DEVELOPING A DNA MARKER BASED SCREENING METHOD TO IDENTIFY SUSCEPTIBLE PEOPLE FOR NON-DIABETIC CHRONIC KIDNEY DISEASE IN DRY ZONE, SRI LANKA: A PILOT STUDY

H.B.D. Jeewantha

Postgraduate Institute of Science, Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka

Non Diabetic Chronic Kidney Disease (NDCKD) is a devastating disease in North Central Province (NCP), Sri Lanka. Currently there is no specific treatment apart from kidney transplantation and if person diagnosed for disease, it is 99.9% sure that patient dies within few years. However, for the treatments like dialysis and other simple in-house treatments to delay the death of patients, Sri Lankan Health Sector spends enormous amount of money; for example in year 2005, three and half million US Dollars (4.6% of the annual health budget) were spent on management of patient with NDCKD and it is a huge burden to the Economy of Sri Lanka.

As shown in the human diseases like Diabetic Mellitus, Hypertension and Ischemic Heart Disease, there is strong evidence that kidney disease also based on quantitative genetic factors, which come in to the expression with the influence of environment factors. Genome wide association studies have been used to identify the genetic loci underlying End Stage kidney Disease (ESKD) in African Americans. DNA markers have been developed that are linked to ESKD and these DNA markers can be tested for NDCKD patients and healthy people in Sri Lanka to establish a screening system to identify the people at high-risk groups for NDCKD. The present study was conducted to test the association of DNA markers linked to ESKD for NDCKD.

Venous blood samples were collected from 100 patients with their records of medical history from epidemiology unit North Central Province and 10 control blood samples were collected from volunteering healthy people in the North Central Province. Ethical clearance for using human subjects was obtained from the Faculty of Medicine, University of Perdeniya Ethics committee. Blood samples from 10 NDCKD patients and from 10 healthy people were used to screen DNA markers. DNA was extracted from leucocytes of collected blood by using QIAamp DNA purification system. PCR was carried out for 10 markers with genomic DNA as the template and the primer annealing temperature was 55^0 C. PCR products were size separated by using 1% agarose gel electrophoresis and 6% polyacrylamide gel electrophoresis (PAGE). The presence or absence of bands for each patient and healthy persons were recorded.

Out of 11 DNA markers tested, only seven yielded scorable bands in agarose gels. For the markers *D8S2324* and *D13S306* each, two bands were detected whereas for other five markers each single band was detected. There were no specific alleles in diseased group or control subjects. However, the second allele detected for *D13S306* marker was only seen in diseased subjects.

A total of 26 alleles/bands detected for the four markers tested using PAGE. The markers *D1S3728* and *D10S1426* yielded 11 bands each and the markers *D8S2324* and *D13S306* yielded two bands each. All these bands were very highly polymorphic. Seven alleles detected which were only specific to diseased group where as two alleles detected which were only specific to control subjects. However, within the groups, these alleles were only found in few individuals. Especially in marker, *D1S3728*, multiples alleles detected within individuals.

These polymorphic bands provide a platform to analyze the pedigrees of families where NDCKD is frequent and have possible inheritance pattern of the trait. This kind of screening enables us to figure out the marker-trait association in order to screen people for susceptibility to this disease as a prevention method.