(3) GENERAL

3.1 CHRONIC KIDNEY DISEASE OF UNCERTAIN ETIOLOGY OF SRI LANKA

National Research Programme

Chronic Kidney Disease of Uncertain Etiology of Sri Lanka

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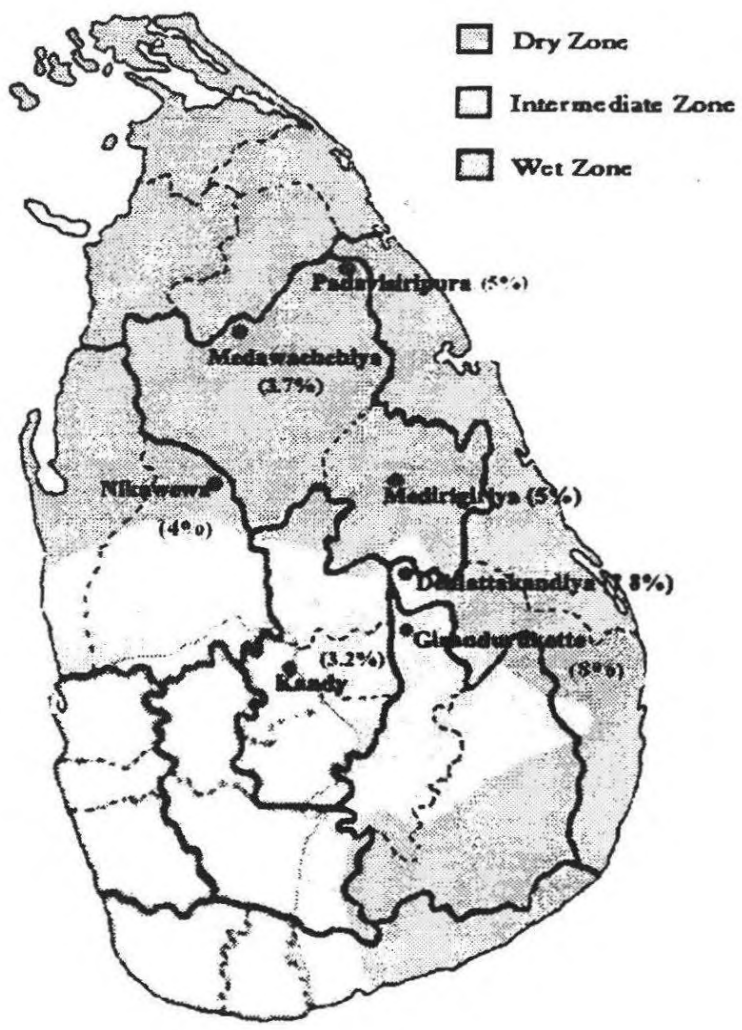
1. Hypothesis

The CKD of uncertain etiology prevalent in the north central region of Sri Lanka is due to an environmental / geological nephrotoxin causing slowly progressive chronic fibrosis of kidneys in susceptible populations of the region.

2. Background and significance of the research

The prevalence of Chronic Kidney Disease (CKD) is on the increase world over and it is attributed to the rising incidence of diabetes and hypertension. [1-5] In Sri Lanka, the prevalence has increased in same intensity. As per the hospital data and systematic investigations conducted during the last 5 years by the authors, the prevalence of CKD in Sri Lanka varies from 3 - 9% [6,7] (unpublished data). It was observed in the regions where the prevalence is high, majority (>90%) does not have an association with known risk factors such as diabetes, long standing hypertension, infections etc. [3] Furthermore, it is noteworthy that the CKD of uncertain etiology (CKD of UE) has a strong geographical bias. [7,8]

Figure I: Map of Sri Lanka indicating the geographical distribution of the disease



The geographical distribution of CKD of UE appears to be biased towards the North Central Province (NCP) of the country. The populations at risk are scattered in the NCP with high prevalence observed at Medawachchiya, Padavisiripura, Dehiattakandiya and Medirigiriya (Figure I). The affected population mostly belongs to the low socioeconomic class with preponderance among the male paddy farmers. It is concerning to note that the highest

prevalence is observed among settlers of 20 years duration in a geographical area (Girandurukotte) closer to the southern border of the NCP of the country. The prevalence of the disease in this area could be more than 10% (ongoing research programme).

With the preliminary investigations revealing a tubulo-interstitial nephritis with minimum inflammation and early fibrosis among the early disease patients of the migrant population, it is possible the disease is due to exposure to environmental toxin/s among populations at risk. [6,7]

The NCP is in the dry zone of the country where the annual temperature ranges from 29 - 35^{0}C. The mean annual rainfall ranges from 1250 mm to 2000 mm. About 40% of the people living in NCP are below the poverty line of Sri Lanka. [9] Geologically, the NCP is in the Fluoride belt of the country which is seen extending up to the southern parts of the country. The farming community uses fertilizer and agrochemicals heavily in the farming process. Contamination of water with these chemicals with potential nephrotoxic effects is a possibility. The population of the NCP has a very close association with the geo-environment. The main source of the drinking water is the ground well which is situated in close proximity to the dwellings. Further, it is well known that the farmers at the field do drink water from the water tanks and other sources of water. Therefore it is reasonable to assume that any material from the surface soil layer could leach into the well water which could be ingested.

The NCP was affected by malaria for many years and the control programmes included spraying of DDT and malathione for a long period. Even though such chemicals are not used presently, these could have contaminated drinking water in low concentrations which could lead to a chronic disease.

Management of CKD patients who present in late stages is carried out in two teaching hospitals of the country, in Colombo and Kandy. The six nephrologists (for 20 million population) of the country are responsible for the management of more than 2000 patents / year who develop End Stage Renal Disease (ESRD). Haemodialysis is the only mode of treatment available for these patients. The public sector hospitals experience a severe dearth of dialysis machines to manage such patients. The requirement being 700 machines but the number available is around 50. Only a few patients could afford renal transplantation.

Therefore, primary prevention with early diagnosis is the only option available for a developing country like Sri Lanka. Thus investigations are required to identify the risk groups

and possible etiological factor/s. Further, population screening programmes require more efficient patient identification markers.

3. Preliminary data

The research team conducted hospital based, population based and case control studies in selected geographical areas of the country during 2000- 2007.

3.1 Hospital Based Studies

3.1.1 Study 1 (2000): A cohort of patients (n= 146), registered at the clinic of the Renal unit, General Hospital, Kandy for a period of one year from 1999 - 2000 was studied. There was clear evidence that majority of patients were from the administrative division of North Central Province (NCP) with a preponderance towards male farmers aged 30 - 60 years and of low socioeconomic class. Further it was noted that the majority were seeking health care at very late stages of the disease. [6]

3.1.2 Study 2 (2001): The Teaching Hospital of NCP is Anuradhapura General Hospital. The patients attending the Renal Clinic of this hospital were studied (n = 246) to identify the high risk areas in the NCP to conduct a population based study. Medawachchiya was identified. The patients' characteristics differed from those found in Kandy in that the disease appeared to be in early stages.

3.2 Population Based Studies

3.2.1 Medawachchiya (North Central Province) Study 2003: Medawachchiya is a rural area in the NCP where majority are paddy farmers. The whole population of four administrative divisions that were randomly selected out of 37 divisions (n= 4107 of a total population 43,000) were screened for proteinuria. The presence of proteinuria persistently in 3 early morning samples was considered as an indicator of kidney disease in this population screening programme. Those who found to be positive (n=156) were further investigated for kidney disease. Haematological and biochemical investigations and, ultrasonography were done for all these patients, while kidney biopsy was done on selected patients.

The point prevalence of the disease for the population screened was 3.7% and for those over 18 years, it was 4.8%. Those below 5 years appeared to be healthy. The disease was common among male paddy farmers of 40 - 60 years. The investigations revealed that the disease was a tubulo-interstitial disease with low proteinuria and possibly slowly progressive. Majority were asymptomatic and had normal haematological and biochemical profile (other

than those who were in late stages of the disease). There was no association between diabetes and/or hypertension, and the CKD found in Medawachchiya. [7,8]

3.2.2 Yatinuwara (Central Province) Study 2004: A similar population based study was conducted in a semi urban area in the Central province where majority were paddy farmers. A sample from randomly selected clusters (n=253) was screened. The study revealed 3.2% of people screened having proteinuria and all were chronic diabetics / hypertensive. None of the patients had the clinical features of those patients found in Medawachchiya. [10]

3.2.3 Girandurukotte (Uva Province) Study 2005: Uva province is located bordering the NCP to the south. It consists of two districts Badulla in the North and Monaragala in the south. The Provincial Ministry data of 2005 revealed that the number of CKD patients reported from the north (Girandurukotte and surroundings) was 40 times higher than that from the south. A population based study was conducted in 8 administrative divisions using cluster sampling method. Early morning urine sample of 1345 persons was examined for proteinuria and those found positive were referred for similar investigations conducted in Medawachchiya. In this study, comparatively younger people were found to have proteinuria. Further those with trace proteinuria were proven to have kidney disease histologically [11] (unpublished data).

The prevalence was approximately 8 - 9%. However this could be an under estimate as trace proteinurics with ultrasonographic changes consist of 15%. There was no difference between those who have migrated and have been living in Girandurukotte for long time. The clinical features observed were similar to those found in Medawachchiya. Histological findings were also similar to Medawachchiya kidney disease.

3.3 Case Control Study

A case control study was conducted to find out the possible risk factors of the disease. 84 CKD patients who were regularly followed-up at the clinic and 155 controls (age, sex and area matched) were studied. We were able to observe an association with male paddy farmer and the disease. There were no associations with diabetes, hypertension, agrochemicals, snake bite, alcohol, medicinal drugs, traditional medicines, water source, fluoride level of water, and usage of aluminum utensils for cooking. [12]

4. Disease Profile and progression

Identification of patients in later stages of the disease at population screening programme is an evidence for the insidious nature of the disease. There were no specific symptoms and at the time of detection, both systolic and diastolic BPs of most of the patients were within

normal range. Urine analysis showed minimum proteinuria without any active deposits. The biochemical and haematological profiles were normal except for creatinine clearance. All 156 patients identified at Medawachchiya in 2003 were referred to Base Hospital, Medawachchiya for follow up.

Causes of death of patients identified at Medawachchiya (2003 – 2006): Within 3 years, 18 patients out of 156 died probably due to end stage kidney disease or its complications. The actual causes of death were not known. This needs further investigations. One patient died of severe leptospirosis.

5. Research to identify risk factors/etiology (2007)

5.1 Preliminary analysis of kidney tissues for metals

Kidney tissues of five patients from the ongoing research area (i.e. Girandurukotte) were sent to the University of Kanazawa, Japan for electron microscopy and metal mapping. The methodology used for the analysis was STEM - EDX (Scanning Transmission Electron Microscopy and Energy Dispersive X-ray Spectroscopy). The nucleus and lysosomes of the proximal tubular epithelial cells showed Cr, U, Hg, Ni, Fe and Mn appearing together in different concentrations. All tissue samples showed the presence of above metals indicating the possibility of common source exposure. [13-16] As all these metals are attracted to phosphates, we investigated the phosphate fertilizer for the presence of these metals.

5.2 Analysis of fertilizer

Commonly used fertilizers for paddy farming were analyzed for metals using "Inductively Coupled Plasma Mass Spectrometry (ICP-MS)" at the University of Erlangern, Germany and "Energy dispersive X ray Fluorescence Spectrometry (XRF)" in Sri Lanka. Fertilizers Triple Super Phosphate (TSP), NPK, Urea, Potash used in the NCP and Giradurukotte were analyzed for metals and radioactivity. When compared with other fertilizers, TSP showed presence of high levels of Mg, Ca, Mn, Fe, Ni, Cu, Zn, U and Cd in the ICP-MS analysis. Radioactivity analysis of fertilizers using Gamma Spectrometry with High Purity Germanium Detector (GS-HPGD) showed the presence of significant amounts of radioactive K and Radium.

The soil was also analyzed using Energy Dispersive X-Ray Fluorescence Spectrometry. This revealed presence of Mn, Sr, Cr, and Fe in significant amounts.

5.3 Analysis of drinking water

Analysis of drinking water revealed high concentration of fluoride ranging from 0.7-1.5 ppm. Currently, analysis of water for organic compounds is ongoing. Gas Chromatography and Mass Spectrometric analysis confirmed the presence of residual pesticides in all 10 samples of drinking water (diazenone, dimethoate and 3,4 - DPA).

6. Objectives and methodology for the proposed studies

6.1 Histological analysis

Objective: To carry out systematic in-depth histological analysis of kidney tissues using electron microscopy and metal mapping and to observe changes in liver, heart and aorta in post-mortem specimens

Method: The kidney biopsy tissues of patients who have evidence of CKD of uncertain etiology (mild to moderate) of the North Central Region of the country will be histologically analysed at the Department of Pathology, University of Peradeniya. Seven biopsy samples of patients from each affected area will be tested for metals using STEM - EDX method (total number of biopsies would be 35). Samples would be obtained from patients of different age groups.

Age and area matched control samples of tissues will be obtained from postmortem specimens of kidneys of those who have died due to accidents and there were no other known pathology. The Judicial Medical Officer will be collaborating with the research team in this regard.

Histological studies of postmortem specimens (kidney, liver, large blood vessels and heart) of those who have died of CKD of uncertain etiology from NCP would be carried out. Macroscopic and microscopic assessments would be conducted. If there is evidence of damage to organs other than the kidney, electron microscopy and metal mapping of those organs would also be carried out. Collaboration with the University of Kanazwa is expected to conduct the study. We expect to seek assistance from a histopathologist with expertise in renal electron microscopy for final diagnosis.

6.2 Disease marker

Objective: To identify a disease marker that could detect the disease in early stage (proposed study of a research fellow).

6.3 Focus group discussions

Objective: To carry out well designed focus group discussions among comparative groups to identify susceptibility to CKD.

Method: To identify exposure to suspected etiological agents and the susceptibility to develop CKD, focus group discussions will be conducted among the high prevalent populations. A focus group will consist of patients and normal individuals. Nutritional and anthropometry assays will also be conducted both in early disease patients identified by screening of population and in a comparative group to identify susceptible factors. Assistance would be sought to train the research assistants to conduct focus group discussions from the Department of Sociology, University of Peradeniya. The data obtained would be qualitative. Thus analysis would be carried out by calculating the proportions.

6.4 Inorganic toxins

Objective: To analyze fertilizer, food, soil and water for possible inorganic toxins and its relevance to CKD.

Method: Dried soil, fertilizer and food samples at 40^0 will be screened through a 1mm polyethylene sieve and finely grind to <63 µm. After mixing soil samples with HNO3:HCLO4:HF (2:2:1) in PTEF containers are to be left in a digestion block in 150^0 for 24 hrs. After repeating the same procedure several times, final residue will be dissolved in 5 ml of HNO3. Final solution will be analyzed by Inductively Coupled Plasma Mass Spectrometry (ICS – MS)

Samples of drinking water will be collected from the disease prevalent areas in dark brown colored glass containers. Control samples will be collected from the Central Province for comparison. Using gas liquid chromatography for organic material and Pre concentration and X ray fluorimetry will be used for inorganic substances (heavy metals). Duplicate soil from agricultural and non-agricultural sites will be obtained from areas with high prevalence and low prevalence of the disease.

6.5 Animal experiments

Objective: To conduct parallel animal experiments to identify environment and geological factors.

Hypothesis: The sources of drinking water of CKD of UE high prevalent regions contain nephrotoxins and the high fluoride content enhance the nephrotoxicity

Method: Five groups of young healthy male rats (7 in each group) will be identified. All will be fed with laboratory animal food. The water will be different as follows. Group 1 and 2: drinking water (fluoride 0.7 ppm and 1.7 ppm) from two areas where the disease is prevalent. Group 3: water from Central Province & fluoride 0.7 ppm added. Group 4: water from Central Province & fluoride 1.7 ppm added. Group 5: water from Central Province. Routine weighing will be done. The rats will be monitored for a period of 3 months to see any early changes in the kidney. 3/7 would be sacrificed and macroscopic and microscopic examination of kidneys will be conducted. The kidneys will be specially examined for fibrosis. At the end of 4th month the rest will be sacrificed and similar examinations will be conducted. Metal mapping will also be conducted.

6.6 Epidemiological studies

Objective: To conduct country-wide epidemiological studies to understand the prevalence of the disease in different geographical localities.

To understand the disease prevalence and to identify risk factors it is essential to conduct the programme in the Eastern Province which is in the dry zone with similar geo-environmental conditions and paddy farmers with low prevalence of the disease (personal communication, no data available). To understand the geographical distribution of the disease, rural communities in the south, western part of the North West Province and northeastern area will be screened.

Method: Due to ongoing conflicts in the eastern region, we may have to carry out an epidemiological study in limited area using randomly selected cluster sample method. Seven administrative divisions out of 15 would be randomly selected and clusters within the administrative divisions will be again randomly selected to screen the >18 population of about 4000 people. Using proteinuria as a disease marker tested using a dipstick, the sample populations will be screened on three occasions. A pre-tested questionnaire will be used after obtaining informed consent. Those who have persistent proteinuria would be examined clinically and investigated for CKD. Patients will be referred to the main hospital in the province for follow-up.

Further using Global Positioning System and Geographic Information System, we propose to map the geographical areas affected by the disease in and around the NCP.

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