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Discussion

5. DISCUSSION:

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) worldwide, and it is estimated that ~20% of type 2 diabetic patients reach ESRD during their lifetime (Ayodele et al., 2004). Kidney disease in diabetic patients is clinically characterized by increasing rates of urinary albumin excretion, starting from normoalbuminuria, which progresses to microalbuminuria, macroalbuminuria, and eventually to ESRD. Microalbuminuria is the earliest clinically detectable stage of diabetic kidney disease at which appropriate interventions can retard, or reverse, the progress of the disease. According to the estimates, India has the largest number of diabetic patients in the world, ~40.9 million in the year 2007 and expected to increase to ~69.9 million by the year 2025. Type 2 diabetes in Asian Indians differs from that in Europeans in several aspects: the onset is at a younger age, obesity is less common, and genetic factors appear to be more common (Mohan and Alberti, 1997). Some studies conducted in migrant Asian Indians in the U.K. and Europe has reported increased prevalence of diabetic nephropathy compared with white Caucasians (Samantha et al., 1986; Mather et al., 1998; Chandie Shaw et al., 2002). The few studies published on the prevalence of diabetic nephropathy in India have all been clinically based (Vijay et al., 1994; Mohan et al., 2000). Indeed, this does not list a single population-based study on diabetic nephropathy from South Asia.

Racial differences in the prevalence of diabetic renal disease have been reported. Asian subjects have significantly (p<0.01) higher
prevalence (52.6%) of diabetic end stage renal disease (ESRD) when compared with the Caucasians (36.2%) (Young et al., 2003). Migrant Asian Indians had 40 times greater risk of developing ESRD when compared with the Caucasians (Chandie Shaw et al., 2002). The prevalence of diabetic nephropathy in type 2 diabetic subjects is reported to be 5-9% from various Indian studies (Acharya and Chawla, 1978; Chugh et al., 1989; John et al., 1991). Patients with diabetic nephropathy, especially with type 2 diabetes, have a high cardiovascular risk. The risk for cardiovascular disease (CVD) was 3 fold higher in South Indian NIDDM subjects with nephropathy when compared with their non-nephropathic counterparts (Viswanathan et al., 1998). Thus, in type 2 diabetes, many patients may not reach end stage renal disease due to premature death from CVD. Current study reports on the first community based data on the prevalence of diabetic nephropathy in India, particularly in coastal region of the Andhra Pradesh, where maximum number of selected Settiyaar (Vysya) community included in the present study are residing. Recent reports demonstrate that, this community is becoming more susceptible to diabetic nephropathy due to their habits, life style and even genetically modifications.

Obesity is the main cause for several life threatening diseases including diabetes. Particularly in countries like India, various forms of people live with unity in diversity. Some communities were more susceptible to diabetes due to their sedentary life style and habits. In Andhra Pradesh, specifically the vysya community (Settiyaar) is more
prone towards obesity due to their sedentary lifestyle. People belong to this community frequently suffering from diabetes and in addition to renal failures. Settiyaar (Vysya) community was the leading community in selected area with a huge population who are involved in the business. Due to their habituations, food practices and sedentary life style they were prone to become diabetic which latter progresses to renal failure. This was the initiative to start the current research in Nellore and Prakasam districts of the coastal region in Andhra Pradesh. Studies related to specific community in Andhra Pradesh are very limited. In addition to that prevalence of diabetic nephropathy by means of ACE polymorphism is no more in any specific community. Since the selected community is reported to have more registered cases of diabetes attention was paid on this area. Hence this study focused on the changes and development of diabetic nephropathy in Settiyaar (Vysya) community in selected districts of coastal region.

Present study was started by distributing the questionnaires to identify the samples in and around the selected areas. The families were identified with the present problem and further analysis was conducted. Around 200 families were screened for type II diabetic patients in Settiyaar (Vysya) community in Nellore and Prakasam. A door to door survey with face-to-face interviews were carried out in the same community group to find out the known diabetic (KD) and newly diagnosed diabetic (NDD) subjects. A total of 820 subjects were identified in to two different groups. Selected subjects were in the age group of 30-
50 years. Among them 630 were males and 190 were females. In this study 408 belonged to KD group and the remaining 412 belonged to NDD group. A detailed explanation was given to the subjects regarding the research and consent was also taken from the participants. The questionnaire included the personal details like age, sex, duration of stay in the specified area, drinking water source, and parental history and present or previous experience of diseases like diabetes and hypertension. It helped us to screen the people suffering with hypertension or diabetes, where they were further divided to KD and NDD. After screening the data 820 people were selected, who were suffering with diabetes.

Preliminary analysis and basic demographic examinations were conducted for specific group disposition (Table 3 Pg no 70). After knowing the physical parameters, biochemical studies focused on blood glucose level in the patient population. As hyperglycemia is the preliminary biomarker for the identification of diabetes, and developing disease progression, studies were explored in the selected populations under fasting and postprandial visits in terms of blood tests (Table 4 Pg no 71). Renal functioning was assessed in terms of serum creatinine levels and the selected subjects showed no renal alterations. This analysis also provided some support for the separation of two sub groups, included known diabetic and newly diagnosed diabetic. This also clearly explains the prevalence and disease progression of the diabetes in the selected population. As part of their family history and lifestyle they were
more prone to diabetes. Elevated blood glucose is the indicator of diabetes and also the altered metabolic pathway, which ultimately targets the vital organs of the body. Hypertension or diabetes can develop renal failures due to their high frequency as well as extreme blood flow (Marshall, 2004). The best indicator of kidney function is considered to be the rate at which blood is filtered by glomerulus, or glomerular filtration rate (GFR). Chronic kidney disease can then be quantitatively defined as a GFR<60 mL/min/1.73m² for three months or more, irrespective of the cause (National Kidney Foundation, 2002). While the GFR can be measured directly by clearance studies of exogenous markers, such as inulin, iohexol, iothalamate, and Cr⁵¹-EDTA, these procedures are costly and time consuming and are not suited to the routine detection of kidney disease. Even to measure the clearance of endogenous substances, such as urea and creatinine, requires both serum and an accurately timed urine collection, so efforts have been directed at more convenient “urine-free” estimates of GFR (Cockcroft and Gault, 1976).

Creatinine is a non-protein waste product of creatine phosphate metabolism by skeletal muscle tissue. Creatinine production is continuous and is proportional to muscle mass. Creatinine is freely filtered and therefore the serum creatinine level depends on the Glomerular Filtration Rate (GFR). Renal dysfunction diminishes the ability to filter creatinine and the serum creatinine rises. Hence creatine was measured in the test and control samples. If the serum creatinine level doubles, the GFR is considered to have been halved. A threefold increase is considered to
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reflect a 75% loss of kidney function. Present study reveals that there was a drastic increase, almost doubled the control value which indicates the loss of renal function and symptoms of renal failure (Table 4 pg no 71). The results indicates a significant (p<0.001) increase in the serum creatinine content. From the literature it is clear that increases serum creatinine levels are seen in: Impaired renal function, chronic nephritis, and urinary tract obstruction, muscle diseases such as gigantism, acromegaly, and myasthenia gravis, congestive heart failure or even with Shock.

After knowing the serum creatinine, the studies were further extended to know the alterations at hematological levels. Complete blood picture can provide a clear picture of the cellular as well as chemical components of the living system. Hence, studies were conducted in the control as well as selected community peoples (KD and NDD). Selected objects in blood samples were used for the analysis. Assessment of haematological parameters can be used to determine the extent of deleterious effect of toxic substances on the blood of an animal (Ekstrand, 1978; Ashafa et al., 2009). It can also be used to explain blood relating functions of a metal or its chemical products (Guy et al., 1976). Such analysis is relevant to risk evaluation as changes in the haematological system have higher predictive value for lower animals or human toxicity, when the data are translated from animal or human studies (Olson et al., 2000). Table 5 (Pg. No.74) shows some of the important haematological parameters like Hb, TLC, neutrophils, lymphocytes, monocytes,
eosinophils, basophils, MCV, MCH and platelets. Haemoglobin levels in the selected families were optimal in range, compared to those in the control population. Obesity and aging are inversely related with Hb level in blood (Kawada, 2004). Results showed that there were normal parameters observed in both KD and NDD (Table 5 Pg No 74). In the case of WBC, RBC, HCT, MCHC, RDW-SD, RDW-CV, PDW, MPV, P-LCR and MXD there was a different response with the exception of MXD the remaining mentioned parameters showed to be significantly (P<0.001) decreased (Table 5 Pg No 74).

In patients with diabetes mellitus, albuminuria is associated with significantly reduced life expectancy and quality of life, due to the dual complications of end-stage renal failure and atherogenic cardiovascular disease. The condition is the most serious complication of diabetes mellitus, accounting for a high proportion of new cases of end-stage renal failure, and a 40±100-fold excess mortality from cardiovascular disease in these patients (Broch-Johnsen et al., 1985). Possibly as a result of improvements in glycaemic control and blood pressure therapy, the incidence of diabetic nephropathy appears to be declining, and overall prognosis is improving (Parving et al 1995). Although recent insights into the natural history of diabetic nephropathy have enabled treatment strategies to be considered earlier to modulate the progression of the condition (Mogensen, 1997), the pathogenic mechanisms involved in initiation are as yet unknown. Subjects with diabetic nephropathy had a longer duration of diabetes (P for trend <0.0001). A sum of 308 KD and
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224 NDD (n=532) patients belonging to the class of normoalbuminuria and 100 KD and 188 NDD (n=228) patients belonging to microalbuminuria (Table 6 Pg no 81). BMI, Waist circumference (cm), Systolic and diastolic blood pressure and fasting plasma glucose values were highest among the nephropathy group, followed by microalbuminuric and normoalbuminuric subjects (P for trend <0.0001). Prevalence of hypertension was higher among subjects with microalbuminuria and nephropathy compared with the normoalbuminuric group (P < 0.001). Subjects with microalbuminuria had higher BMI and waist circumference with higher fasting plasma glucose and longer duration of diabetes. Other parameter like blood pressure did not vary significantly between the study groups (Table 6 Pg. no 81).

The control and prevention of high lipid levels are central to the prevention of stroke, coronary heart diseases, cardiovascular diseases and other lipid associated health hazards including renal failures. In India, systematic epidemiological studies of lipid profiles among selected group of populations are limited. And, there is need for the detection of risk factors of several health problems and to develop and evaluate the low cost methods of effective treatment in selected population. Many researchers emphasized that in India there is a need to organise a community based program for identifying individuals with elevated lipids and bring them into medical facilities for further evaluation and maintaining a high proportion of them in a long term control program. To diagnose renal failure, a further analysis of lipid profile was conducted.
Any changes in the body will reflect immediately in lipid content of the biological system. Lipid profile, also known as coronary risk panel or lipid panel, is the collective term given to the estimation of, typically, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides, used to assess risk of coronary heart disease.

An extended lipid profile may include very low density lipoprotein cholesterol, LDL and HDL. Particularly alterations in any metabolic activity reflect in the altered lipoprotein content as well as the cholesterol. In the case of diabetic nephropathy in Settiyaar community the results indicated that there was a drastic enhancement in lipid parameters except in HDL and LDL (Table 7 Pg no 83). In the case of triglycerides control subjects showed 122.72±36.78 mg/dL, whereas the selected group of patients showed 202.14±59.98 mg/dL for KD and 206.96±66.76 mg/dL for NDD which shows a drastic increase. This indicates a direct relation between the obesity (diabetes) and the lipid metabolism and accumulation of fat content in the blood stream. There was also an enhanced level of cholesterol can be observed in both of the groups. In the case of VLDL and LDL there was also an increase when compared to that of controls (Table 7 Pg no 83). In the case of LDL and HDL it was lower and located within the normal range. Results showed comparative altered lipid profile in the control as well as selected group of subjects. But in all cases except for HDL, increased lipid metabolism indicate the synthesis and accumulation of lipid metabolism. This proved
that obese people with diabetes in Settiyaar community are more prone towards getting heart disorders.

After conducting lipid analysis studies were made to assess the important and vital organ of the body, i.e., the liver. Liver function tests (LFTs or LFs), which include liver enzymes, are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver. Most liver diseases cause only mild symptoms initially, but it is vital that these diseases be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. Some tests are associated with functionality (eg. albumin); some with cellular integrity (eg. transaminase) and some with conditions linked to the biliary tract (gamma-glutamyltransferase and alkaline phosphatase). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to (1) detect the presence of liver disease, (2) distinguish among different types of liver disorders, (3) gauge the extent of known liver damage, and (4) follow the response to treatment. Table 8 (Pg No:85) explains about the detailed analysis of LFTs in Settiyaar community. Here we can find altered LFT values when compared to the controls. Almost all parameters are within the normal range reference values. Total bilirubin (direct and indirect) was within the normal range. There was an elevated levels of liver functioning enzymes viz, ALP, SGOT and SGPT when compared to control values. The increased levels of serum transaminases in diabetic individuals suggest alteration in liver function. These levels
increase several times if cellular damage occurs in the liver, so these enzymes are markers for assessing liver function. The total protein content was decreased in the selected group. Since there was a loss of protein through renal function the total and individual protein contents were decreased when compared to the controls. The A/G ratio also decreased compared to the control.

Since diabetes and altered thyroid hormones are metabolic disorders that affect the levels of carbohydrates, proteins and lipids. The effects of iodothyronine on the various metabolic pathways are assessed by specific tests, such as TSH, T4, T3 and FT3. The thyroid hormones, triiodothyronine and tetraiodothyronine are insulin antagonists that also potentiate the action insulin indirectly (Granner et al., 2000). TRH synthesis decreases in diabetes mellitus (de-Greef et al., 1992; Suzuki et al., 1994). These facts could be responsible for the occurrences of low thyroid hormone levels in some diabetics. The level of TSH in the study was not clinically significant in diabetics than in non-diabetics. This finding is not consistent with the report of Celani et al (1994), Smithson (1998) who recorded varied levels of thyroid hormones in diabetic subjects. The large SD observed among the diabetics may be due to the influence of the oral hypoglycaemic agents some of the diabetics were receiving since some of the diabetics were Type 2 subjects. When compared to the control group KD and NDD showed decreased activity, with slightly lower with the normal range. Total T3, KD and NDD was with in the normal range. Similarly  T4 in the KD and NDD was within the normal range. But
in case of TSH it was altered. The control group showed a value of 3.1 ± 0.98, whereas in the Settiyaar group was altered drastically with a value of 22.0 ± 3.23 in case of KD and 25.12 ± 4.21 in case of NDD (Table 9 Pg No 88). This indicates the altered thyroid metabolism in the diabetic patients of selected Settiyaar community. The elevated levels of TSH is not able to express the T3 and T4 levels.

Changes in the homeostasis can alter the electrolyte concentration in the blood. Where sodium and potassium are the two important electrolytes, they maintain the homeostasis, acid base balance and also different biological functions in the membranes. Hence studies were undertaken to determine the level of two important electrolytes (sodium and potassium) in the serum of the control as well as the selected group of peoples. Sodium levels increased significantly (P<0.001) in the selected subjects (Table 10 Pg no 90). Potassium showed similar results to that of sodium. This clearly suggests that diabetes effects the electrolytes by altering the sodium and potassium levels. Differential distribution of these two cations is essential for normal membrane function and integrity. Similar augmentation in electrolyte levels was demonstrated in rats fed with sodium fluoride (Chinoy et al., 1993). Serum potassium is an indicator of cell damage. Increased levels suggest cell deterioration.

The routine classical evaluation of nephropathy (any type of renal problems) includes the identification of glomerular and tubular markers in the patient’s serum and urine. The normal individual doesn’t contain this
content elevated in their urine or in serum samples. These glomerular and tubular markers include: transferring, Ig G, antitrypsin, β-2-microglobulin and ACE. Recent studies also have demonstrated that, there were tubular components in renal complications of disease conditions as shown by the detection of renal tubular enzymes and low molecular weight proteins in the urine as well as in serum. In fact, tubular involvement may precede glomerular involvement because several of these tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and rise in serum creatinine (Catalano et al., 1993).

Thus studies were conducted to evaluate the glomerular and tubular marker in urine as well as serum of the control and selected community people. Table 11Pg no 92 shows the analysis of serum glomerular and tubular markers in the control and test samples. Low transferrin can impair hemoglobin production (since to make hemoglobin, iron is required) and so lead to anemia. In the present study the level of transferring was low when compared to that of control (Table 11 Pg. no 92). Also the low levels of serum IgG, but with in the normal range indicated altered renal function (Table 11 Pg. No 92). In the present case there was no difference with the control value. It was almost equal, indicating normal functioning of liver (Table 11 Pg. No 92). β2m is normally cleared by the kidneys at a rate comparable to GFR (Karlsson et al., 1980; Gautier et al., 1984), then reabsorbed and catabolized in the tubules, and serum levels are inversely related to GFR (Kamsson et al., 1980). Clearance by conventional dialyzers is negligible as these
membranes are impermeable to β2M. Production of β2M in normal individuals is 9 mg/hr/70 kg (Karlsson et al., 1980). Production may be increased in proliferative disorders (Schildun et al., 1980) and rheumatoid arthritis (Manicourt et al., 1978) as indicated by high serum levels in the presence of normal renal function.

Beta 2-microglobulin is a protein found on the surface of many cells. Testing is done primarily when evaluating a person for certain kinds of cancer affecting white blood cells including chronic lymphocytic leukemia, non-Hodgkin’s lymphoma, and multiple myeloma or kidney disease. In the present study a rapid enhancement of β2M was noticed. The control subjects showed 2.60 ± 0.99 g/ml, where as the KD patients showed a maximum increase of β2M to 10.89 ± 2.08 g/ml and NDD patients showed 11.56 ± 1.88 g/ml. This shows a drastic increase which indicates altered renal activity in the selected group of people (Table 11 Pg. no 92). A significant (P<0.001) change was noticed compared to the normal. This altered range is more supportive for further analysis for the diabetic nephropathy in Settiyaar community.

*Angiotensin-converting enzyme* gene (ACE) is a risk factor for DN. Its plasma levels have been reported to be associated with DN but not with diabetic retinopathy in type 1 diabetes patients (Marre et al., 1994). ACE modulates the generation of angiotensin II, which increases intraglomerular hydraulic pressure (Hall et al., 1977), leading to glomerulopathy. ACE inhibition strongly modifies renal hemodynamics in
animals (Zatz et al., 1986), and the course of DN can be considerably improved by treatment with ACE inhibitors, in patients with type 1 diabetes (Lewis et al., 1993). Plasma ACE concentrations are stable in individuals (Alhenc-Gelas et al., 1991) and are partly under genetic control (Cambien et al., 1992). The ACE gene is located on chromosome 17, at q23, and contains 26 exons that span a total of 21 kb, and ACE levels are controlled primarily by the ACE region that maps to an 18-kb interval that is flanked by two intragenic ancestral recombination breakpoints (Soubrier et al., 2002). An insertion/deletion (I/D; rs1799752) polymorphism in intron 16 of ACE accounts for a large proportion of interindividual variation for ACE serum concentrations (Rigat et al., 1990). Several case-control studies have examined the association between the ACE I/D polymorphism and DN. As shown in a recent meta-analysis, a deleterious effect of the D allele was evidenced (Ng et al., 2005). Longitudinal follow-up studies recently provided further evidence for the deleterious effect of the D allele (Rudberg et al., 2000; Boright et al., 2005). However, one family-based study discordantly showed that the I allele was over transmitted in cases of DN (Krolewski, 1988). Here in the present study the ACE level decreased when compared to that of control individuals (Table 11 Pg. no 92). Control individuals had a concentration of 44.97±8.72, and selected group of people belonging to KD had 37.07±12.68 and NDD group are showing had a concentration of 32.51±10.23 indicating a significant (P<0.001) decrease. This indicates the accumulation of angiotensin I.
After identifying the glomerular and tubular markers in the serum, studies were undertaken to determine the status of the same in the urine to confirm the diabetic nephropathy in Settiyaar community people. From the Table 12(Pg. no 93) it was clear that transferrin levels were hiked in the Settiyaar community people. Transferrin concentration was low in the serum and increased in the urine indicating the loss of renal function (Table 11 Pg. no 92 and 12 Pg. no 93). Similar results were found in the case of IgG in the urine as well as in serum. Here also the decreased concentration of serum IgG and increased levels of urinary IgG indicates the renal alterations. Similarly AIAT was also changed with slight modifications, where the serum AIAT did not show any significant change. But there was altered values of AIAT. β2M also showed similar pattern of over excretion. Hence, it can be concluded that, these values are drastically increased in the serum as well as in urine of the selected community (Settiyaar). The same was also observed with ACE levels where the control value was 11.46±0.84, in the KD group it was 13.77±1.46 and in the NDD group it was 16.74±0.89 over excretion indicates the renal problems (Table 12 Pg. no 93).

Individual differences in renal ACE activity predict the susceptibility for proteinuria-associated renal damage in experimental conditions (Huang et al., 2001; Rook et al., 2005). Furthermore, Ang II is increased in damaged tubules and is suggested to be a possible mediator of renal damage in experimental and human renal disorders (Wolf and Ritz,
2005; Ruiz-Ortega et al., 2006). Blockade of the actions of Ang II by ACE inhibitors or AT$_1$ receptor blockers has been proven to effectively reduce blood pressure and proteinuria (Anderson et al., 1986), thereby providing renoprotection. A disrupted balance between intrarenal ACE and ACE2 with consequent low levels of Ang II and high levels of Ang(1–7) might contribute to the renoprotective mechanisms of ACE inhibitors (Campbell et al., 1991; Ferrario and Iyer, 1998; Ferrario et al., 2005; Kocks et al., 2005).

In recent years a vast amount of data has been published on the association between the insertion/deletion (I/D) polymorphism of the gene coding for angiotensin-converting enzyme and renal disease. It has become clear that the polymorphism does not affect the prevalence of renal disease. However, data on the association with progression of renal disease and therapy response are still contradictory. Moreover, sufficient data on the physiological significance of this polymorphism are still lacking. This contribution provides an overview of the available studies and the potential pitfalls in interpreting the data. In the present study the putative mechanisms for the association between the DD genotype and progression of renal disease was discussed and directions for the future that might be employed to further clarify the role in renal pathophysiology is suggested.

Renal failure is an outcome of complex pathophysiological process resulting from multiple etiologies with contribution from both genetic and environmental factors. A large variation abounds in the frequencies of
ACE I/D polymorphism in different ethnic groups. It is evident from table 13 pg.no 96 that the D allele frequency of our controls was intermediate to most reported Caucasian (Hubacek et al. 2000; Tarnow et al. 1995; Chowdhury et al. 1996; Schmidt and Ritz, 1997) and Asian (Hsieh et al. 2000; Wang et al. 2005; Tamaki et al. 2002; Oh et al. 1996; Gesang et al. 2002; Ergen et al. 2004) populations. However, two Caucasian (Doria et al. 1994; Powrie et al. 1994) and an Asian (Tamaki et al. 2002) population are reported to have comparable allele frequencies. The failure to find statistically significant differences in the distribution of ACE gene I/D genotypes and their allele frequencies between the selected community and the controls suggest that this polymorphism is not a risk factor for the development of renal failure in the studied population. These observations find support in the work of Tamaki et al. (2002) and Ergen et al. (2004). In conclusion, the present study suggests that the ACE I/D polymorphism are not associated with advanced form of renal failures due to diabetic nephropathy within the selected (Settiyaar) population.