05	0.220031	0.012072	0.020884	0.014486	0.173514
		Ingestion (Grain)	0.489726	2.17334	0	0.313927	0.048973	3.767123	2.825342	0.320205	0.080051	0.878995	0.232306
Gaisilat	NDNCR Soil	Ingestion	0.001952	0.008035	0.001952	0.0004	3.08E–05	9.25E–05	0.05137	0.000514	0.013065	0.001941	0.024092
		Dermal	0.001013	0.004168	0.001013	0.000207	1.6E–05	4.8E–05	0.029606	0.026646	0.006777	0.001007	0.012497
		Inhalation	1.44E–06	5.91E–06	1.44E–06	2.94E–07	2.27E–08	6.8E–08	3.78E–05	6.3E–07	9.61E–06	1.43E–06	0.000531
	DNCR	Ingestion (Water)	0.000201	6.04E–05	3.62E–05	3.62E–05	0.000109	9.26E–05	0.219709	0.020764	0.261176	0.396764	0.003622
		Ingestion (Grain)	0.414384	1.883562	0.113014	0.188356	0.263699	0.113014	2.699772	0.339041	0.098887	1.004566	0.244863

Hcle heptachlor epoxide, *Chld* Chlordane, *Dichl* Dichlorovus, *NDNCR* Non-Dietary Non-Carcinogenic Risk, *DNCR* Dietary Non-Carcinogenic Risk found to be highest in the CKDu group among farmers, i.e., 62.26%. Conversely, cases of normocytic anemia were most common among farmers in the CKD cohort. Macrocytic anemia, observed across the groups, exhibited the lowest prevalence compared with the other types of anemia. None of the individuals with CKD were diagnosed with macrocytic anemia. The highest prevalence of aplastic anemia was observed among farmers in the CKDu group (71.15%). Overall, the percentage prevalence of aplastic anemia was found to be predominant among individuals affected by CKDu. In contrast, the prevalence of anemia was markedly lower among healthy individuals, being found at 25.3%, than in the other two groups, as illustrated in Supplementary Table 3.

Discussion

The present study identified five blocks of Bargarh as hotspots with CKD/CKDu prevalence higher than the national average of 8.02%. The overall prevalence of CKDu was estimated to be 13.82%, whereas that of CKD was found to be 6.60%, suggesting a higher prevalence of CKDu in the hotspot blocks than in the rest of the district. Interestingly, four out of five hotspot blocks with higher prevalence of CKDu were intense agricultural zones. A previous study by our laboratory reported that sixteen villages in the Bargarh district presented a number of CKD cases that was twice the national average [7]. A similar higher prevalence of CKD/CKDu was observed in agriculturally intensive areas of Central America, Mexico, Egypt, Tunisia, North Central Province of Sri Lanka, and some parts of India such as Andhra Pradesh (Uddanam endemic nephropathy), Chattisgarh (Supebeda), and Odisha (Cuttack). The high proportion of CKDu cases reported in the studied area suggests that CKDu is endemic in this region. In the present study, a higher proportion of males was found to be affected with CKD compared to females, in keeping with previous findings [13]. This may be due to their socio-behavioral and occupational practices with longer exposure to heatwaves, heavy work load, direct exposure to pesticides in agricultural fields and consumption of locally made alcoholic drinks. Previous studies reported that the average age of the people effected with CKD in Karnataka and Odisha was 52.73 ± 17.08 and 46 ± 11.82, respectively [14]. Interestingly, the present study showed that many individuals between 40 and 45 years of age were affected by the disease. CKDu cases were found to be more prevalent among the younger population in lower socio-economic settings.

The study showed that 65% of the individuals directly engaged in farming and related activities across the hotspot blocks were affected with CKD compared to other occupational groups. The highest number of CKD cases was found in Attabira blocks. Attabira block has 30,150 hectares of irrigated land, and 76% of the total population is involved in farming activities, followed by Bheden (28,758 hectares), Bhatli (16,280 hectares), Bijepur (16,003 hectares) and Gaisilat (11,469 hectares) (District Statistics Office, Bargarh). The major crop grown in these blocks is paddy, which requires heavy use of fertilizers, continuous supply of water and frequent use of pesticides to protect the crops. Most of the pesticides used in paddy cultivation, such as 2,4-D, paraquat dichloride, dichlorvos, DDT, captan, cypermethrin, glyphosate and DBCP are reported to be nephrotoxic [2, 15]. The intensity of agricultural activities among the blocks under study is, in decreasing order, Attabira > Bheden > Bijepur > Bhatli > Gaisilat. The increased incidence of CKDu among farming communities in the blocks with high intensity of agricultural activities indicates a possible association of pesticide exposure with disease onset and progression. In support of our findings, studies carried out on Sri Lankan Agricultural Nephropathy reported higher prevalence of CKDu among farming communities which could be linked to cyclic dehydration, direct exposure to pesticide residue and heat [16]. Further, Bargarh shares agroclimatic condition similar to Andhra Pradesh, which is one of the neighboring states of Odisha, and is known for Uddanam Endemic Nephropathy drastically affecting agricultural communities. It is well known that most CKD patients develop anemia with disease progression due to compromised production of hepcidin and erythropoietin. However, in individuals with CKDu in whom environmental toxicants such as heavy metals and pesticide residue are reportedly associated with disease onset and progression, the question of whether pesticides/heavy metals induce CKD, which subsequently leads to anemia, or if toxicants directly cause anemia is not clear. The predominant form of anemia among CKDu patients in our study area was aplastic anemia which is known to be associated with exposure to environmental toxicants such as heavy metals and/or pesticides [17]. Epidemiological studies conducted worldwide recorded a higher prevalence of anemia among CKD patients, namely 53.4% in the USA, 53.5% in Ethiopia, 44.9% in Korea, 33% in Tanzania, and 6.76% in the UK. Similar studies in Indian settings by Zaawari et al., reported an 82.4% prevalence of anemia among CKD patients[5]. The trend of anemia was found to differ with regard to gender; in the current study 60% of the males suffering from CKDu were found to be anemic whereas only 40% of the females having CKDu were anemic. This difference in gender may be due to greater occupational exposure of males to pesticides in agricultural areas. As pesticides are known to have potential nephrotoxic effects that cause CKDu, the majority of the study population practicing farming were found to be affected with CKDu. Individuals with lower income and low educational status were found to be more affected with these disease conditions, which corroborated with the findings of the previous studies showing higher incidence of CKD and anemia among people with poor socio-economic status [18].

We observed more cases of microcytic anemia in the CKDu group than in the CKD group. Organochlorine pesticides such as aldrin, DDT and dieldrin have also been reported to cause aplastic anemia. Cases of aplastic anemia are more prevalent among the farmers affected with CKDu [19]. In line with our findings, Malhotra et al., reported a higher prevalence of aplastic anemia among agricultural workers with low socio-economic status in India [20]. The detection of organochloride pesticide residues, such as aldrin, dieldrin and DDT, in the blood samples may explain the development of aplastic anemia. In keeping with our findings, Ahamed et al. detected organochlorine pesticide residues in the blood samples of anemic patients in a case control study conducted at Lucknow, India [21]. Interestingly, the prevalence of anemia among non-affected individuals residing in the same village was much lower than that among individuals affected with CKD.

The present study found a higher hazard quotient for heptachlor epoxide i.e. 3.042, and dieldrin i.e. 1.017 when exposed through the dietary route compared to the non-dietary route in Attabira block. The maximum oral LD_{50} value for dieldrin in mammals is 38 mg/kg body weight, while for heptachlor epoxide it is 100 mg/kg body weight, indicating that dieldrin is more toxic to mammals than heptachlor epoxide. However, the concentration of heptachlor epoxide was found to be greater than dieldrin in all three matrices, indicating higher non-carcinogenic risk. A similar trend was observed in all blocks studied, indicating heptachlor epoxide as a major concern in this area. In support of our findings, the study carried out by Singh et al. reported a closer association of heptachlor epoxide with renal dysfunction leading to interstitial fibrosis in the rat [22]. Pesticide residue such as DDT, dichlorvos, and dieldrin in water, soil and rice grain was found to be highest in Attabira among all the hotspot blocks, corroborating its higher prevalence of CKDu cases. Researchers investigating CKDu in Sri Lanka have suspected that contaminated drinking water, particularly with heavy metals and/or pesticides, is a primary factor contributing to the disease [23]. Prolonged use of dichlorvos has been shown to damage the structure of the renal cortex, potentially leading to kidney failure. Organochlorine pesticides such as dieldrin cause oxidative stress and can accelerate kidney injury, leading to the onset and progression of CKD.

In addition to pesticide residues, agricultural activities significantly influence heavy metal pollution in agricultural soils and plants [24]. In the present study, chromium, arsenic and lead are the prominent heavy metals found above permissible limits in soil, water and rice grains in the hotspot blocks of Bargarh. Due to their bioaccumulative nature, these heavy metals very often reach the community through trophic transfer and biomagnification at elevated concentrations. In the present study, higher hazard quotient values were observed for arsenic and mercury exposure compared to all other heavy metals in the Attabira block. The observed higher non-carcinogenic risk for arsenic, i.e., 6.718, could be because of the low oral LD_{50} value which ranges from 15–293 mg/kg body weight against mammalian system compared to other heavy metals. A higher prevalence of CKD was observed among farmers in hotspot blocks reporting elevated concentrations of heavy metals, indicating a closer association between heavy metal exposure and onset of CKD. In line with the present findings, studies carried out in an urban community industrial region of Greece reported a strong correlation between prolonged exposure to chromium in drinking water and an increased incidence of kidney diseases among residents [25]. A cross-sectional study of patients in Taiwan showed a positive correlation between urinary levels of arsenic and the incidence of CKD [26]. Lead has a broad spectrum of negative impacts causing anemia, renal dysfunction, and hypertension. Similar studies carried out by Tyagi et al. in India reported hazard quotient values exceeding the permissible level (set at 1) for exposure to arsenic [27]. Agrochemicals and heavy metals which are absorbed through various pathways, can accumulate in the bodies of individuals living in endemic regions and are often found in high concentrations in their urine. In the present study, the concentrations of nephrotoxic heavy metals (lead, arsenic and cadmium) and pesticides (aldrin, dieldrin and DDT) in the blood were significantly higher in the exposed CKDu patients than in unexposed patients. The present findings gain support from the study carried out on Sri Lankan Agricultural Nephropathy reporting the implication of multiple heavy metal and pesticide residues in inducing kidney disorders [23]. Animal studies have shown that all heavy metals found at high levels in the urine of Sri Lankan Agricultural Nephropathy patients can cause oxidative damage to the kidneys.

Heavy metals occur naturally and are released primarily from various types of rocks influenced by environmental conditions and human activities, such as mining, agriculture, and contaminated wastewater. Igneous and metamorphic rocks, including granite, basalt, sandstone, limestone, and shale, are known sources of cadmium, which can leach into water sources. Lithological studies in Bargarh district have revealed that the presence of meta-sedimentary rocks mainly shale, calcareous shale and granitic rocks with varying composition such as tonalite, medium grained granodiorite, porphyritic granodiorite, to alkaline-granite could contribute to the high levels of these heavy metals into water and soil. Arsenic is found in over 200 mineral species, with arsenopyrite being the most common. Human activities, such as mining, non-ferrous metal smelting, and fossil fuel combustion, significantly contribute to arsenic contamination in air, water, and soil. Additionally, the use of arsenic-containing pesticides has left extensive agricultural areas contaminated. Inorganic fertilizers, such as phosphate fertilizers, liming materials, and bio-fertilizers, are significant contributors to heavy metal release in agricultural soils, which are then absorbed by plants. Chromium is present in minerals such as chromite, magnesiochromite, stichtite, and fornacite rock. Commercially available nitrogen fertilizers can contain heavy metals, including arsenic (2.0–6.5 mg/kg), copper (12.5–26.3 mg/kg), iron (16.5–1442 mg/kg), manganese (20.3–5290 mg/kg), nickel (6.2–27.8 mg/kg), and zinc (1.4–166.0 mg/kg).

Exposure to multiple heavy metals can intensify kidney damage due to combined effects, even at lower concentrations. Sanchez et al. reported that when rats are exposed to both mercury and uranium, they experience significantly more necrosis in the proximal tubules compared to controls [28] Similarly, the combined exposure to lead and cadmium has been shown to worsen renal tubular dysfunction, leading to more severe kidney impairment than when each metal is encountered alone [29]. Therefore, it is crucial to assess both individual and combined toxicities to better understand the impact of heavy metal mixtures on kidney health. Additionally, previous research has shown that exposure to multiple pesticide compounds can also have additive toxic effects. A World Health Organization (WHO) study group reported excessive levels of multiple pesticides and pesticide residue in the urine samples of individuals from CKDu endemic areas [30]. Further studies are necessary to explore the combined effects and interactions of multiple pollutants in contributing to chronic kidney disease of unknown etiology among exposed individuals.

Conclusion

The present study identified five blocks in the Bargarh district under Hirakud Command area as hotspots for CKD and CKDu, with a prevalence rate twice the national average. Four of the five hotspot blocks are located in intense agricultural zones with extensive use of pesticides and chemical fertilizers. CKDu was found to be more prevalent among populations directly involved in agriculture and related activities, indicating an association of agrochemicals and heavy metals with CKDu disease onset and progression. Nephrotoxic pesticide residues such as DDT, dichlorvos, dieldrin and chlordane and heavy metals like arsenic, cadmium, chromium and lead were found to be above permissible limits in water, soil and rice grains. A higher concentration of pesticides and heavy metals was found in water, soil and rice grains. Our study further detected traces of heavy metals and pesticide residue in the blood and urine samples of patients occupationally exposed to the agrochemicals in the study area. A higher prevalence of anemia was observed among people suffering from CKD/CKDu compared to the non-affected population of the region. Most of the patients suffering from CKDu were affected by aplastic anemia which could be associated with pesticide residue and heavy metal exposure. However, our study should not be generalized to the entire Indian population as it deals with a single region. Additional cohort-based studies are needed to investigate the cumulative risks faced by communities exposed to multiple nephrotoxic environmental toxicants, as well as their role in the onset and progression of chronic kidney disease.

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Data availability All the data related to the paper are available in the manuscript or as supplementary material.

Declarations

Conflict of interest Authors have no conflict of interest to declare.

Ethical Approval The study was approved by the Institutional Ethical Committee of Sambalpur University (IEC-SU), Odisha vide letter number 16/IEC-SU/2023, dated 29/07/2023.

Inform consent to participate Informed consent was obtained from all the individuals participating in the study.

References

1. Tatapudi RR, Rentala S, Gullipalli P et al (2019) High prevalence of CKD of unknown etiology in Uddanam India. Kidney Int Reports. https://doi.org/10.1016/j.ekir.2018.10.006
2. Ghosh R, Siddarth M, Singh N et al (2017) Organochlorine pesticide level in patients with chronic kidney disease of unknown etiology and its association with renal function. Environ Health Prev Med. https://doi.org/10.1186/s12199-017-0660-5
3. Jamil K, Shaik AP, Mahboob M, Krishna D (2004) Effect of organophosphorus and organochlorine pesticides (monochrotophos, chlorpyriphos, dimethoate, and endosulfan) on human lymphocytes in-vitro. Drug Chem Toxicol. https://doi.org/10.1081/DCT-120030725

4. Desai SN, Desai PV (2008) Changes in renal clearance and renal tubular function in albino mice under the influence of Dichlorvos. Pestic Biochem Physiol. https://doi.org/10.1016/j.pestbp.2008.03.008
5. Zaawari A, Tejaswini KL, Davina GD, Singanaveni A (2022) Prevalence of anemia among chronic kidney disease patients in India: a single-centre study. Int J Basic Clin Pharmacol. https://doi.org/10.18203/2319-2003.ijbcp20222135
6. Levin A, Stevens PE, Bilous RW et al (2013) Kidney disease: improving global outcomes (KDIGO) CKD work group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int 3(1):1–150. https://doi.org/10.1038/kisup.2012.73
7. Biswas P, Ashish Kumar S, Soumya RN et al (2024) Prevalence of chronic kidney disease and its association with pesticide exposure in Bargarh District, Odisha, India. Indian J Nephrol 34(5):467–474. https://doi.org/10.25259/ijn_188_23
8. Hsieh YP, Chang CC, Kor CT et al (2017) Mean corpuscular volume and mortality in patients with CKD. Clin J Am Soc Nephrol. https://doi.org/10.2215/CJN.00970116
9. Gutiérrez-Serdán R, López-Karpovitch X (2016) Acquired aplastic anemia: a demographic, clinical, and therapeutic survey of a single institution in Mexico City. Rev Hematol Mex 17(2):81–89
10. Iqbal KJ, Khan N, Fatima M et al (2023) Determination of heavy metals (Pb, Cr, As, Hg, and Cd) into the body organs of selected fish, water, sediment, and soil samples from Head Punjnad and Head Taunsa, Punjab. Pakistan PLoS One. https://doi.org/10.1371/journal.pone.0288163
11. Wang Y, Xu W, Li J et al (2022) Assessing the fractionation and bioavailability of heavy metals in soil–rice system and the associated health risk. Environ Geochem Health. https://doi.org/10.1007/s10653-021-00876-4
12. Siddharth M, Datta SK, Bansal S et al (2012) Study on organochlorine pesticide levels in chronic kidney disease patients: Association with estimated glomerular filtration rate and oxidative stress. J Biochem Mol Toxicol. https://doi.org/10.1002/jbt.21416
13. Mohanty NK, Sahoo KC, Pati S et al (2020) Prevalence of chronic kidney disease in Cuttack district of Odisha, India. Int J Environ Res Public Health. https://doi.org/10.3390/ijerph17020456
14. Palo SK, Swain S, Chowdhury S, Pati S (2021) Epidemiology & attributing factors for chronic kidney disease: Finding from a case-control study in Odisha, India. Indian J Med Res. https://doi.org/10.4103/ijmr.IJMR_2148_18
15. Jacobson MH, Wu Y, Liu M et al (2021) Organophosphate pesticides and progression of chronic kidney disease among children: A prospective cohort study. Environ Int. https://doi.org/10.1016/j.envint.2021.106597
16. Herath C, Jayasumana C, De Silva PMCS et al (2018) Kidney diseases in agricultural communities: a case against heat-stress nephropathy. Kidney Int Rep 3(2):271–280. https://doi.org/10.1016/j.ekir.2017.10.008
17. Prihartono N, Kriebel D, Woskie S et al (2011) Risk of aplastic anemia and pesticide and other chemical exposures. Asia-Pacific J Public Heal. https://doi.org/10.1177/1010539511403605
18. Kumar P, Sharma H, Sinha D (2021) Socio-economic inequality in anaemia among men in India: a study based on cross-sectional data. BMC Public Health. https://doi.org/10.1186/s12889-021-11393-5
19. Asif Syed M, Rahman AAU, Siddiqui MI, Arain AA (2021) Pesticides and chemicals as potential risk factors of aplastic anemia: a case–control study among a Pakistani population. Clin Epidemiol. https://doi.org/10.2147/CLEP.S304132
20. Malhotra P, Gella V, Murthy GSG et al (2016) High incidence of aplastic anemia is linked with lower socioeconomic status of Indian population. J Public Heal (United Kingdom). https://doi.org/10.1093/pubmed/fdv027
21. Ahamed M, Anand M, Kumar A, Siddiqui MKJ (2006) Childhood aplastic anaemia in Lucknow, India: Incidence, organochlorines in the blood and review of case reports following exposure to pesticides. Clin Biochem. https://doi.org/10.1016/j.clinbiochem.2006.03.021
22. Singh N, Siddarth M, Ghosh R et al (2019) Heptachlor-induced epithelial to mesenchymal transition in HK-2 cells mediated via TGF-β1/Smad signalling. Hum Exp Toxicol. https://doi.org/10.1177/0960327119828136
23. Jayasumana C, Gunatilake S, Senanayake P (2014) Glyphosate, hard water and nephrotoxic metals: Are they the culprits behind the epidemic of chronic kidney disease of unknown etiology in Sri Lanka? Int J Environ Res Public Health. https://doi.org/10.3390/ijerph110202125
24. Xiao R, Wang S, Li R et al (2017) Soil heavy metal contamination and health risks associated with artisanal gold mining in Tongguan, Shaanxi China. Ecotoxicol Environ Saf. https://doi.org/10.1016/j.ecoenv.2017.03.002
25. Karagiannis D, Deliveliotis C, Papadimitriou E et al (2015) Oral exposure to hexavalent chromium through drinking water and urologic morbidity in an industrial area of Greece. J Public Heal. https://doi.org/10.1007/s10389-015-0681-8
26. Tsao DA, Tseng WC, Chang HR (2017) RKIP expression of liver and kidney after arsenic exposure. Environ Toxicol. https://doi.org/10.1002/tox.22291
27. Tyagi N, Raghuvanshi R, Upadhyay MK et al (2020) Elemental (As, Zn, Fe and Cu) analysis and health risk assessment of rice grains and rice based food products collected from markets from different cities of Gangetic basin, India. J Food Compos Anal. https://doi.org/10.1016/j.jfca.2020.103612
28. Sánchez DJ, Bellés M, Albina ML et al (2001) Nephrotoxicity of simultaneous exposure to mercury and uranium in comparison to individual effects of these metals in rats. Biol Trace Elem Res. https://doi.org/10.1385/BTER:84:1-3:139
29. Wang X, Cui W, Wang M et al (2021) The association between life-time dietary cadmium intake from rice and chronic kidney disease. Ecotoxicol Environ Saf. https://doi.org/10.1016/j.ecoenv.2021.111933
30. Jayatilake N, Mendis S, Maheepala P, Mehta FR (2013) Chronic kidney disease of uncertain aetiology: Prevalence and causative factors in a developing country. BMC Nephrol. https://doi.org/10.1186/1471-2369-14-180