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TO EXPLORE THE ROLE OF TGF AND SMAD4 IN DIABETES ASSOCIATED KIDNEY DISEASE

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ABTRACT

Diabetes, both type 1 and type 2, is a significant risk factor for the development and progression of chronic kidney disease (CKD). The relationship between diabetes and CKD is multifaceted and involves various pathophysiological mechanisms. Chronic kidney disease (CKD) is an important public health concern in developed countries because of both the number of people affected and the high cost of care when prevention strategies are not effectively implemented. Prevention should start at the governance level with the institution of multisectoral polices supporting sustainable development goals and ensuring safe and healthy environments. The TGF-beta family of cytokines are ubiquitous, multifunctional, and essential to survival. They play important roles in growth and development, inflammation and repair and host immunity. The mammalian TGF-beta isoforms (TGF-beta 1, beta 2 and beta 3) are secreted as latent precursors and have multiple cell surface receptors of which at least two mediate signal transductions. The role off tgfbr1, tgfbr2 in chronic kidney disease, in ambiguous in association with the smad4, smad6, epo production. Diabetes plays a crucial role in the kidney failure in this study we examine the involvement of the TGF Beta and SMAD gene protein. Targeting of other type of the SMAD protein in the various disease provide the salient finding and their role illustrated till date.

INTROUCTION

Transforming Growth Factor-beta (TGF-β) plays a significant role in chronic kidney disease (CKD) by modulating various cellular processes, including inflammation, fibrosis, and extracellular matrix deposition. SMAD proteins are intracellular mediators of TGF-β signalling. Upon activation by TGF-β, receptor-regulated SMADs (R-SMADs), such as SMAD2 and SMAD3, are phosphorylated and form complexes with the common mediator SMAD (co-SMAD), SMAD4. Targeting the TGF-β/SMAD pathway represents a potential therapeutic strategy for CKD by inhibiting renal fibrosis and inflammation, thereby slowing down disease progression and preserving renal function. Dysregulation of the TGF-β pathway in diabetes contributes to renal structural and functional abnormalities, including biochemical change, glomerular damage, fibrosis, inflammation, and oxidative stress, all of which are central to the pathogenesis of DKD. Targeting the TGF-β pathway represents a potential therapeutic approach for preventing or slowing the progression of CKD in diabetic patients. TGFβ-Smad signaling is an important pathway, with established role in many types of cancer, but with scarce data in CKD. This pathway is essential regulator of cellular proliferation, differentiation, apoptosis, extracellular matrix remodelling of the cell, angiogenesis, and inflammation. The TGFβ super family consists of more than 30 related members in mammals, including three kinds of TGFβs, 4 kinds of activins and over 20 kinds of bone morphogenetic

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proteins (BMPs). Subversion of TGFβ family signaling has been implicated in various human diseases including autoimmune 2 disease, vascular disorders and cancer [11]. Main components of TGFβ-Smad pathway are TGFβ1 protein, which is a ligand, TGFβR1 and TGFβR2, are the two ligand receptors, Smad 2 and Smad3 are the receptor regulated Smad (R-Smad), Smad4 is the common mediator Smad (co-Smad), whereas Smad 6 and Smad 7 acts as inhibitory Smad (I-Smad) (Su E, et al, 2010). Binding of extracellular TGFβ1 protein to the TGFβ type2 receptor on the cell surface initiates the signaling pathway dimerize type 1 receptor, which recruits and phosphorylates Smad2 and Smad3. Activated Smad2-Smad3 complex recruits Smad4, which forms higher order complex and translocate into the nucleus where they act as transcription factors. Inhibitory smads, Smad6 and Smad7, have suppressive effects on the TGFβ-Smad signaling pathway by interrupting the actions of R-Smad and Co-Smad (Su E, et al, 2010).

Smad4 mutations are known to play important role in different types of solid malignancies (Miyaki M, et al., 2006; Schutte M, et al., 1999). Resistance to homeostatic effects of TGFβ are demonstrated in hematological malignancies, though in some of these malignancies, elevated levels of TGFβ are known to promote myelofibrosis and pathogenesis through their effect on the stroma and immune system [44]. In hematological malignancies, it has been shown that absence of Smad 4 is involved in the AML (Wierenga ATJ, et al., 2002). SNPs and expression levels of this gene are found to be associated with susceptibility and prognosis of various cancers (Yin J, et al., 2011; Mangone FR, et al., 2010; Singh P, et al.,2011; Osawa H, et al., 2004; Kawate S, et al., 2001; Jeon HS, et al., 2008). Lack of direct interaction of diabetes and TGFβ-Smad pathway, despite being important factor in Ckd, makes it even more interesting for the research. Moreover, to the best of our knowledge no pertinent literature is available till date on the role of differential expression and mutations in the genes of this pathway with susceptibility or prognosis of CKD.

MATERIAL & METHODS

3.1. Subjects

All patients (>18 years) diagnosed with diabetes associated CKD were prospectively enrolled for a period of 12 month (Nov 2021-October 2022) at Department of General Medicine in collaboration with nephrology department at, Sir Ganga Ram Hospital, Delhi, India. Sample size and sampling: 100.

Inclusion criteria:

1) Patients with chronic kidney disease with stage I –V disease.
2) Patients with End-stage renal failure on renal replacement therapy in the form Of Hemodialysis and peritoneal dialysis.

Exclusion criteria:

1) Patients with other systemic illness without renal failure
2) Pregnancy
3) Aplastic anemia
4) Known haematological malignancy causing secondary renal failure
5) Patients with the end-stage renal disease treated with renal replacement therapy in the form of renal transplantation
6) History of blood transfusion during the last three months

During this period, all newly diagnosed cases of chronic kidney disease based on the National Kidney foundation definition were included in this study.

1 All the patients were evaluated based on detailed history taking, clinical examination, and laboratory investigations after informed consent were obtained from them. Staging of CKD was

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done based on the National Kidney Foundation (NKF/KDOQI) staging system. GFR was estimated using the abbreviated MDRD (modification of diet in renal disease) formula. The case history was recorded on pro-forma. Data on age, sex, education, occupation, and lifestyle factors, tobacco usage (chewed), smoking and alcohol consumption were collected from all subjects. Detailed medical histories were obtained regarding present complaints. All subjects were also interviewed regarding the history of diabetes, hypertension and other co-morbid conditions Chronic Glomerulonephritis was diagnosed in patients with a history of edema, hypertension and documented the nephritic range of proteinuria. Hypertensive nephropathy was diagnosed in patients with a long history of hypertension and other target organ damage. Diabetic Nephropathy was diagnosed in patients with a long history of diabetes, presence of diabetic retinopathy and proteinuria more than 500mg in 24 hours. Chronic Pyelonephritis was diagnosed on ultra-sonogram when there is the presence of small kidneys with irregular borders. Obstructive Uropathy, Autosomal dominant polycystic kidney disease, and Obstructive nephropathy were diagnosed by ultra-sonogram GFR Peripheral blood sample in EDTA vials and plain vials (for serum) was obtained from patients and controls as well. Serum was collected to compare TGFβ1 levels and stored at-80^{o}C. Peripheral blood RNA was immediately extracted (Nucleospin RNA#740200, Macheley-Nager, Duren, Germany) and stored at -80^{o}C for further use.

Sample size and sampling: *100.*

Inclusion criteria:

1) Diabetic Patients with chronic kidney disease with stage I –V disease.
2) Patients with End-stage renal failure on renal replacementtherapy in the form of Hemodialysis and peritoneal dialysis.
3. GFR <60 ml/min/1.73 m^2 based on estimated GFR using the Modification of Diet in Renal Disease (MDRD) formula

Exclusion criteria:

1. Pregnancy
2. Patient below the age 18 year
3. Aplastic anemia
4. Known hematological malignancy causing secondary renal failure.
5. Patients with the end-stage renal disease treated with renal replacement therapy in the form of renal transplantation.
6. History of blood transfusion during the last three months

During this period, all newly diagnosed cases of chronic kidney disease based on the National Kidney foundation definition were included in this study.[1] all the patients were evaluated based on detailed history taking, clinical examination, and laboratory investigations after informed consent were obtained from them. Staging of CKD was done based on the National Kidney Foundation (NKF/KDOQI) staging system. GFR was estimated using the abbreviated MDRD (modification of diet in renal disease) formula. The case history was recorded on pro-forma. Data on age, sex, education, occupation, and lifestyle factors, tobacco usage (chewed), smoking and alcohol consumption were collected from all subjects. Detailed medical histories were obtained regarding present. complaints. All subjects were also interviewed regarding the past history of diabetes, hypertension and other co-morbid conditions.

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Chronic Glomerulonephritis was diagnosed in patients with a history of edema, hypertension and documented the nephritic range of proteinuria. Hypertensive nephropathy was diagnosed in patients with a long history of hypertension and other target organ damage. Diabetic Nephropathy was diagnosed in patients with a long history of diabetes, presence of diabetic retinopathy and proteinuria more than 500mg in 24 hours. Chronic Pyelonephritis was diagnosed on ultra sonogram when there is the presence of small kidneys with irregular borders. Obstructive Uropathy, Autosomal dominant polycystic kidney disease, and Obstructive nephropathy were diagnosed by ultra sonogram.

Data collection/Analysis:

Investigations for assessment of renal failure

A. Blood urea, Serum creatinine, serum electrolytes.
B. Abdominal ultrasound with KUB
C. Cardiovascular parameters(Blood pressure&Echocardiogram).
D. Estimation of the TGF level in Blood
E. Estimation of the SMAD 4

STATISTICAL ANALYSIS

Data was collected by using pre-tested proforma meeting the objectives of the study, Mean, median and standard deviation for descriptive statistics, Chi-square tests for inferential statistics, Statistical significance if $P < 0.05$ and 95% confidence limit will be used. Fisher's exact test when appropriate was performed to analyze the univariate relations between possible prognostic factors. As it is likely that different prognostic factors are mutually related, the independent effects of prognostic factors were additionally analyzed with multivariate logistic regression Analysis of variance (ANOVA), Student`s `t` test were applied and *p*-value of less than 0.05 was considered significant

RESULTS AND DISCUSSION

Subject

CKD patients (n=83) enrolled Healthy Controls (n=91) with similar age and sex distribution were enrolled. Clinical and demographic details of patients and controls are provided in (Table3). Twenty patients and five healthy controls were selected for sequencing. Mean age of shortlisted patients was 42.11 (SD = $\pm$ 12.76) years, whereas that of controls was 38.40 (SD = $\pm$ 12.76) years ($p = 0.570$). All the patients were in chronic phase at the time of diagnosis. IM responder group had 12 patients (55.55%), while 8 patients (44.45%) were in IM failure group. Mean age of responder group was 35.1 (SD = $\pm$6.74) years, whereas failure was 50.88 ($\pm$ 13.36) years ($p = 0.005$).

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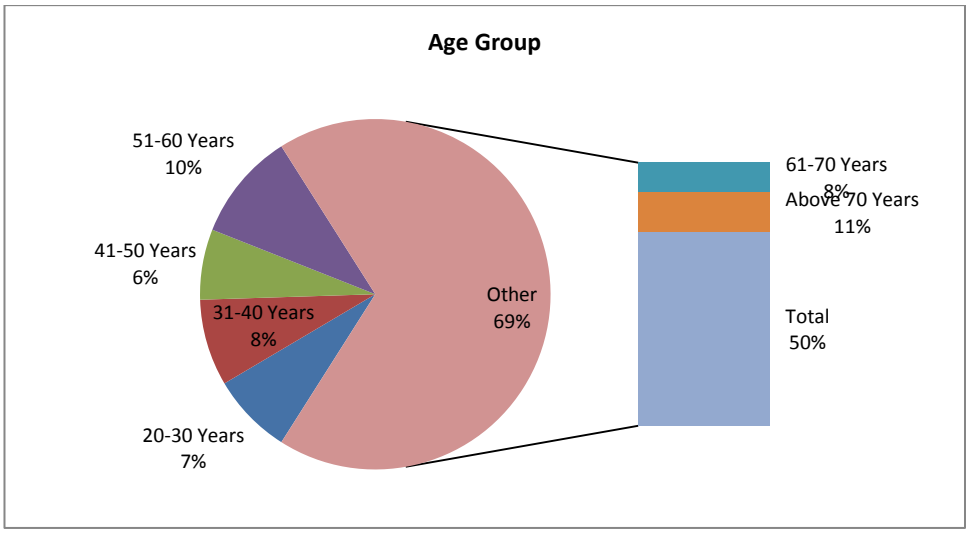
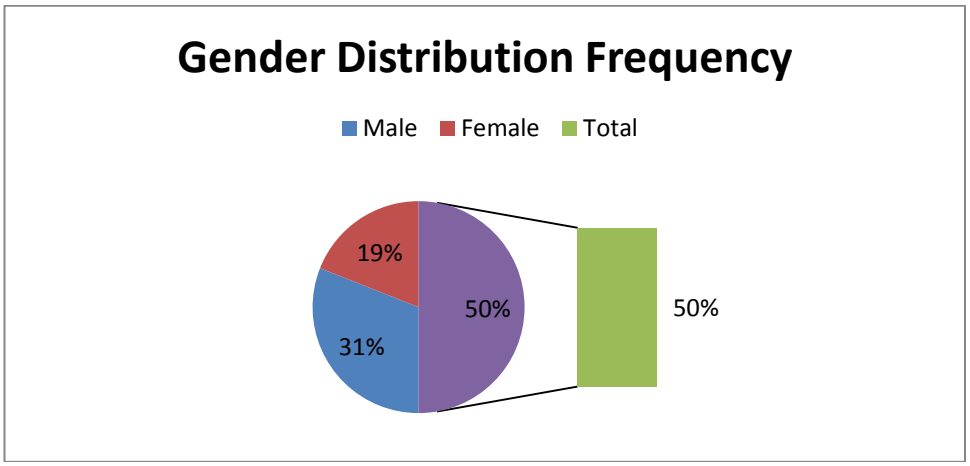
4.1. Age Group Distribution



Graph: 1 Shows the age group distribution. 20years – 70 years above the patients are included in the study. Age group : 20-30 Years 15 patients (15%) 31-40 Years 16 patients(16%) 41-50 Years 13patients (13.%) 51-60 Years 20 patients (20%)61-70 Years 15 patients(15%) Above 70 Years 21 patients (21%)

4.2. Gender Distribution Frequency



Graph: 2 shows the gender distribution Among 100 cases of CKD, there were 62 (62%) male patients and 38 (38%) female patients. The ratio of male to female was 2.63 i.e. males are 2.63 times more susceptible to CKD when compared to females

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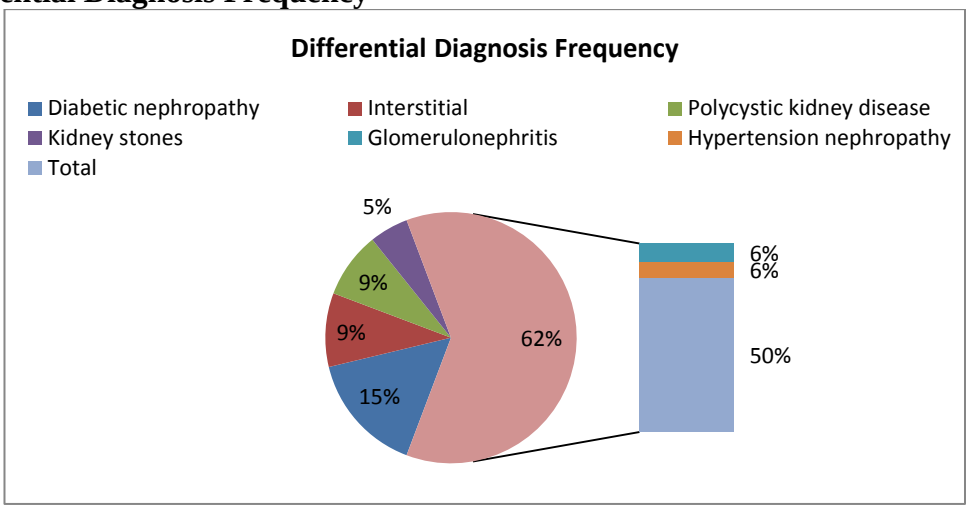
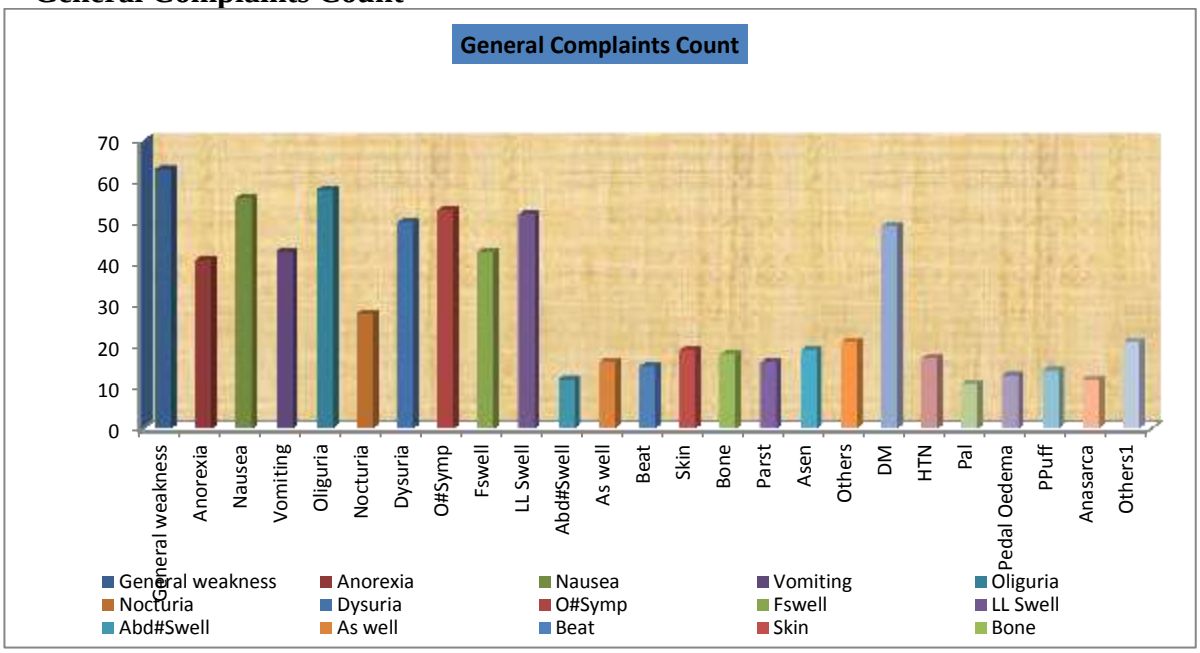
4.3. Differential Diagnosis Frequency



Graph :3 In 100 cases the differential diagnosis were analyzed Diabetic nephropathy was in 31(31%) Interstitial kidney disease was in 19(19%) Polycystic kidney disease diagnosed in 17patients which were (17%) Kidney stones in different types seen in 10 patients (10%) Glomerulonephritis in 12 patients (12%) Hypertension nephropathy in 11(11%). Diabetic nephropathy was more common in the study which was statistically more significant of p-value <0.005>

5. General Complaints Count



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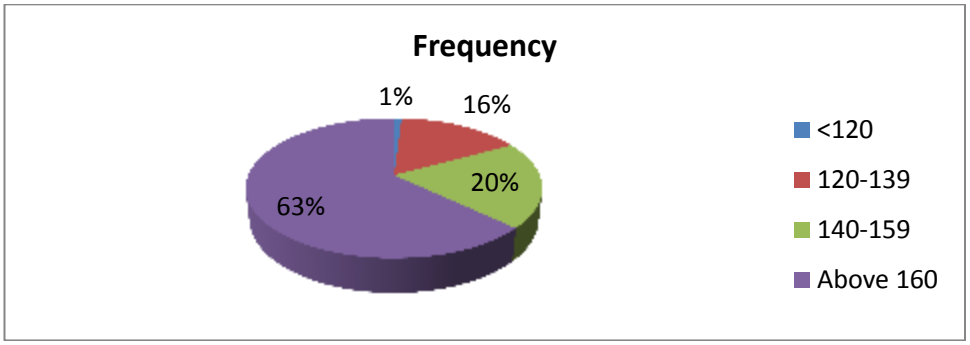
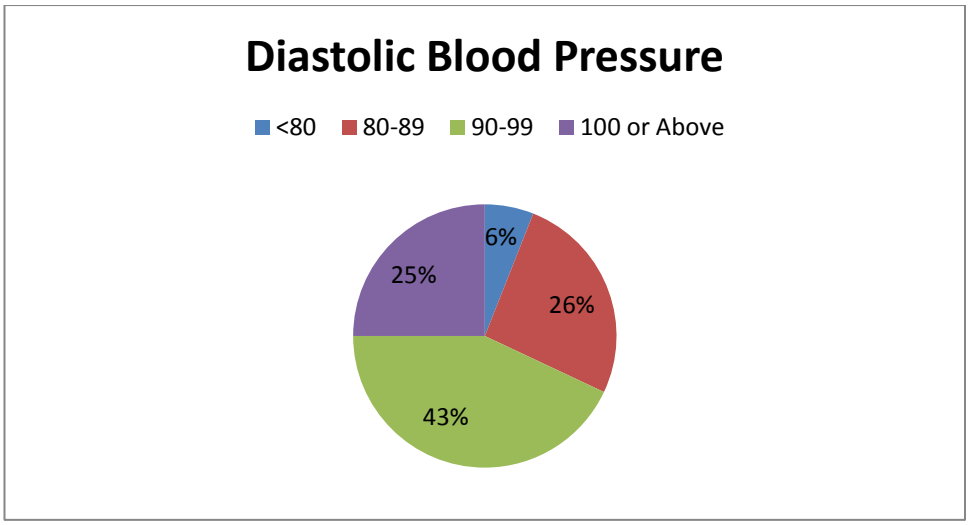
6. Systolic Blood Pressure



Graph: 5 In 100 patients blood pressure was recordedby standard method .systolic blood pressure was <120 in 1patients which was (1%), systolic blood pressure between 120-139 in16 patients which was(16%). Above 140-159 were observed in 20 patients were (20%) Above 160 were observed in 63 patients (63%) most of the patient were hypertensive in CKD due to decreased renin release which was statistically more significant of p-value <0.005>

7. Diastolic Blood Pressure



Graph: 6 In 100 patients blood pressure was recorded by standard method .diastolic blood pressure was <80 seen in 6 patients(6%) diastolic blood pressure between 80-89 observed in 26 patients (26%) Above 90-99 was in 43patients(43%)were observed in 43 patients Above 100 were observed in 25 patients (25%) most of the patient were hypertensive in CKD due to decreased cardiovascular stability which was statistically more significant of p-value <0.005>

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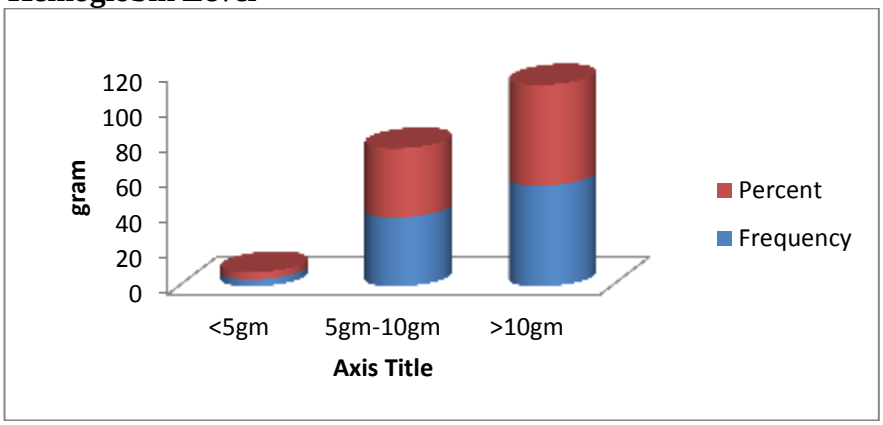
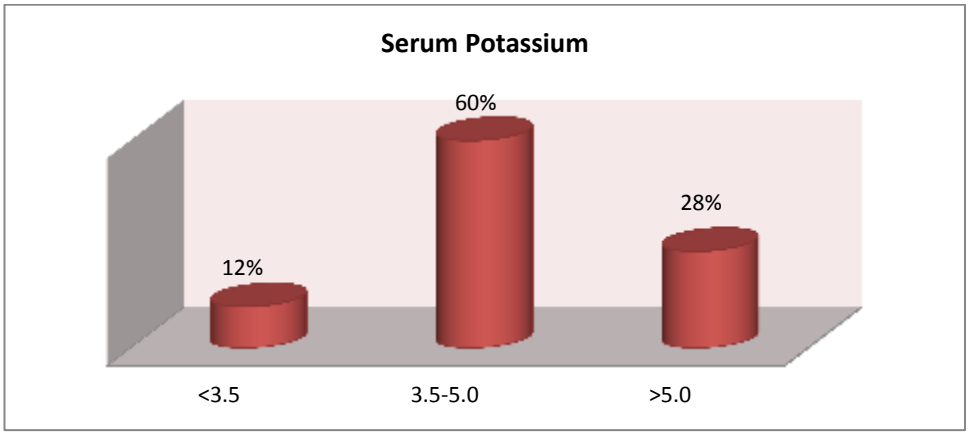
8. Graph: 7 Hemoglobin Level

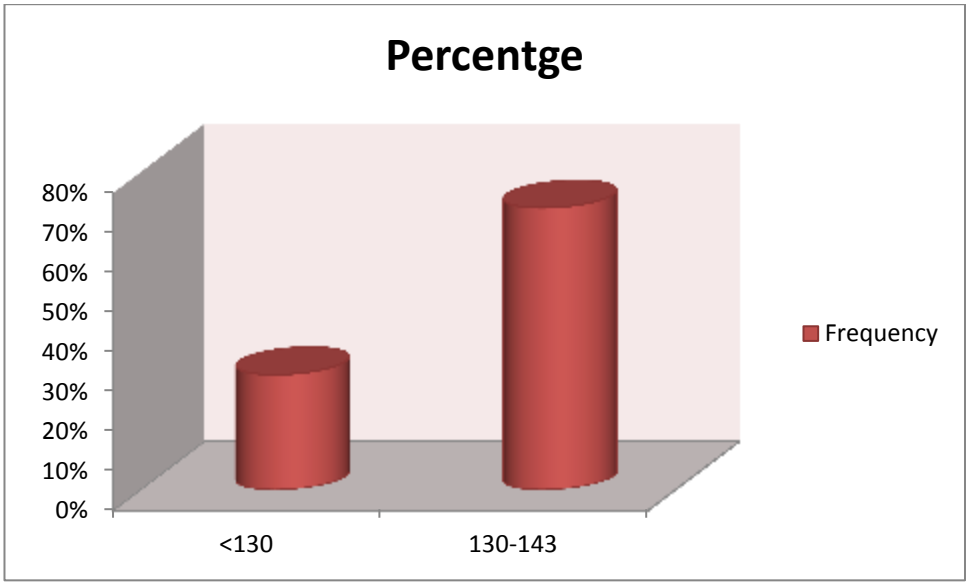


Graph: 6 The above table reveals that 39% of the patients have their hemoglobin level in the range of 5-10 gm%. Only 4%of the patients have their value below 5mg%, but 57% of the

9. Graph: 8 Serum Potassium



Graph: 8 28% of patients have Hyperkalemia. 60% had the value within normal limits (3.5-5 meq/1). Only 12% had the value of less than 3.5 meq/1. Serum Sodium Level



Graph: 9 It appears that hyponatremia (Serum sodium level < 130 meq/1) is present in 29% of patients. Further, in 71% cases, this value lies between the normal limits (130-143 meq/1).

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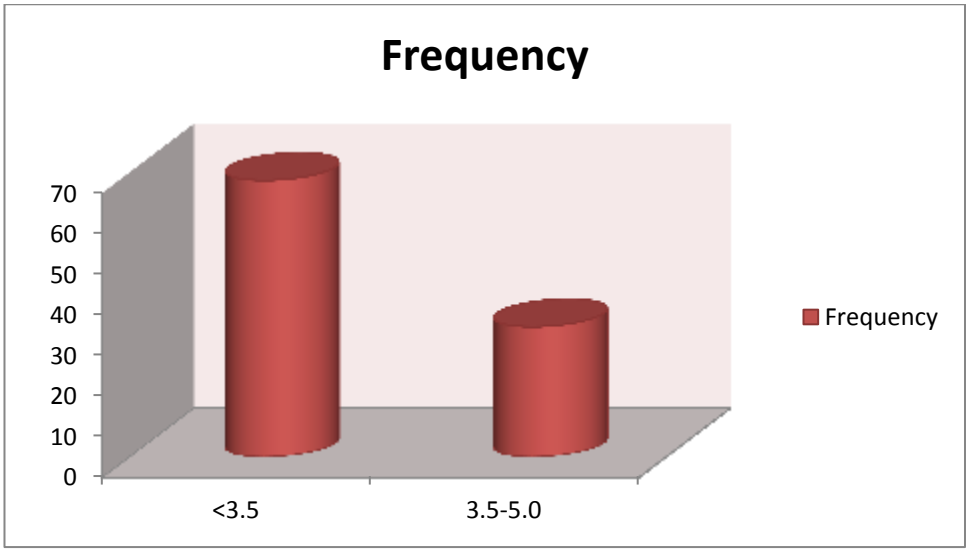
1. Table :1 Serum Albumin Level

Serum Albumin	Frequency	Percent
<3.5	68	68.0
3.5-5.0	32	32.0
Total	100	100.0

10. Serum Albumin Level



Graph :10 Hypoalbuminemia (Serum Albumin < 3.5g/dl) can be seen in 68% of cases. 32% of cases have this value within normal limits (3.5 - 5 g/dl).

Table 10: Kidney Size

Kidney Size	Frequency	Percent
Small (<8.5 cm)	61	61.0
Normal	29	29.0
Increased >12 cm	10	10.0
Total	100	100.0

Graph: 10 Kidney Size

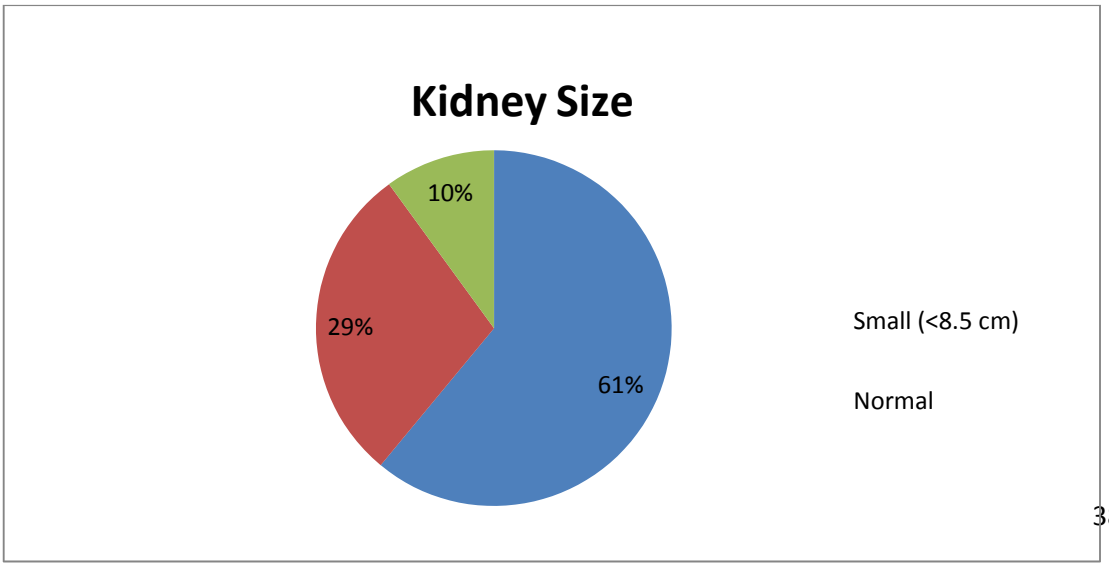


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Table &Graph: 10 61% of the cases seem to have decreased kidney size and 29% appears to have an increased kidney size. Whereas 10% of the patients have increased normal size.

TABLE :2 Echo Findings

Echo	Frequency	Percent
Not done	21	21.0
LVH	41	41.0
Ischaemic dilated cardiomyopathy, Hypokinesiaof wall or septum	12	12.0
Diastolic dysfunction	8	8.0
Normal	14	14.0
Pericardial effusion	4	4.0
Total	100	100.0

Graph: 11 Echo Findings

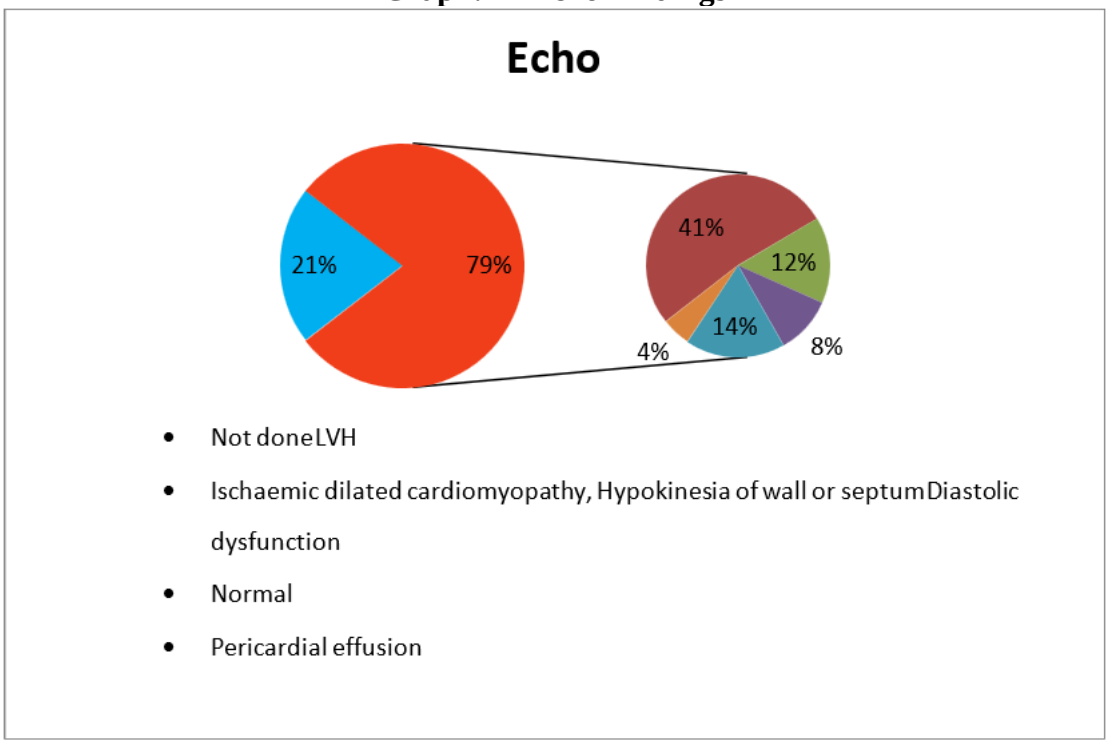

Table &Graph: 11 Echo Cardiogram Was Not done in21patients (21%) LVH was present in 41 cases (41%).Ischaemic dilated cardiomyopathy, Hypokinesia of wall or septum is seen in 12 patients (12%).Diastolic dysfunction.8 patients (8%).Normal findings in echocardiography were in14 patients (14%) Pericardial effusion was seen in 4patients (4%) LVH was more common among CRF patients as it reflects on cardiac efficiency

Table: 3 USG Parenchymal Changes

USG Parenchymal Changes	Frequency	Percent
Grade II parenchymalchanges	38	38.0
Grade III parenchymalchanges	12	12.0
b\I shurken Kidneys	18	18.0
Grade I parenchymalchanges	18	18.0
Normal	14	14.0
Total	100	100.0

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Graph: 12 USG Parenchymal Changes

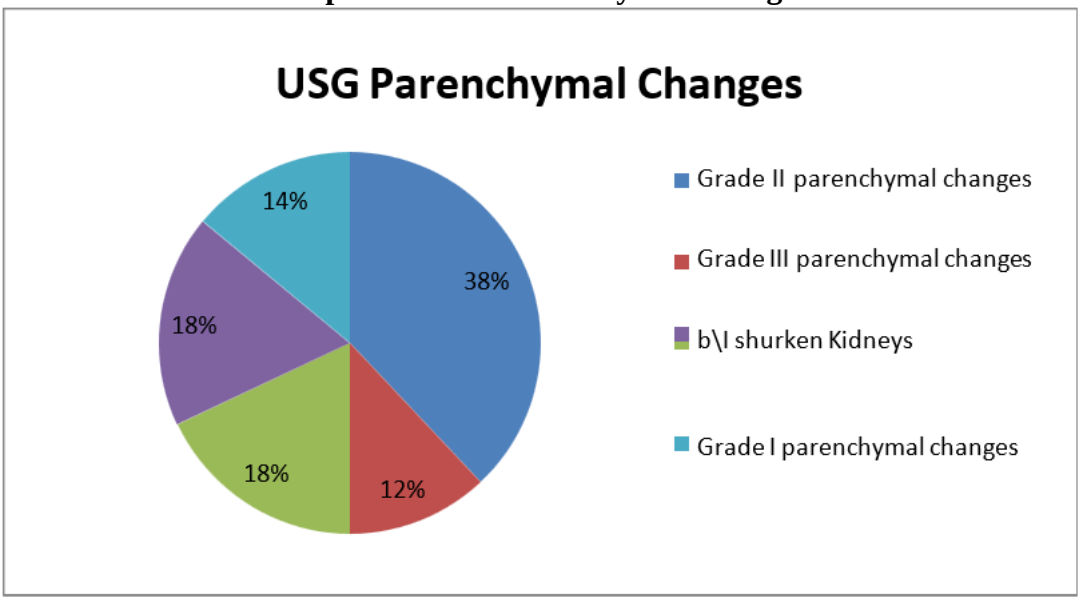

Table &Graph: 12 Grade II parenchymal changes seen in 38 patients (38%).Grade III parenchymal changes in 12patients (12%).b\I shurken Kidneys in 18(18%).Grade I parenchymal changes seen in 18 patients (18%).Normal parenchymal seen in 14 patients (14%)

Table :13 Comparsion of Sex Distribution In Diabetic And NonDiabetic Nephropathy Patients

			DM		Total
			Non Diabetic nephropathy	Diabetic nephropathy	
Sex	Male	Count	45	17	62
		% within DM	65.2%	54.8%	62.0%
	Female	Count	24	14	38
		% within DM	34.8%	45.2%	38.0%
	Total	Count	69	31	100
		% within DM	100.0%	100.0%	100.0%

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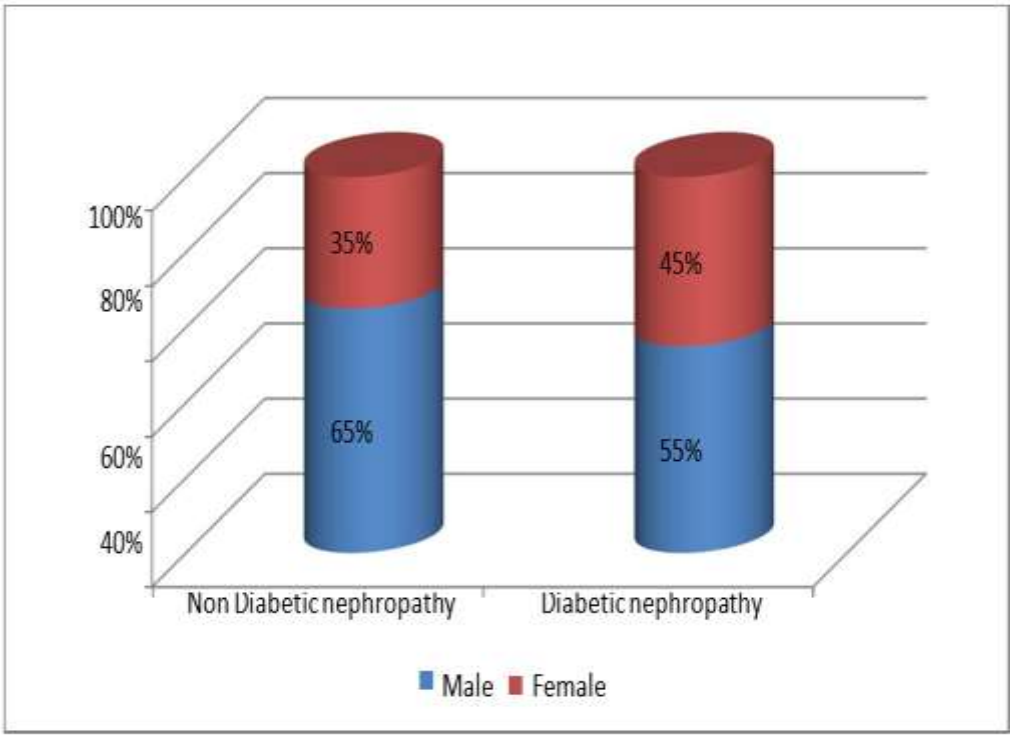
Graph :13 Comparison of Sex Distribution In Diabetic And NonDiabetic Nephropathy Patients



Pearson Chi-Square=0.978 p=0.323

Table &Graph: 13 In 100 patients 62 patients were male and38females.Male non-diabetic patients were 45(65.2%) male diabetic nephropathy patients were 17 (54.8%) female non-diabetic patients were 24(34.8%) female diabetic nephropathy patients were 14 (45.2. Male diabetic nephropathy patients were more in our study which correlates with increased duration of diabetes . %) Pearson Chi-Square=0.978p=0.323 were statically more significant

Table :14 Shows Comparsion Of Diabetic Nephropathy With Age,Creatinine,Creatinine Clearance

Group Statistics							
	DM	N	Mean	Std.Deviation	Std. ErrorMean	t value	Pvalue
Age	Non-Diabetic Nephropathy	69	51.5362	17.82726	2.14615	0.914	0.363
	Diabetic Nephropathy	31	55.0323	17.36372	3.11862		
creatinine	Non-Diabetic Nephropathy	69	9.2478	3.41471	.41108	2.480*	0.015
	Diabetic Nephropathy	31	7.3326	3.90280	.70096		
CCL	Non-Diabetic Nephropathy	69	13.7570	5.13656	.61837	0.233	0.816
	Diabetic Nephropathy	31	14.0313	6.09128	1.09403		

In non diabetic nephropathy 69(13.75) when compared to diabetic nephropathy 31(14.03) The patient in whom diabetic nephropathy was the cause of chronic kidney disease were of an older age group and were having better creatine clearance compared to non diabetic etiology.off pvalue (0.816)

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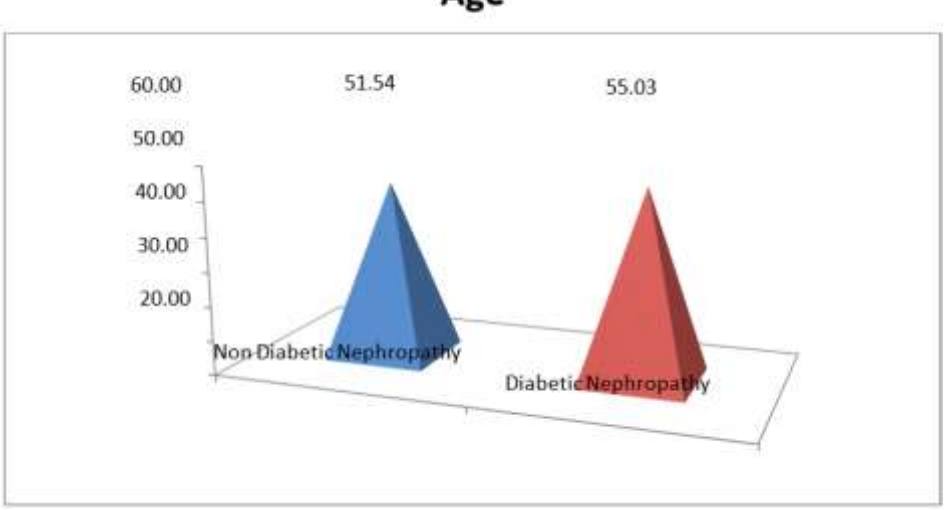
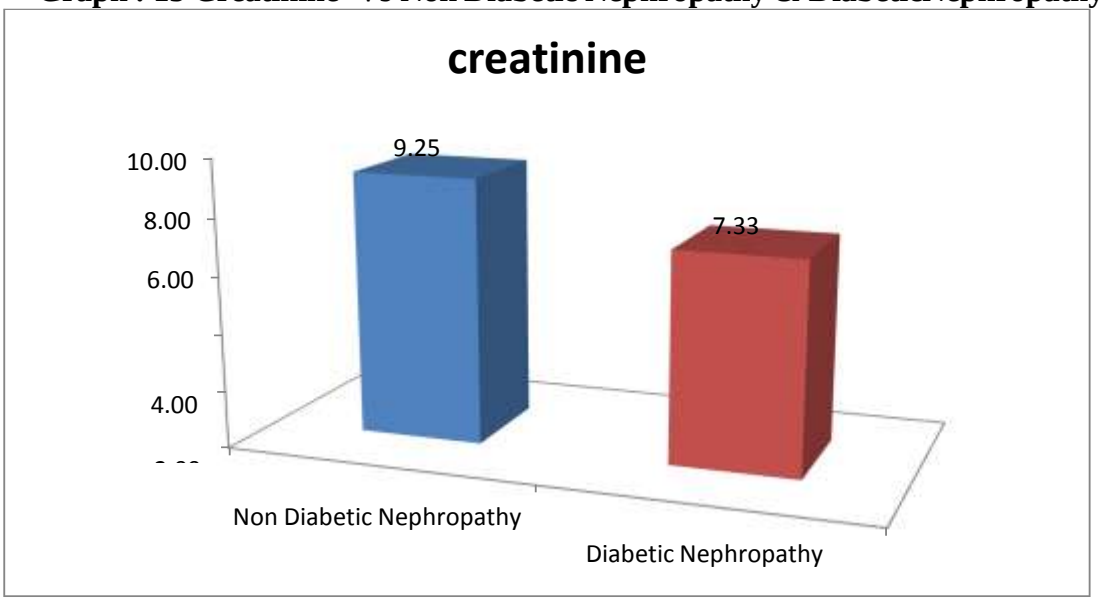
Graph : 14 Age Vs Non Diabetic Nephropathy & DiabeticNephropathy



Graph : 15 Creatinine Vs Non Diabetic Nephropathy & DiabeticNephropathy



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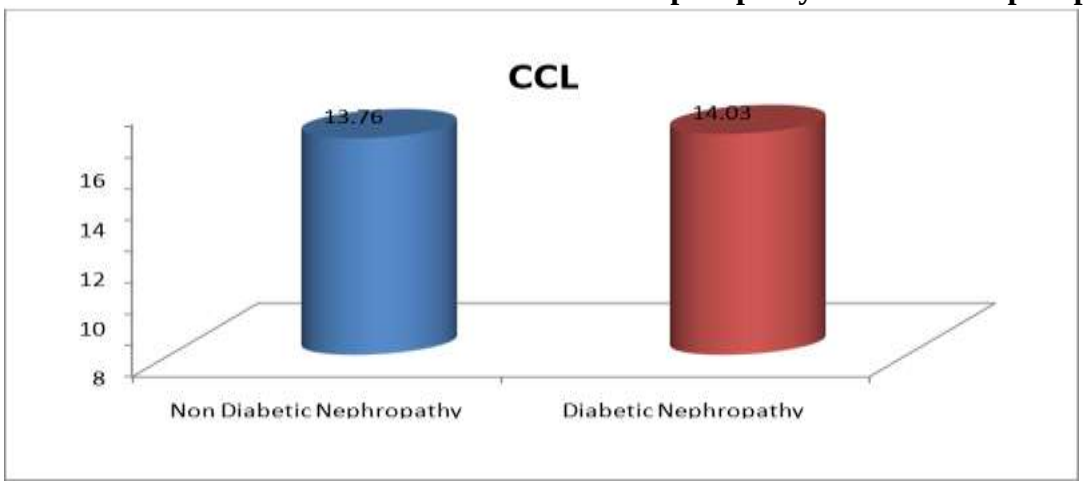
Graph : 16 Creatinine Clearance Vs Non Diabetic Nephropathy &Diabetic Nephropathy



To examine the differential expression of key genes of TGFβ-Smad pathway in chronic kidney disease.

Expression of TGFβ-Smad Pathway genes

Differential levels of TGFβ1 and TGFβR2 in CKD

To see if TGFβ1 was differentially expressed in CKD patients compared to healthy individuals, serum levels of TGFβ1 were evaluated in diabetic CKD patients and healthy controls. The levels were significantly elevated (1.2-fold) in patients as compared to non-diabetic healthy controls (p=0.020) (Figure9a).. The data indicates a trend of increased TGFβ1 levels in the serum of Ckd patient.

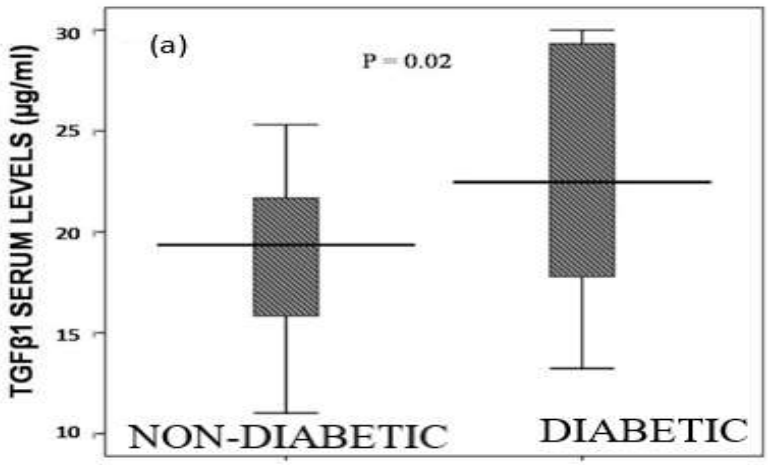


Figure9: Box-plot representation of (a) TGFβ1 serum levels in CKD patients and healthy controls (b) Comparison of TGFβ1 serum levels in Resistant CKD patient with Responders In the graph,

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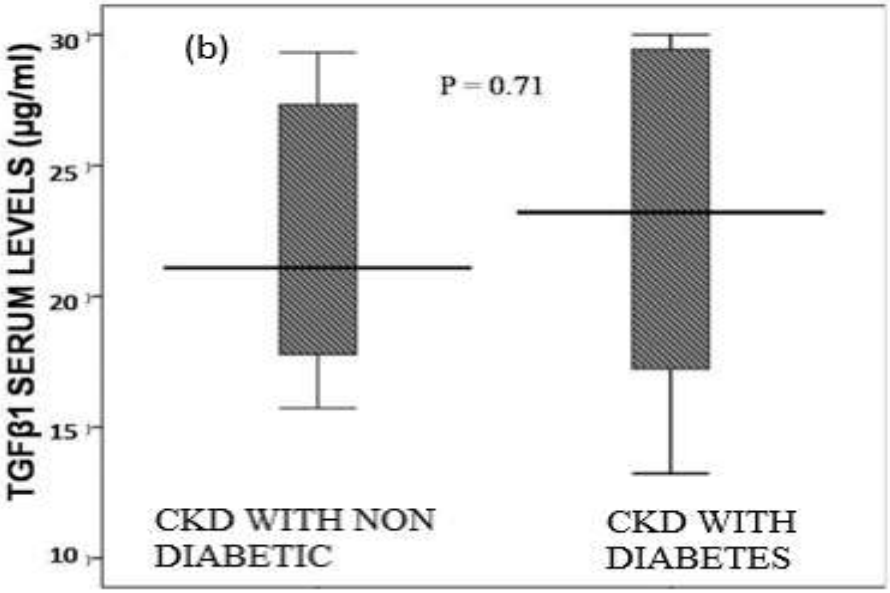
central line represents median, boxes represent 25th-75th percentile and whiskers indicate minimum and maximum values. p values <0.05 considered significant.



To determine if TGFβ1 receptors are differentially expressed in the blood cells of CKD patients, we evaluated RNA expression of TGFβR1 and TGFβR2 in Ckd patient and compared with healthy individuals. TGFβR1 transcript levels did not show any difference in Ckd patient as compared to controls (Figure10a, 10b). TGFβR2 expression was significantly low in Ckd patient, compared to healthy controls (p=0.012) (Figure10c). To see if there is any difference in the levels of TGFβR2 in response to IM, we further compared the levels in responders and resistant patients. However, we did not observe any significant difference of TGFβR2 levels in the two groups (Figure10d).

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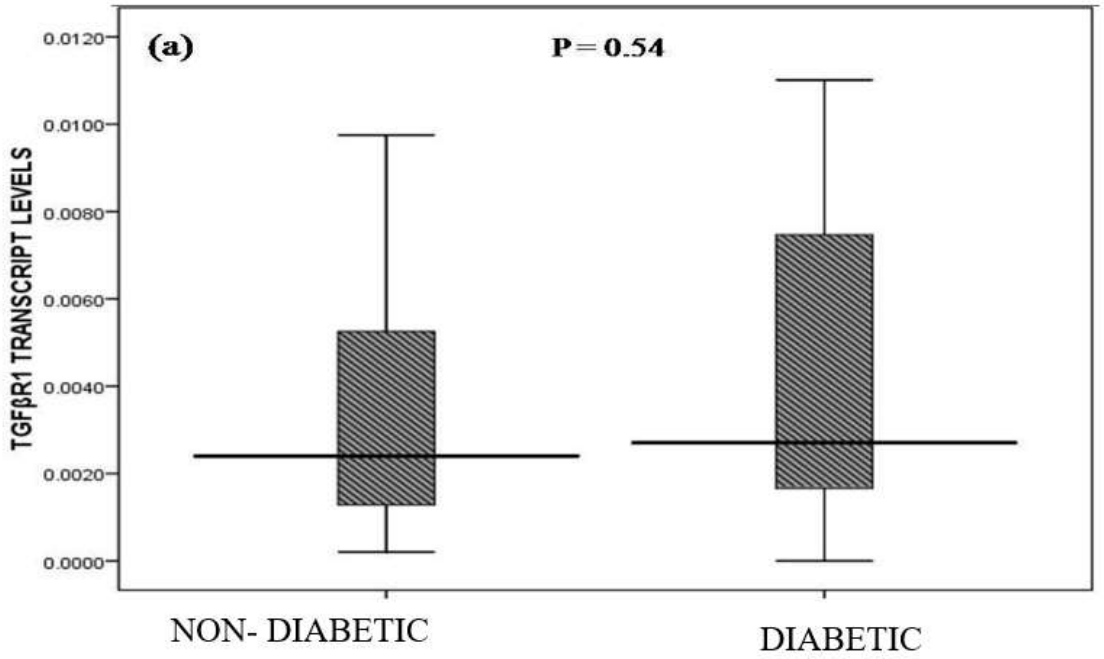

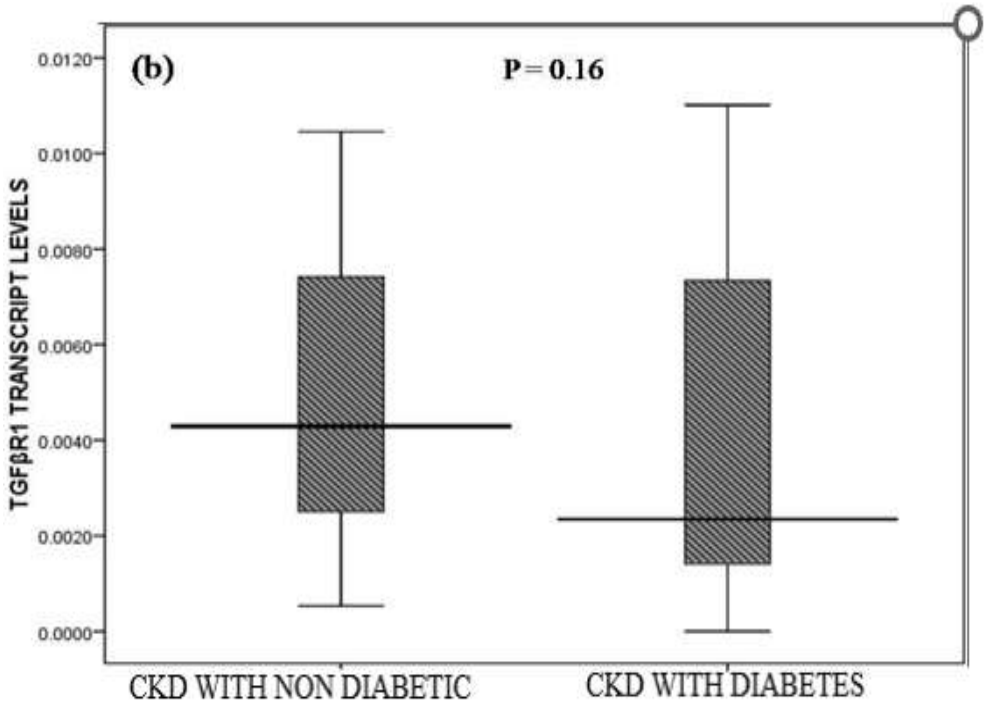

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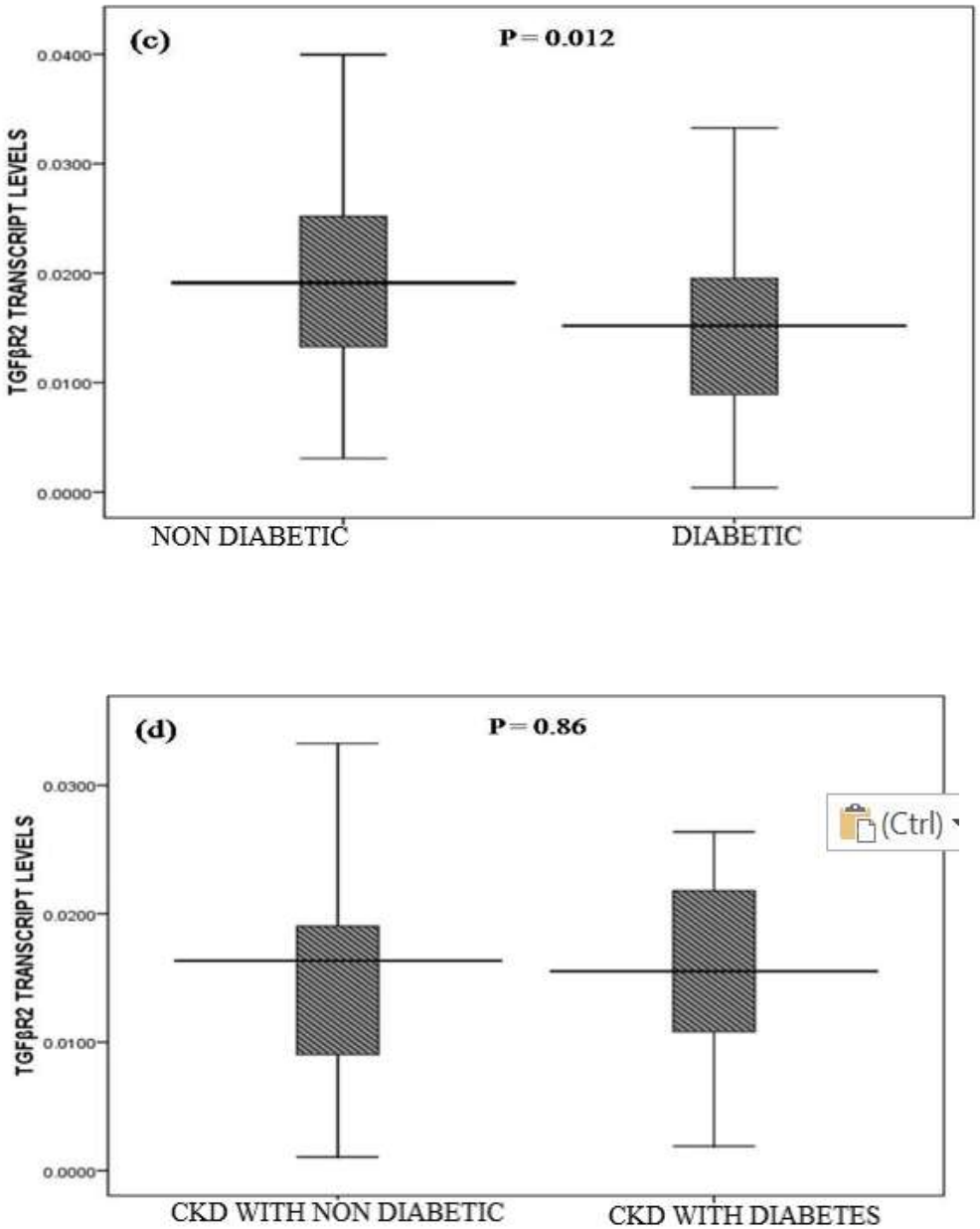

Figure10: (a) Comparison of TGFβR1 transcript levels in diabetic patient with healthy controls and (b) Comparison of TGFβR1 transcript levels in CKD patients with diabetic and non-diabetic patient (c) Transcript levels of TGFβR2 in in diabetic patient with healthy controls (d) Comparison of TGFβR2 transcript levels in CKD patients with diabetic and non-diabetic patient 25^{th}-75^{th} percentile and whiskers indicate minimum and maximum values. p values <0.05 considered significant.

Reduced levels of SMAD4 in CKD

Since SMAD4 is a co-SMAD downstream of TGFβR2, we examined the transcript levels of SMAD4 in CKD patients along with healthy, Diabetic and non-diabetic individuals. SMAD4 RNA transcript levels were significantly reduced as compared to controls (p=0.043) (Figue11a).

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However, no major difference was observed in SMAD4 expression levels between IM resistant and responding patients (Figure11b).

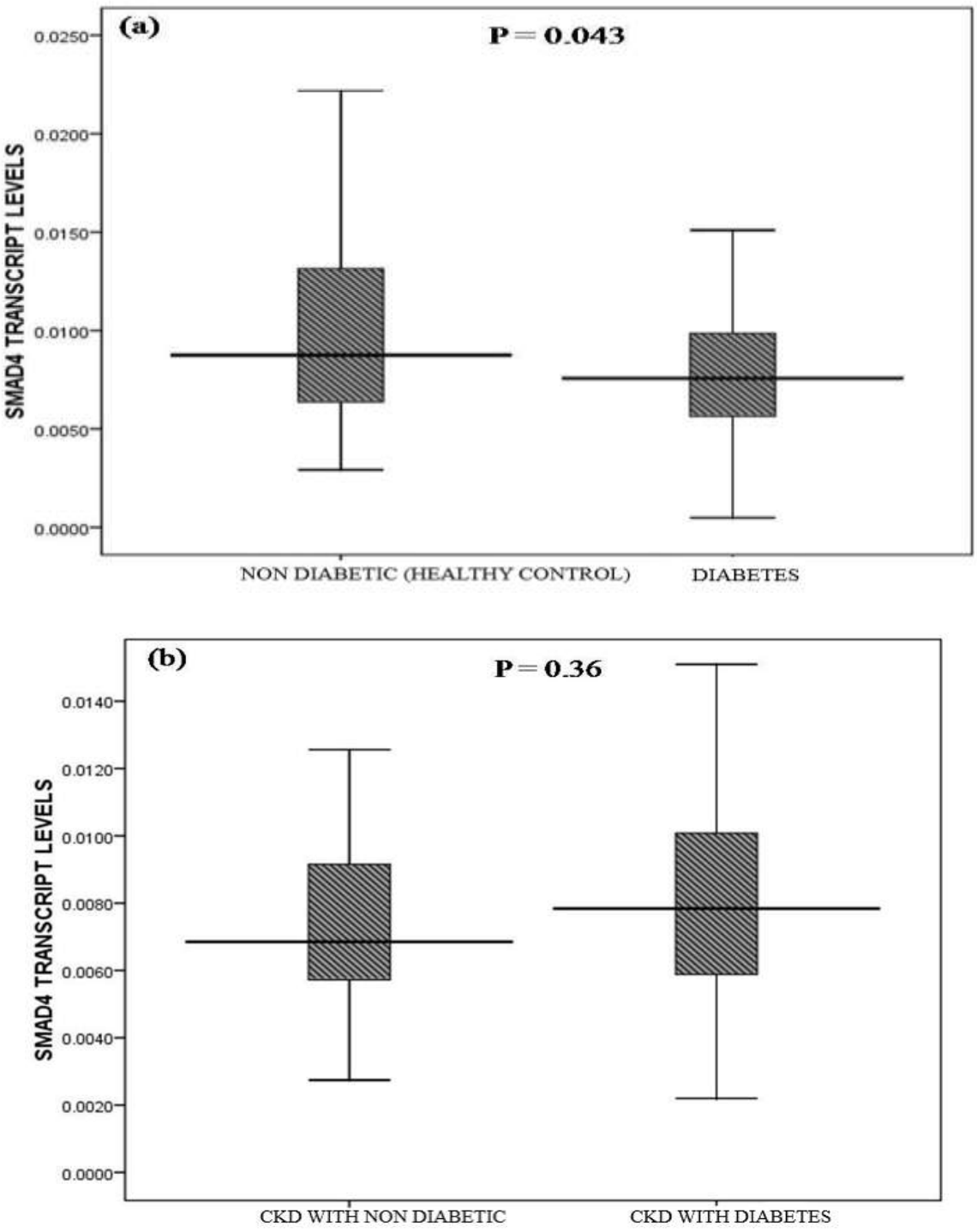

Figure11: Transcript levels comparison of SMAD4 gene between (a) Diabetic patients and healthy controls, (b) ckd with diabetic and non-diabetic patients. In the graph, central line represents

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median, boxes represent 25th- 75th percentile, and whiskers indicate minimum and maximum values. p values <0.05 considered significant

DISCUSSION

4.1. TGFβ-Smad pathway in CML

CKD is diagnosed by the presence of BCR-ABL gene and treated by Imatinib mesylate (TKI) in first line setting. Alterations in BCR-ABL dependent and independent pathways are the cause of resistance to IM in CKD(Hamad A, et al, 2013). TGFβ-Smad is one of the key BCR-ABL independent pathways, which has been extensively studied in normal and abnormal hematopoiesis (Kim SJ, et al, 2003). Alterations in this pathway have been implicated in lymphocytic (DeCoteau, JF, et al, 1997) and myeloid leukemias (Le Bousse-Kerdiles MC, et al, 1996) but its role in CKDis not well established so far. TGFβ-Smad signaling is known to increase the hyper-responsiveness of CKDcells leading to better response through BCR-ABL inhibition (Møller GM, et al, 2007). Though, this pathway inhibits the activation of AKT, which is a downstream component of BCR-ABL pathway, leading to release of inhibitory sequestration of FOXO that promote quiescence in CKDstem cells, ultimately resulting in TKI resistance (Zhu X, et al, 2011; Naka K, et al, 2010). Present study attempted to explore more direct links between alterations in TGFβ-Smad signaling pathway and Ckd patient. TGFβ1, cytokine, is a strong inhibitor of progenitor cell growth and differentiation, and its autocrine production maintains immature hematopoietic progenitor cells in quiescent state. Significant elevation was observed in TGFβ1 serum levels in CKDpatient group as compared to controls group. Higher levels of TGFβ1 have been observed in hematological malignancies (Liu X, et al, 2013) and solid tumors (Ciftci R, et al, 2014; Choi YJ, et al, 2015), which corroborate with our findings. Circulating TGFβ1 protein concentration levels were associated with mutation c.29C>T (rs1800470) in exon 1 of TGFβ1 gene (Wong TY, et al, 2003; Singh P, et al, 2013). We discovered this mutation in 50% of patients of the cohort selected for sequencing. Interestingly, elevated TGFβ1 levels were observed in 3 patients (serum levels available) harboring this mutation, though due to small number, the correlation between serum levels and 29C>T mutation couldn't be clearly demonstrated in our study. It lies in the conserved region and expected to be damaging by in silico analysis. It is speculated that Proline to Leucine (P10L) change modifies the peptide polarity, leading to change in protein transport rate (Wood NA, et al, 2000). We are the first to report this mutation in CKDto the best of our knowledge. A recent in-vitro study suggest BCR-ABL expression enhance TGFβ1 levels and TGFβ signaling activity in CKDcell lines (Smith PG, et al, 2012), which prompted us to inquire whether increased serum levels in our cohort are also leading to increased signaling activity. Evaluation of TGFβ1 receptor transcript levels showed significantly reduced TGFβR2 expression, which probably hamper tumor suppressive effect of TGFβ1 in Ckd patient. The finding was similar with an earlier study, where decreased TGFβR2 levels were reported in Ckd patient compared to healthy individuals (Rooke HM, et al, 1999). The attempt to correlate the reduced transcript levels with genetic mutations in our cohort couldn't reveal significant observation as no mutation was present in enough number of patients to suggest such association. However, some important genetic variants were observed in TGFβR1 gene. Genetic variant, c.69A>G (rs868), present in 3' UTR TGFβR1, was found in 20% (4/20) patients. In silico analysis of this variant shows the mutation site to be the target for miRNA Let7f/miRNA98 (Figure13c). The Let7f/miR98 family is known to reduce TGFβR1 expression during embryogenesis and mutation in the binding region of this miRNA further reduces expression of gene (Tzur G, et al, 2009). Analysis of transcript levels in 4 patients having this

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mutation demonstrated reduced TGFBR1 transcript level, however no significant change in expression was observed in overall patient group (Figure13b). Out of these 4 patients, 3 were IM resistant and showed first relapse after consuming standard dose (400mg O.D.) of Imatinib Mesylate for 6 years or more. The fourth patient harboring this variant completed sixth year of standard IM treatment and was a good responder till the time of sample collection (Figure13b). Correlating this finding with clinico-demographic characteristics, this variant may play a role in late relapse. Though this claim requires concrete evidence in a larger cohort, the hint is worth attracting the attention. Another variant, c.1024+24G>A (rs334354) in intron 6 of TGFβR1, discovered in 40% (8/20) of our patients is an established genetic marker for increased susceptibility for cancer (Liu X, et al, 2013; Wu W, et al, 2015).

SMAD4, is key component of TGFβ-Smad signaling and an important marker in Colorectal cancer (CRC). Down regulation of SMAD4 in CRC is due to increased miRNA responsible for its controlled expression (Liu L, et al, 2013). SMAD4 deficient cells were observed in malignancies of diverse origins like oral epithelial cells, keratinocytes, mammary cells, bile duct, and odntoblasts (Bornstein S, et al, 2008; Qiao W, et al, 2006; Yang L, et al, 2005; Li W, et al, 2003; Xu X et al, 2006; Gao Y, et al, 2009) and leukemic cells of Chinese patients (Zhang Y, et al, 2006). Our study findings also revealed significantly reduced SMAD4 levels along with low TGFBR2 levels. SMAD4 is essential for the formation of heterologous complex with SMAD2 and SMAD3 and its translocation into the nucleus for expression of target genes. Its low expression can be another potential reason for containment of this tumor suppressor pathway. Ckd patient have elevated TGFβ1 serum levels and c.29C>T is the major genetic variant among TGFβ1 gene mutations. Lower transcript levels of TGFβR2 can be the possible reason of decreased signaling activity that abolishes the tumor suppressor effect of the increased TGFB1 levels. Though no significant change in the transcript levels of TGFBR1 was observed in patients compared to control, TGFBR1 levels were reduced in the patients with c.69A>G variant. We also reported low levels of SMAD4 in CML. Previous studies have also reported similar findings in various other cancers including hematological malignancies such as acute myeloid leukemia and T-cell lymphoma (Singh P, et al, 2011; Go JH, et al, 2008). Although our results are encouraging but being a single centric study sample size was the limitation. Multi-centric studies with more number of patients and detailed research on TGFβ - SMAD signaling pathway in different CKD models is required to substantiate our findings.

CONCLUSION

Chronic kidney disease is the irreversible condition whichprogresses relentlessly leading sooner or later to the end stage renal failure. The diagnosis of this stage can be achieved by eliciting the history carefully, discovering co-morbid factors, utilizing imaging techniques, interpreting histological material and placing this in the context of probability derived from epidemiological data. Screening of high risk individuals- those with hypertension, diabetes mellitus, cardiovascular and other risk factors, lifestyle modification, physical exercise, abstinence from smoking will retard the progression to ESRD This will help in bringing down the huge burden due to mismatch between demand and availability of resources for renal replacement therapy in developing countries like India, especially for patients belonging to lower socioeconomic group. The assessment of clinical profile of these patients showed the most common aetiology as diabetes mellitus (36.9%). Hypertension being a cause and a complication of CKD was present in 64.6% of patients. Early detection and effective management of these illnesses can delay the onset, progression of CKD and subsequent morbidity and the requirement of renalreplacement therapy, if any. Another manageable condition obstructive uropathy found in 10% of these patients, if treated at an early stage prevents

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progression to irreversible kidney damage. Cardiovascular disease in Chronic kidney disease is more common in the presence of Diabetes mellitus, Hypertension, hypernaterima, increased kidney size, hypoalbuminuria In patients with anemia, cardiovascular disease was more common when hemoglobin levels were less than 5g/dl. Cardiovascular diseases as a morbidity was identified in 28.5 % patients. Being the leading cause of mortality in CKD it would be imperative to monitor patients for this morbidity Cardiac structural as well as functional abnormalities are common in patients with ESRD, more so in those with hypertension and anaemia. LVH is the commonest cardiac abnormality in ESRD patients, followed by diastolic dysfunction. Bothconditions are more marked in hypertensive patients and anaemic patients.LVH has got prognostic implications, because this group of ESRD patients have propensity of diastolic dysfunctionor sudden cardiac death

SUMMARY

Chronic kidney disease is definedas abnormalities of kidney structure or function, present for>3 months, with implications for health. Kidney damage refers to a broad range of abnormalities observed during clinical assessment, which may be insensitive and non-specific The present Descriptive study was done in the Department of General Medicine in collaboration with the nephrology department at Karpaga Vinayaka institution of medical sciences. The study was carried out during the period of October -2017 to June -2019 (20months) . Totally 100 patients were included in the study who diagonized CKD and staging The age group distribution was 20years – 70 years above the patients are included in the study. Age group : 20-30 Years 15 patients (15%) 31-40 Years 16 patients(16%) 41-50 Years 13 patients (13.%) 51-60 Years 20 patients (20%)61-70 Years 15 patients (15%) Above 70 Years 21 patients (21%) Gender distribution Among 100 cases of CKD, there were 62 (62%) male patients and 38 (38%) female patients. The ratio of male to female was 2.63 i.e. males are 2.63 times more susceptible to CKD when compared to females. In 100 cases the differential diagnosis were analysed Diabetic nephropathy was in 31(31%)Interstitial kidney disease was in 19(19%) Polycystic kidney disease diagnosed in 17 patients whichwere (17%) Kidney stones in different types seen in 10 patients (10%) Glomerulonephritis in 12 patients (12%) Hypertension nephropathy in 11(11%). Diabetic nephropathy was more common in the study which was statistically more significant of p-value <0.005> The presences of various symptoms observed in 100 patients are presented in the above table. We see that 79%of the cases had pedal edema followed by the most common urinary symptom Oliguria that is 73%. The Gastrointestinal symptom namely anorexia is found in 33 cases. 28% had general weakness and 44% were having vomiting as a symptom. The numbers of cases having facial edema were 25 and 70% of the cases exhibited breathlessness as a symptom. Puffiness of face 62 (77.50%), swelling over feet 58 (72.50%) and breathlessness 56 (70.00%) were the next predominant symptom. Nausea and vomiting were present in 54 (67.50%) and tingling and numbness of extremities were complained by 40 (50.00%) patient. Joint pain 24 (30.00%), were the other less common presenting symptom in this study.

Blood pressure was recorded by standard method. systolic blood pressure was <120 in 1patients which was (1%) systolic blood pressure between 120-139 in16 patients which was(16%) Above 140-159 were observed in 20 patients were (20%) Above 160 were observed in 63 patients (63%) most of the patient were hypertensive in CKD due to decreased renin release which was statistically more significant of p-value <0.005>

Diastolic blood pressure was <80 seen in 6 patients(6%) diastolic blood pressure between 80-89 observed in 26 patients (26%) Above 90-99 was in 43patients(43%)were observed in 43 patients Above 100 were observed in 25

patients (25%) most of the patient were hypertensive in CKD due to decreased cardiovascular stability which was statistically more significant of p-value <0.005>

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39% of the patients have their hemoglobin level in the range of 5-10 gm%. Only 4% of the patients have their value below 5mg%, but 57% of the patients exhibit that their hemoglobin level more than 10 mg%.28% of patients have Hyperkalemia. 60% had the value within normal limits (3.5-5 meq/1). Only 12% had the value of less than 3.5 meq/1. hyponatremia (Serum sodium level < 130 meq/1) is present in 29% of patients. Further, in 71% cases, this value lies between the normal limits (130-143 meq/1). Hypoalbuminemia (Serum Albumin < 3.5g/dl) can be seen in 68% of cases. 32% of cases have this value within normal limits (3.5 - 5 g/dl). 61% of the cases seem to have decreased kidney size and 29% appears to have an increased kidney size. Whereas 10% of the patients have increased normal size.

Echo Cardiogram Was Not done in21patients (21%) LVHwas present in 41 cases (41%).Ischaemic dilated cardiomyopathy, Hypokinesia of wall or septum is seen in

12 patients (12%). Diastolic dysfunction.8 patients (8%). Normal findings in echocardiography were in14 patients (14%) Pericardial effusion was seen in 4patients (4%) LVH was more common among CRF patients as it reflects on cardiac efficiency

Grade II parenchymal changes seen in 38 patients (38%). Grade III parenchymal changes in 12 patients (12%).b\I shurken Kidneys in18(18%).Grade I parenchymal changes seen in 18 patients (18%).Normal parenchymal seen in 14 patients (14%)

In non-diabetic nephropathy 69(13.75) when compared to diabetic nephropathy 31(14.03) The patient in whom diabetic nephropathy was the cause of chronic kidneydisease were of an older age group and were having better creatine clearance compared to non-diabetic etiology. off p value (0.816)

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