Supplementary File 1

Method and instruments of lab tests:

Serum creatinine was measured by modified kinetic Jaffe's method and urinary protein and urine albumin were estimated by colorimetric and immune-turbidimetric methods, all by using fully automated chemistry analyzer (Beckman Coulter). HbA1c was analysed by ion-exchange high-pressure liquid chromatography method using a D10 Haemoglobin testing system (BioRad Laboratories).

Extraction and clean-up of pesticide residues

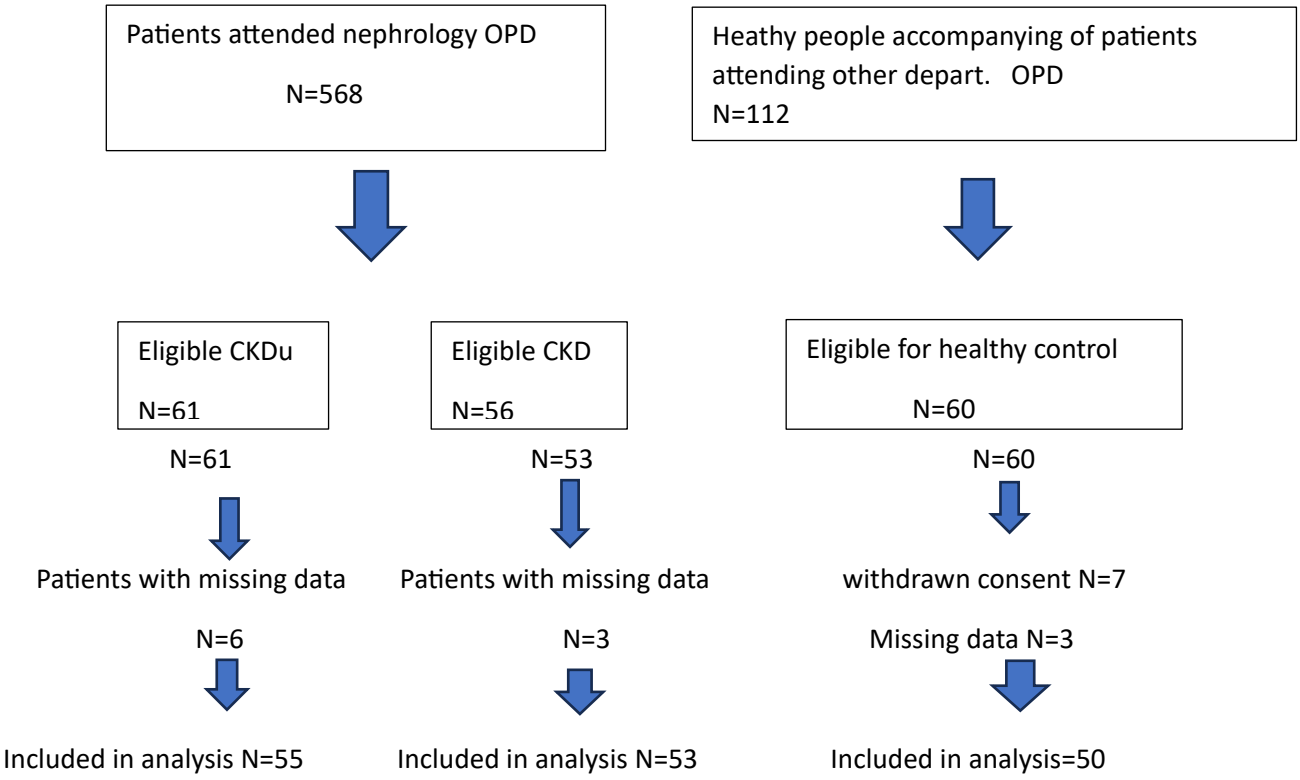
One millilitre of serum sample was mixed with 0.4 g of MgSO4 and 3 mL of 2% acidified ethyl acetate in a 15-mL polypropylene centrifuge tube. Concentrated acetic acid was added to ethyl acetate to make it acidic. After adding the solvent and $MgSO_4$, the polypropylene tube was vortexed thoroughly for 5 minutes after that centrifuge at 8000 rpm for 10 min. The organic layer was transferred to a fresh polypropylene tube and evaporated to dryness with the use of a nitrogen evaporator. For sample clean-up, the residue was reconstituted in 1 mL of ethyl acetate and combined with 50 mg of PSA. This mixture was shaken at 50 rpm for five minutes, then centrifuged at 8000 rpm for ten minutes. Further, the supernatant was separated, evaporated, and reconstituted in 100 µL of ethyl acetate, and one µL was injected into the GC–MS/ MS system for analysis.

Method Validation parameters

The developed analytical method was validated for different parameters, including Selectivity, accuracy, precision, linearity, limit of detection (LOD), and limit of quantification (LOQ). Calibration curves were plotted for each analyte to demonstrate the linearity of method concentration ranging from 5 to 200 mL-1. Recovery of all analytes was in ranged from 70 to 110%. The limit of detection and limit of quantification of the method ranged from 0.34 to 3.89 ng mL-1 and 1.03 to 11.67 ng mL-1, respectively. The LOD calculation was performed using the Student's t value, 3.14 (t value at 99% confidence level, six degrees of freedom), multiplied by the standard deviations obtained for the measured concentration of spiked samples (n = 7). By multiplying the standard deviation by 10, the limit of quantification was obtained.

Used Chemical grades and quality Acetone, n-hexane, ethyl acetate, acetic acid (MERCK), and Acetonitrile (FINAR) were of HPLC grade. Magnesium sulfate and sodium chloride (Sigma-Aldrich, Bangalore, India), primary-secondary amine (Agilent Technologies-USA), and pesticide standard mixture (RESTEK) were also of high-quality analytical grade.

Flow Diagram: Patient flow in study(Fig-S1)



Drinking water source according to diagnostic categories: Table-S1

Drinking water source	**Diagnosis**			**Total**	**P value Chi square**
	CKDu	**CKD**	**Healthy**		**0.00**
Ground	**28**	**42**	**40**	**110**	
Surface	**27**	**11**	**10**	**48**	
Total	**55**	**53**	**50**	**158**	

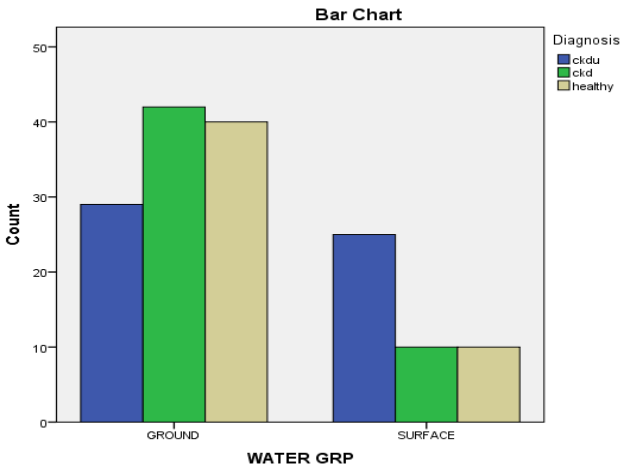


Fig –S2 Bar chart of drinking water source as per diagnostic criteria

Table-S2 Blood pesticide levels (undetected) between different groups:

S. No.	pesticide	CKDu N=53	CKD N=55	Healthy N=50	P – Value 1&2	Ckdu Vs healthy	Ckd Vs healthy	Ckdu Vs ckd
1	Alpha HCH	0.00(0.00-0.00)	0.00(0.00-0.00)	0.00(0.00-0.00)	1.000			
2	Beta HCH	0.00(0.00-0.00)	0.00(0.00-0.00)	0.00(0.00-0.00)	1.000			
3	Chlorpyrifos methyl	0.00(0.00-0.00)	0.00(0.00-0.00)	0.00(0.00-0.00)	1.000			
4	Heptachlor	0.00(0.00-0.00)	0.00(0.00-0.00)	0.00(0.00-0.00)	1.000			
5	Malathion	0.00(0.00-0.00)	0.00(0.00-0.00)	0.00(0.00-0.00)	1.000			
6	Phorate sulfone	0.00(0.00-0.00)	0.00(0.00-0.00)	0.00(0.00-0.00)	1.000			
7	Heptachlor epoxide	0.00(0.00-0.00)	0.00(0.00-0.00)	0.00(0.00-0.00)	1.000			
8	P'P-DDE	0.00(0.00-0.00)	0.00(0.00-0.00)	0.00(0.00-0.00)	1.000			
9	O'P-DDE	0.00(0.00-0.00)	0.00(0.00-0.00)	0.00(0.00-0.00)	1.000			
10	Endrin	0.00(0.00-0.00)	0.00(0.00-0.00)	0.00(0.00-0.00)	1.000			
11	Endrin-Aldehyde	0.00(0.00-0.00)	0.00(0.00-0.00)	0.00(0.00-0.00)	1.000			

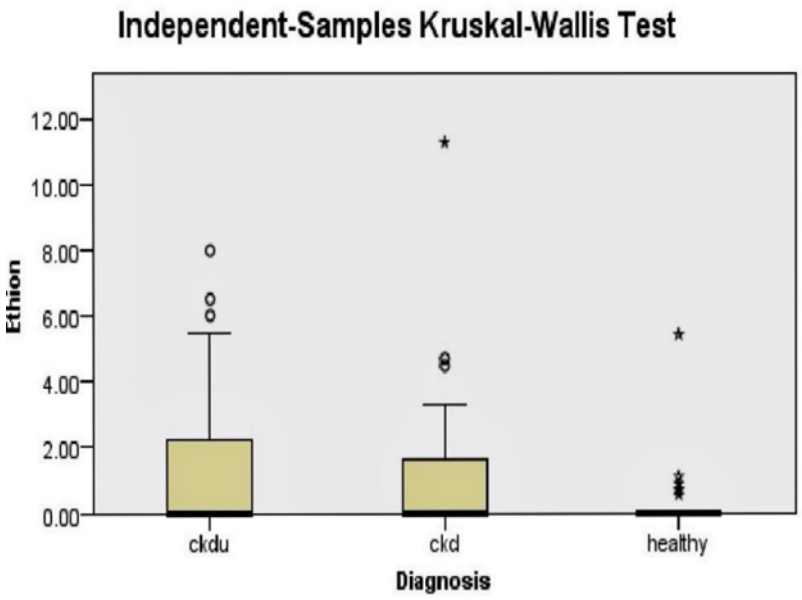
[1] Median (IQR); %-percentage [2] Pearson's Chi-squared test; [2] Fisher's exact test; [2] Kruskal-Wallis rank sum test; **=p<.05. All values of serum pesticides in ppb (parts per billion or micrograms/litre). CKD-chronic kidney disease, CKDu-chronic kidney disease of unknown cause.

Multi-nominal regression analysis of univariate associated factors with CKDu, CKD with reference to healthy subjects.

Unadjusted: Table-S3

S.No.	Parameters	OR CI(95%)	P value
CKDu			
1	Serum Chlorpyriphos	2.707 (1.812 4.046)	0.000
2	Serum Ethion	2.103 (1.243 3.558)	0.006
CKD			
1	Serum Chlorpyriphos	2.892 (1.931 4.331)	0.000
2	Serum Ethion	1.880 (1.105 3.199)	0.020
Goodness of fit (Pearson-0.989); Model fitness-(p-0.000)			

Fig-S3; Serum Ethion between CKDu, CKD, and healthy subjects



CKDu-Chronic kidney disease of unknown cause; CKD- Chronic kidney disease.