SUMMARY
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Polycystic kidney disease (PKD) is the most common genetic, life-threatening disease, affecting more than 12.5 million people worldwide. It has been characterized by the accumulation of fluid-filled cysts in the cortex and medulla (Igarashi et al., 2002; Somlo et al., 2002; Grantham et al., 2003; Harris and Torres, 2006). Therefore, the current study is focused on polycystic kidney disease among South Indian (Madurai) population.

There are two forms of PKD:

(i) **Autosomal Dominant Polycystic Kidney Disease (ADPKD)**

(ii) **Autosomal Recessive Polycystic Kidney Disease (ARPKD).**

**Autosomal Dominant Polycystic Kidney Disease (ADPKD):**

Autosomal dominant polycystic kidney disease occurs worldwide and in all races. ADPKD disease is one of the most commonly inherited conditions in human with a ratio of 1:500 to 1:1000 (Persu et al., 2002; Grantham and Calvet, 2001). It is genetically heterogeneous with two genes, identified as PKD1 (16P13.3) and PKD2 (4q21) (Ravind et al., 1990 Iragashi et al., 2002). The proteins encoded by the PKD1 and PKD2 genes are polycystin-1 (PC-1) and polycystin-2 (PC-2), that interact with each other in the primary cilia of renal epithelial cells and participate in complex signal transduction pathways, which seems to be involved in chemosensory/mechanical functions and has some role in cell proliferation and maturation (Igarashi et al., 2002). Defects in the genes encoding PC1 or PC2 lead to aberrant gene transcription, cell proliferation, and ion secretion, which in turn result in the formation of fluid-filled cysts. These cysts lead to displacement of the normal renal parenchyma and the formation of a cyst-filled kidney with reduced functional capacity.

Till now there is no gene polymorphism report available among South Indian (Madurai) population. Hence the current study is focused on PKD1(C/T) and PKD2 (G/C) gene polymorphisms in ADPKD patients and age and sex matched control group among South Indian (Madurai) population.
Major objectives of the study:

♦ To estimate the level of total cholesterol (T.Chol.), triglycerides (TGL), low density lipoproteins (LDL), high density lipoproteins (LDL),and very low density lipoprotein (VLDL) in autosomal dominant polycystic kidney disease (ADPKD) patients and control group.

♦ To analyze the concentration of serum minerals (Na, K, Ca and Fe, Mn, Zn, and Se) in ADPKD patients and control group.

♦ To study the association between PKD1 and PKD2 gene polymorphism and autosomal dominant polycystic kidney disease (ADPKD) in patients and control group among South Indian (Madurai) population.

♦ To analyse SNP database using Bioinformatics tools.

♦ Designing 3D structure of polycystin 1 and 2 proteins using protein structure database e tool

Three hundred clinically proven Autosomal dominant polycystic kidney disease (ADPKD) patients (Equal number of males and females) within the age group of 10- 80 were surveyed using questionnaire. Blood samples were collected by from the Department of Nephrology, Madurai Government Rajaji Hospital, and Madurai kidney transplantation and research Centre, Madurai, Tamil Nadu after obtaining ethical clearance. The age and sex matched healthy individuals as control subjects were selected from the general population.

1. The lipid profile levels were analyzed using commercially available span diagnostic kit and the levels were measured using semi- autoanalyzer.

The calcium (Ca), sodium (Na), potassium (K), iron (Fe), Manganese (Mn), Zinc (Zn) and Selenium (Se) concentrations were estimated by Flame Photometry and Atomic Absorption Spectroscopy (AAS).

2. In genetic study, the genomic DNA was isolated, which would be subjected to Polymerase chain reaction (PCR) and Restriction fragment length polymorphism (RFLP) analysis. The amplified PCR fragment was sequenced and confirmed the polymorphism.
3. The human C/T polymorphism at position 4058 in exon 45 and G/C polymorphism at position 83 in exon 1 polymorphic and wild sequences were analyzed and confirmed with SNP data base, Online Mendelian Inheritance in Man (OMIM) available at National Centre for Biological Information (NCBI) website.

It was observed that the males (50%) and females (50%) were equally affected by ADPKD. This might be due to the fact that gene inheritance in both the sexes takes place equally and that some of the normal individuals are also affected by ADPKD. It might follow simple Mendelian coinheritance (Constantinides et al., 1997). The present study also revealed that most of the patients fall under the age group of 30-50 years. The study reveals that 72% of the patients have high blood pressure (hypertension). The ADPKD patients also showed complications like hematuria (12%), renal calculi (10%), urinary tract infection (13%), and diabetic nephropathy (17%), cardiovascular problems (21%), renal osteodystrophy (13%) and anemia (14%). From the results it was observed that most of the patients had hypertension, diabetes and cardiovascular problems and anaemia. The patients’ body mass index (BMI) is showed that, the patients fall in normal or under weight. The study also revealed that the patients were non- vegetarians (35%), betel/ tobacco chewers (31%), alcoholies (16%) and smoker (18%).

The results reveal a significant increase (p<0.0001) in the level of T. Chol., TGL, LDL and significant decrease (p<0.0001) in the level of HDL in ADPKD patients than in control subjects among South Indian (Madurai) population. The total cholesterol level shows less significant increase in patients than in control subjects. Dumm et al (2003; 2000) have also reported that the level of total cholesterol and low density lipoprotein fall within the normal range in ADPKD condition or renal failure.

It was also observed that the levels of calcium (Ca), sodium (Na), potassium (K), iron (Fe), zinc (Zn), manganese (Mn) and selenium (Se) were altered in ADPKD patients. Significantly (p<0.001) increased levels of Ca and K were observed in ADPKD subjects when compared to the control subjects. The level of Na, Fe, Zn, Mn and Se were significantly (P<0.0001) lowered in ADPKD Patients than in the control group.
C/T polymorphism at position 4058 in exon 45 of the PKD1 gene among South Indian (Madurai) population with ADPKD revealed that the “TT” “CT” genotype and the frequency of “T” mutant allele are significantly (p<0.05) higher in the ADPKD subjects compared to control subjects. The study has also demonstrated that ADPKD subjects had higher frequencies of “T” allele and lower frequency of “C” allele than the control group.

G/C polymorphism at position 83 in exon 1 of the PKD2 gene among South Indian (Madurai) population with ADPKD revealed that the “CC” and “GC” genotype and the frequency of “C” allele was found to be significantly higher in the ADPKD compared to control subjects. The study also demonstrates that ADPKD subjects have higher frequency of “C” allele and lower frequency of “G” allele. The work also coincides with the work of Koptides et al., (1999), who identified a polymorphism at position 83 which was occupied by either G or C encoding either arginine or proline (R28P) This polymorphism enabled us to verify whether the disease was co-inherited with allele “C”.

Mutations in PKD1 and PKD2 genes include changes in single DNA building blocks (base pairs) and deletions or insertions of small number of base pairs in the gene. PKD2 gene mutations are predicted to result in the production of an abnormally small, non-functional version of the polycystin-2 protein. It leads to the cyst formation which likely disrupts the protein’s interaction with polycystin-1 and alters signaling within the cell and in primary cilia. As a result, cells lining the renal tubules might grow and divide abnormally which leads to the growth of numerous cysts characteristic of polycystic kidney disease (Gratham and Calvet, 2001). The present study has also revealed lower heterozygocity and there was a significant difference in alleles between the affected and normal individuals.

The study revealed an association between PKD1 (Ala/Val4058, C/T) and PKD2 (G/G) polymorphism and the severity of the ADPKD in ADPKD subjects among South Indian (Madurai) population.

The structural study of the protein by biocomputing method, confirmed the absence of structural significance of a protein and such protein might affect an important domain in the protein structure (Gomez et al., 2009). In silico methods are mainly harnessed to reduce the time, cost and risk associated with Drug discovery
(Shoba et al., 2010). Hence the present study focuses on structure prediction of protein sequence of polycystin-1 and 2 using POLYVIEW server and MUSTER.

The study has also demonstrated that single nucleotide polymorphism (SNP) in PKD1 gene at position 4058, the amino acid change is alanine (A) to valine (V), and C to T nucleotide variation and in PKD2 at position 28, the amino acid change is arginine (R) to proline (P), and G to C nucleotide change. Both these (SNPs) are missense mutations. The mutations and disease association were demonstrated by Polyphen, SNPs & GO, Variant effect predictor, SNPs3D, SIFT. The prediction on the encoded pathogenicity using amino acid sequences which are conserved among different species was done using multiple sequence alignments. The prediction results also suggest that this mutation in a conserved domain region of polycystin-1 has a high potential to affect the structure and function of the protein.