Application to KHDC-ISN Clinical Research Program

Section A

1. Country where the study takes place: **India**
2. Project title: **Study of the associations of heavy metals and environmental toxins with Chronic kidney disease in a rural population in South India**
3. Name and Address of the co-ordinating Institution:

   a) Legal Name: Nanjappa Hospital

   b)Address: Kuvempu Road, Shivamogga-577201,Karnataka, India c)Head of the Institute:Mr.D G Benakappa

4. Name of the local co-ordinator**: Dr Anupama Y J**

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5. Duration of the project in months**: 12 (twelve)**

Section B:

Title: Study of the associations of heavy metals and environmental toxins with Chronic kidney disease in a rural population in South India

1).Introduction and rationale:

1. Background:

The prevalence of Chronic kidney disease is rapidly increasing in the world as has been documented in numerous studies[1,2].It is a global health problem and a key determinant of poor health outcomes[3]. The prevalence of CKD varies in different studies and is estimated to be between 8-16% .This is because there is marked heterogeneity in the methods used for screening, the proteinuria assays, the methodology of creatinine and GFR estimation and the composition of the studied population. Most of the global increase in the prevalence of CKD is traditionally linked to the increasing prevalence of major lifestyle diseases such as diabetes mellitus, hypertension, obesity.

The burden of untreated and unrecognised CKD is disproportionately more in disadvantaged populations, such as the lower socio-economic class or racial and ethnic minorities.This has been linked to the low education and awareness levels, reduced access to health care and sociocultural beliefs, low birth weight, genetic differences and exposure to environmental toxins[3]. Particularly curious is the disproportionate increases in the prevalence of CKD in some geographic locations in the world which has been suspected to be related to the occupational environment. Collectively known as Chronic kidney disease of uncertain etiology(CKDu), the

etiology is not clear and several epidemiologic studies have linked them to various heavy metals, pesticides and heat stress to name a few[4,5,6.]

The prevalence of Chronic kidney disease in India has not been well studied. Although there are several hospital based studies on the prevalence of CKD in India, population based studies are few and mostly done in urban India[7,8]. There is one large community based study from South India, which studied the prevalence of CKD in the rural population.The prevalence of CKD was estimated to be 1.39% in this study[9]. These studies employed different measures for estimating the prevalence of CKD and cannot be directly compared.In a study done in urban Delhi, using criteria of serum creatinine more than 1.8 mg/dl as the cut-off, Agarwal et al. have recorded prevalence of 0.79%[8].In another study, Singh et al. employed random cluster sampling method to screen 5252 subjects aged more than 20 years across the city of New Delhi [7]. Using a cut-off of MDRD GFR of 60 ml/min/1.73 sq m, the prevalence of decreased GFR was 4.2%. In the recent multicentric SEEK -India study,where people attending camps in both urban and rural areas were included, the prevalence of CKD was estimated to be 17.2% with ~6% having CKD stage 3 or worse. CKD risk factors were similar to those reported in earlier studies[10].

In a previous study done in the rural population under consideration, near Shivamogga in Karnataka state, the prevalence of CKD was 6.3%[11]. In this study, a door-to-door screening of all adults in the villages of Hosakoppa, Indiranagar and Gajanur villages about 8 km away from Shivamogga was carried out and 2091 subjects were screened for CKD with serum creatinine and urine dipstick examination for protein. 131 subjects were found to have CKD (eGFR <60 ml/min/1.73 sq m).Further analysis of this CKD cohort led to some interesting observations. 79

of these subjects were agricultural laborers, only 10% of them had diabetes. Nearly 60% of them had hypertension, but most had stage I hypertension. 45% of these subjects were younger than 50 years and majority were males. 54% of these CKD had no documented proteinuria. These observations were indicative of the need to look beyond the traditional risk factors for CKD. Since the subjects were predominantly agricultural laborers exposed to various pesticides and heavy metals in the course of their occupation, it is possible that environmental toxins have had a role to play in the causation of their illness.

Studies of CKDu from Sri Lanka have shown that their cases of CKDu also tend to affect the young farmers and have focussed on the possibility of environmental toxic exposure either in the drinking water or while working in the farms. Scientists have shown that there is a significant association of CKD cases in that country with cadmium and arsenic[5,12,13]. They have hypothesised that the probable exposure to heavy metals is through contamination of water or soil with heavy metals.Widespread use of harmful pesticides with prolonged complexation of heavy metals in the layers of the soil has been proposed to be the mechanism of causation[14].

Lead is another heavy metal that has been found to be ubiquitous in the environment. Environmental lead levels vary from place to place depending on a number of factors that includes proximity to a smelting factories and other industrial facilities that release lead into the environment. Leaded gasoline,and lead in storage batteries seep into the environment and affect human beings. Water pipes contain lead soldering in some areas. Lead has also been found in household paints, children's toys and cosmetics. Lead is associated with numerous health hazards. It is associated with unexplained anaemia[15], cognitive defects and attention

disorders in children[16]. It is also associated with the development of hypertension[17], gout and nephrotoxicity[18]. Sanchez –Fructuoso et al had in their study of 296 subjects/patients without history of lead exposure reported normal body lead in renal failure patients of known aetiology but elevated lead levels in 56 % of renal failure patients of unknown aetiology[19]. In the analysis of NHANES-III data, Muntner et al concluded that in the United States population with hypertension, exposure to lead, even at low levels , is associated with CKD[20].

There have been no systematic study in our country linking these heavy metals in the environment and chronic kidney disease. Preliminary analysis of the urine of 8 of the CKD subjects in our cohort for heavy metals including lead, arsenic and cadmium showed significant lead levels in 4 of them, which raises the possibility of lead exposure in this area. This would probably explain the increased prevalence of nonproteinuric CKD. Further the villages under consideration are irrigated areas and the villagers grow the crops all round the year, that is 2-3 crops per year. Rice, sugarcane and areca are the chief produce grown.Unlike areas which depend entirely on rainfall for agriculture, these villagers use the fertilizers 2-3 times more often.We suspect that this may be another reason for the increased predisposition to CKD

The purpose of this study is to systematically examine the probable role of heavy metals and environmental toxins including pesticides and fertilizers in the etiology of Chronic kidney disease in this rural population.

1.2 Research question:

Is there any association between exposure to pesticides and heavy metals-cadmium, lead and arsenic with the prevalent chronic kidney disease in the people of Hosakoppa, Indiranagar and Gajanur villages in Shivamogga district

2)Objectives of the program

a. To determine if there is any association between environmental heavy metals and the pesticides with the prevalent CKD in the villages mentioned above.

b. To determine the strength of the association between the two, if any

3)Plan and methodology:

3.1 **Study setting**

Adult population of Hosakoppa,Indiranagar,Gajanur villages of Shimoga district ,Karnataka

3.2 Study population

People in the age group of 18 and above who are living in the villages of Hosakoppa,Indiranagar and Gajanur villages of Shimoga District

3.3 Study design

It is a matched case control study. Participants will be recruited from the cohort of 2091 subjects who formed the study cohort in the prevalence study that was conducted earlier in 2012-2013 in the same area.Our reference period is 2012-2015.

3.4 Operational definitions:

3.4.1 Case: Adult residing in the villages mentioned above and who had participated in the earlier study of 2012-2013 and who had evidence of chronic kidney disease(CKD). CKD is defined as presence of proteinuria and/or MDRD eGFR <60 ml/min/1.73 sq m.

3.4.2 Control: Adult residing the villages mentioned above who had participated in the earlier study of 2012-2013 and had no evidence of chronic kidney disease

3.4.3 High blood lead level: Blood lead level more than 10 microgram/dl[21]

3.4.4 Urinary lead level (random)-1-23 mcg/litre

3.4.5 High blood Cadmium levels:Blood cadmium levels >6mcg/dl[21]

3.4.6 High urine cadmium levels(random) : 0-2.6 mcg/litre

3.4.7 High blood arsenic levels: >2 mcg/dl[21]

3.4.8 High urine arsenic levels(random): 0-35 mcg/l

3.4.9 **Exclusion criteria:**

1. Elderly >75 years
2. Pregnant women or women in postnatal period up to 40 days after delivery
3. Chronic kidney disease stage 5 , already undergoing dialysis or has undergone renal transplantation.

3.5 **Duration of the study**: 1 year

3.6 **Sample size**: We will conduct matched pair case control study where they will be matched for age and sex.

3.7 **Sample procedure:**

1) 60 cases will be randomly selected from line list of 131 people with chronic kidney disease as recognised in the prevalence study 0f 2012-2013[1]

2) 60 Controls will be selected from subset of line list of cohort which participated in the prevalence study of 2012-2013 and were found to have no signs of Chronic kidney disease, after matching age and sex to the cases.

3.8 **Data collection procedure**: Data will be collected by using a questionnaire designed for the study containing the demographic details and details of symptomatology of kidney disease.Questions related to their work environment and the environment of their house, the sources of water, will be taken. Examination of blood pressure and anthropometry will be done for all participants. Informed consent will be taken before biological samples are collected. Blood will be collected for Haemoglobin percentage, blood sugar, creatinine and uric acid estimations. Blood and random urine samples will be collected for quantitative heavy metal estimation and for qualitative pesticide residue analysis and will be sent to toxicology lab using the required transport precautions.

3.9 **Data Analysis procedure**:The data will be entered and analysed using appropriate statistical measures.

3.10 **Quality Assurance**: Quality will be maintained in the stage of data collection as well as data analysis stages. Data will be collected by specially trained nursing volunteers from Nanjappa Institute of Nursing Sciences College of Nursing and they will be trained to collect all the necessary information in an unobtrusive manner. Anthropometry and basic examination including Blood pressure will be recorded by the nurses. The Principal Investigator and the other co-investigators will examine all the cases for any evidence of heavy metal exposure. They will also randomly cross-check the examination done earlier by the nurses. Blood will be collected by the laboratory technicians and transported to the laboratory in Nanjappa Hospital which is 8 km away from the villages being studied. Blood will be analysed for creatinine and uric acid in a fully automated analyser (Biosystems, Spain) using standard methods and reagents.Urine analysis will be carried out by 5-parameter dipstick for protein and blood. Spot urine albumin excretion rate will be estimated by dipstick. Blood and random urine samples collected for quantitative heavy metal estimation will be sent to toxicology lab where it will be analysed by Inductively coupled plasma atomic energy spectroscopy(ICP-AES) method. Pesticide residue analysis will be done by thin layer chromatography method. Special precautions will be taken to transport the samples for the above testing to the toxicology lab in Amrita Institute of Medical Sciences at Cochin, Kerala in ice pack as per the requirement. Data will be analysed by a qualified biostatistician.

4) Expected results: We expect that there will be a pattern of relationship of heavy metal exposure in the CKD cases based on the results of the preliminary data received from analysis of 8 cases of CKD in the area in which 4 cases had a significant lead excretion in the urine.

5)Expected benefits:

The study aims to find out if there is a pattern of association between the Chronic kidney disease and heavy metal / pesticide exposure in this predominantly agricultural community. It is well known that in many cases of CKD, a definite etiology cannot be established. Analysing the results of the previous KIDS study, we suspect that these subjects are at risk of predominantly tubulointerstitial renal disease, the causes of which may be untreated renal stone disease or renal infections,NSAIDs, Herbal medicine or environmental toxins. If a definite strong association can be established by our study between the environmental toxins and CKD, it has widespread public health ramifications.Steps can be taken to prevent progression of the kidney disease at the individual level and further exposure reduced or minimised. Chelation therapy may offer reduction in the heavy metal levels and that may have beneficial effects on the glomerular filtration rate. The society in general will benefit by interventions aimed at reduction in toxin levels. This may not only be useful in reducing nephrotoxicity, but also have impact on other health parameters such as anaemia, hypertension, diabetes etc., Above all, they will benefit from heightened health awareness and kidney disease awareness which will have a positive impact on their overall health.

6.1) Bias and Limitations: We will be analysing aspect of heavy metals and pesticide residues in these people. There is a possibility of various other etiologies of that may be contributing to the condition that may not be picked up as a detailed assessment is not possible. The cases will be encouraged to visit the nephrologist in the hospital for the detailed evaluation as required. The study is limited to finding the association between the environmental toxins and

CKD. Based on the results of this study, we plan to take up a larger study in the future to test the environmental sources of the pollutants and to study their likelihood analysis.

6.2 Human Subjects protection:

Compensation: No monetary compensation is given to the participants.

Risks: We expect that the participant may fear the following risks - a) of disclosing confidential and personal information, b) discomfort, c) loss of wages d)loss of time, e) forceful participation, f)fear of losing services from the health department if he/she does not participate in the study.

Measures to minimise the risks: a) It will be a voluntary participation.

b)Only the required information will be collected and the information will be kept confidential.

c)The data will be collected in a place and time suitable for the participant

d) We will ensure that the interview does not come in the way of his job and ensure that there will not be loss of wages.

e)All participants will be explained the nature of the study and an informed consent in the local language (Kannada)will be taken.

f) The approval of the institutional Ethics Committee and the local health authorities will be taken.

7) Details about the applicant's institution:

Nanjappa Hospital is a 150-bedded multispeciality hospital run by M/S Nanjappa Trust in Shivamogga,Karnataka State of India. It was started in 1987 and has reached out to the health needs of the people of Shivamogga, Davanagere , Chitradurga, Uttarakannada and Chikkamagalur districts. Care is provided in various specialities such as Internal Medicine, General surgery, Pediatrics, Gynecology, ENT,Orthopedics,Neurology ,Urology and Nephrology. The Trust runs another state-of the art cardiac hospital and critical care unit with facilities for advanced cardiac care namely, Nanjappa LifeCare in an adjoining area. The hospital also has a well-equipped laboratory and and advanced imaging facilities with CT and MRI. Now NANJAPPA HOSPITAL is known to be the institution providing quality health care in different specialities and super speciality care in the department of Urology, Neurosurgery, Plastic Surgery, Cardiology, Nephrology and Neurology. The Diploma course in General Nursing and Midwifery Course started in the year 1994 is recognized by Indian Nursing Council and Karnataka Nursing Council). The Trust has started B.Sc. (Nursing) from the academic year 2004, affiliated to Rajiv Gandhi University of Health Sciences, Bangalore .Traveling with the vision since inception, the institution has been path- finding in improving diagnostic and treatment facilities by upgrading the departments from time to time.Nephrology Services started in 1995 and today we have a 13 bedded haemodialysis unit, full-fledged CAPD center, ICU dialysis. On an average, 1000-1200 haemodialysis is done per month.

The hospital has an active research wing which has conducted many clinical trials. It has collaborated with St Johns Medical Academy and Research wing in conducting international clinical trials in Cardiology such as CREATE,OASIS,ASTOR . The hospital has multiple community

oriented programs and is involved with multiple screening camps at various places for cardiac and kidney diseases.

Section C:-

References:

1. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: Global dimension and perspectives. Lancet 2013;382:260-72.

2. Couser WG, Remuzzi G, Mendis S, Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. Kidney Int 2011;80:1258-70.

3. Garcia-Garcia G, Jha V, Tao Li PK, et al. Chronic kidney disease (CKD) in disadvantaged populations. *Clinical Kidney Journal*. 2015;8(1):3-6. doi:10.1093/ckj/sfu124.

4. Gooneratne I. K, Ranaweera A. K. P, Liyanarachchi N. P, Gunawardane N and Lanerolle R. D. Epidemiology of chronic kidney disease in a Sri Lankan population. International Journal of Diabetes in Developing Countries. 2008; 28(2): 60–64

5. Bandara JMRS, Senevirathna DMAN, Dasanayake DMRSB, Herath V, Bandara JMRP, Abeysekara T, Rajapaksha KH. Chronic renal failure among farm families in cascade irrigation systems in Sri Lanka associated with elevated dietary cadmium levels in rice and freshwater fish (Tilapia).Environ Geochem Health 2008; 30:465-78.

6. Correa-Rotter R, Wesseling C, Johnson RJ .CKD of unknown origin in Central America: the case for a Mesoamerican nephropathy.Am J Kidney Dis. 2014 ;63(3):506-20.

7. Singh NP, Ingle GK, Saini VK, Jami A, Beniwal P, Lal M, et al.Prevalence of low glomerular filtration rate, proteinuria and associated risk factors in North India using Cockcroft-Gault and Modification of Diet in Renal Disease equation: An observational cross-sectional study. BMC Nephrol 2009;10:4.

8. Agarwal SK, Dash SC, Irshad M, Raju S, Singh R, Pandey RM. Prevalence of chronic renal failure in adults in Delhi, India. Nephrol Dial Transplant 2005;20:1638-42.

9. Mani MK. Experience with a program for prevention of chronic renal failure in India. Kidney Int Suppl 2005;94:S75-8.

10. Singh AK, Farag YM, Mittal BV, Subramanian KK, Reddy SR,Acharya VN, et al. Epidemiology and risk factors of chronic kidney disease in India-Results from the SEEK (Screening and Early Evaluation of Kidney Disease) study. BMC Nephrol 2013;14:114.

11. Anupama YJ, Uma G. Prevalence of chronic kidney disease among adults in a rural community in South India: Results from the kidney disease screening (KIDS) project. Indian J Nephrol 2014;24:214-21.

12. Jayatilake N, Mendis S[*], Maheepala P, Mehta F R and On behalf of the CKDu National Research Project Team.Chronic kidney disease of uncertain aetiology: prevalence and causative factors in a developing country. BMC Nephrol. 2013;14:180.

13. Jayasumana M.A.C.S., Paranagama P.A., Amarasinghe M. D., Wijewardane K.M.R.C., Dahanayake K.S., Fonseka S.I. et al. Possible link of Chronic arsenic toxicity with Chronic

Kidney Disease of unknown etiology in Sri Lanka, Journal of Natural Sciences Research, 2013: 3(1), 64-73.

14. Jayasumana C., Gunathilake and Senanayake P. 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. 2014 Feb; 11(2): 2125–2147

15. Jain N B et al.Relation between Blood Lead Levels and Childhood Anemia in India Am. J. Epidemiol. 2005; 161 (10): 968-973

16. Banks EC, Ferretti LE, Shucard DW. Effects of low level lead exposure on cognitive function in children: a review of behavioral, neuropsychological and biological evidence.Neurotoxicology.1997;18(1):237-81.

17. Kopp S J , Barron J T , Tow J P. Cardiovascular actions of lead and relationship to hypertension: a review.Environ Health Perspect. 1988 ; 78: 91–99.

18. Yu CC, Lin JL, Lin-Tan DT. Environmental exposure to lead and progression of chronic renal diseases: a four-year prospective longitudinal study. *J Am Soc Nephrol* 2004; 15: 1016–1022.

19. Sanchez -Fructuoso AI, Torralbo A, Arroyo M, Luque M, Ruilope LM, Santos JL, Cruceyra A, Barrientos A. Occult lead intoxication as a cause of hypertension and renal failure. Nephrol Dial Transplant 1996 11: 1775 -80

20. Muntner P1, He J, Vupputuri S, Coresh J, Batuman V. Blood lead and chronic kidney disease in the general United States population: results from NHANES III.Kidney Int. 2003 ;63(3):1044-50.

21. Singh R ,Gautam N, Mishra A and Gupta R. Heavy metals and living systems: An overview.Indian J Pharmacol. 2011 May-Jun; 43(3): 246–253.

Section D: Budget

Section E: Brief Summary of the project:

Chronic kidney disease is a global health problem with increasing prevalence being seen disproportionately in some disadvantaged populations. The disease is mostly attributed to increasing prevalence of modern lifestyle diseases, such as diabetes mellitus, hypertension and obesity. However, in some areas of the world, studies have shown that there is an association of CKD with various heavy metals, environmental toxins, heat stress etc., in those areas. We conducted a population based study of prevalence of Chronic Kidney Disease in 2012-2013 in a cluster of 3 villages, namely Hosakoppa, Indiranagar and Gajanur near Shivamogga town, Karnataka State of South India where we estimated the prevalence to be 6.3%. Analysing the CKD cases further we noted that these were predominantly agriculturists with the cause of CKD being unclear and not related

to diabetes. Hypertension was also mild in most cases. 8 patients were randomly selected and their urine sent for estimation of heavy metals by ICP-AES method and 4 of these samples showed high levels of lead. Hence we wanted to do a detailed case-control study in the same area in the cases of CKD (diagnosed to have CKD from the earlier cohort) and controls being the age- and sex- matched people without CKD, looking for a possible association between CKD and the environmental toxins. We expect that if there is a significant association between the two, this study will have important public health implications, not only for prevention of CKD, but also other health problems such as anaemia, hypertension that affects these people. We will then pursue the studies further looking for the environmental sources of the culprit toxins and initiate steps to minimise the exposure.

Section F:

Informed consent(in English): Enclosed (Enclosure no.4)

Informed consent (in Kannada): Enclosed (Enclosure no.5)