Diabetic Nephropathy a Major Cause of Renal Failure

Thesis submitted to

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in partial fulfilment of the requirements

for the degree of

Master of Science
in
Clinical Biochemistry

Submitted by
Ms. Jaspreet Kaur
(Regd No. 11304952)

Submitted to
Mr. Rajesh Prasad Jayaswal
Lecturer

LOVELY SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA

May 2015

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Certificate

This is to certify that the present thesis entitled "**Diabetic nephropathy a major cause of renal failure**" is the outcome of the original piece of work carried out by Ms. Jaspreet Kaur (Registration No: 11304952) herself under my guidance and the contents of his thesis did not form a basis of the award of any previous degree to him and to the best of my knowledge to anybody also. The thesis has not been submitted by the candidate for any research degree in any other University.

The dissertation is fit for submission to the partial fulfilment of the conditions for the award of M.Sc. in Clinical Biochemistry. Further certified that the candidate in habit and character is a fit and proper person for the award.

(Mr. Rajesh Prasad Jayaswal)

Supervisor

Certificate

This is to certify that **Ms. Jaspreet Kaur** student of **M. Sc (Clinical Biochemistry),** Lovely Professional University, Punjab has completed her dissertation entitled "Diabetic Nephropathy a major cause of renal failure" from Civil Hospital, Sangrur under mu guidance.

During this period she was found to be sincere and hard working.

I wish her best of luck in her carrier.

30th April 2014

Sangrur (Punjab)

MD Pathologist

DR. INDERJIT SINGLA
MBBS, MD, MO,
PCMS-I
PMC Regd. No. 26765

Declaration

I hereby declare that work embodied in this thesis was carried out by me under the direct supervision of Mr. Rajesh Prasad Jayaswal, Lecturer. This work has not been submitted in part or in full in any other university for any degree or diploma.

Place: Department of Paramedical Sciences
Lovely Professional University, Punjab

Jaspreet Kaur
(Reg. No. 11304952)

Date

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Jaspreet Kaur

List of Contents

Abstract

Diabetes represents serious health problems worldwide. Diabetes causes complications like diabetic nephropathy. Diabetic nephropathy is microvascular complication that causes glomerulosclerosis and leads to end stage renal disease. Diabetic nephropathy characterized by albuminuria and about 20%-30% patients develop nephropathy due to diabetes. After 5 to 15 years onset of diabetes patient develop diabetic nephropathy and after 15 to 25 years diabetic nephropathy converts to overt nephropathy. This study is conducted to see patients how much individuals develop diabetic nephropathy due to diabetes and to see gender difference. In this study 75 diabetic individuals are involved and 75 diabetic individuals tested for kidney disease. This study shows 22 individuals having kidney disease due to diabetes. This shows 29% diabetic patients develop kidney disease. Gender difference in this study shows 17.3% male diabetic individuals develop kidney disease and 12.00% females having kidney disease due to diabetes. From 22 kidney disease individuals 59.09% males and 40.90% females effected with renal disease. This shows great variation between males and females.

CHAPTER 1

Introduction

1.1 Diabetic Nephropathy

Diabetes represents one of the most important health problems worldwide (1). As according to epidermiological studies, 285 million people affected with diabetes worldwide. Diabetes increases day by day worldwide especially in the developing countries. In India about 50 million people suffer from diabetes. In 2030 no of patients with diabetes expected to be 87 million. Diabetes causes macrovascular and microvasular complications (2-3). Diabetic nephropthy is microvascular complication and after diabetes this develops after some years. Diabetic nephropathy is major cause of end stage renal disease that characterized by glomerulosclerosis and kidney interstitial tissue that leads to renal failure (4). Approximately from all diabetic patients one third of patients are affected by DN and number of persons with kidney failure who are treated with dialysis and transplantation is projected to increase from 340 000 in 1999 to 651 000 in 2010(1, 5). About 20-30% patients develop diabetic nephropathy from diabetic patients. Due to longer hyperglycemia, filtering system of the kidney were destroyed, results leakage of large proteins results excretion of albumin in urine. Diabetic nephropathy is many characterized by albuminuria (6). Cotunnius in 1770, Rollo in 1798 find that some diabetic patient's urine contains proteins. Led Rayer in 1840 postulates that some diabetic patients having "Bright's disease". In 1836 Guy's Hospital, Bright gives that albuminuria is a major sign of renal disease. According to WHO, from 1999 to 2005 more than 44% ESRD due to diabetes (7-9).

1.2 Urinary Albumin Excretion(UAE)

Patient with diabetic nephropathy shows UAE and also higher urinary albumin excretion rate (AER) (stage-1). Insulin treatment given to the patient, within 3 to 6 months it shows deceased level and within 1 to 2 years it shows normal level of AER. During poor control, AER temporary rises. In diabetic patients it rises after 5-15 years and AER value 20-200ug/min defined as microalbuminuria and value more than 200ug/min defined as microalbuminuria. Excretion of albumin after 5 to 15 years diabetes shows a sign of glomerular damage and is called incipient nephropathy(stage 3)(10). AER shows variation and microalbuminuria continuously for 3 months also 20% increase in AER per year shows complications (11). After 15 to 25 years of diabetes nephropathy converts to overt nephropathy (Stage-4) (12).

1.3 Glomerular Filtration Rate and Renal Plasma Flow Rate

GFR, RPF are higher in diabetic patients (stage-1). Insulin treatment given to these patients and level of GFR, RPF become normal with weeks to months. About 25-40% cases GFR level raised (stage-2, 3) (13-14). In third stage also level become similar. According to some studies GFR become loss 10-12ml/year. After the onset of 6-10 years microalbuminuria patient reached to ESRD or died (15-17).

1.3 Urea

Non-protein nitrogenous substances measure the renal function. Urea is non-protein nitrogenous substance and almost half of the total constitutes. In liver, it is synthesized from ammonia and carbon dioxide, also from destruction of amino acids. Urea is protein metabolism's excretory product. Plasma carries the urea to the kidney and then filtered by glomerular from plasma. Filtered urea reabsorption done in renal

tubules, about 40% urea reabsorbed. Filtrate urea excreted in the urine and some amount also by skin (18).

1.4 Creatinine

Creatinine is non-protein nitrogenous substance. Metabolism of Creatinine is in the skeletal muscles. Creatinine filtered by the kidney and excreted out through the urine. Creatinine values varies with the gender because males having more muscle mass and due to more muscle mass, more metabolism of Creatinine and it shows variation between males and females (19, 20).

1.5 Natural History of Diabetic Nephropathy

Nephropathy in diabetes described as stages from renal function to end stage renal failure (ESRD). ESRD shows excess amount of albumin in urine (21). At the initiation of diabetic nephropathy, no renal histologic abnormalities has been diagnosed but elevated levels of renal plasma flow (RPF) and glomerular filtration rate (GFR). After 3 years, evident of histologic changes like increased mesengial matrix, glomerular basement membrane thickening and GFR, GRPF same elevated. Within 10 to 15 years of diabetes renal hyperfilteration persists and macroalbuminuria (more than 300mg/day) is detected but normal rate of GFR and RPF. Elevated GFR and RPF returned to normal show the sign of renal insufficiency. After the 15 years of diabetes and 5 years of albuminuria shows 50% reduction of GFR and raised values of Creatinine. About half of these patients go to End Stage Renal Disease (ESRD) after 2 to 4 years. Renal insufficiency also not pervaded by glycemic control (21, 22).

In the early 1980's independent investigations reported that excretion of albumin in urine is not detected by standard laboratory methods and is termed as albuminurea (23). Excretion of albumin by normal person is 10 to 15 mg/day. 30 to 300 mg/day is termed as microalbuminuria. Microalbuminuria detected by using Radioimmunoassay, ELISA techniques are highly accurate techniques for detection (24-26). Microalbuminuria to diabetic nephropathy prevented by tight glycemic control with insulin (27).

1.6 Pathogenesis of Diabetic Nephropathy

1.6.1 Hemodynamic Changes

(Increase of systemic and intraglomerular pressure)

Pressure increases due to activation of hormones rennin, angiotensin, aldosterone (RAAS) especially renal hormone angiotensin II (Ag II). Activation of renal hormone by mesangial cells (28). Mesangial matrix overproduction enhanced by transforming growth factor beta(TGF-β). Hyperglycaemia activates prostanoids, endothelial nitric oxide, vascular endothelial growth factor (VEGF) (28, 29).

1.6.2 Metabolic Pathway

1.6.2.1 Advanced glycation products (AGEs)

Due to Hyperglycemia causes protein present in matrix glycosylated non-enzymatically and change to AGEs and irreversible products. Injury cause by mesangial cells because AGEs attach to AGEs receptors (30).

1.6.2.2 Activation of PKC

Hyperglycemia causes activation of PKC, over production of PGE2, AGEs and NADPH depletion because conversion of glucose to sorbitol in mesangial cells (31).

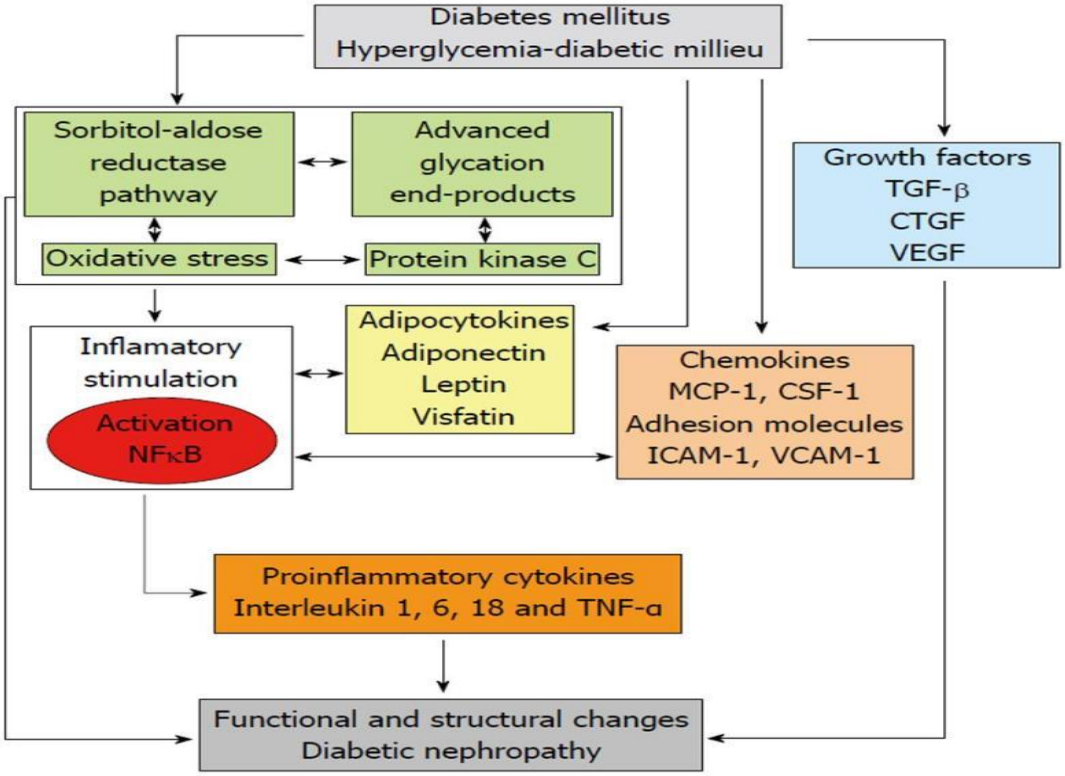


Fig 1. Different pathway shows hyperglycemia leads to diabetic nephropathy

1.6.2.3 PKC (Activation ok protein kinase C)

Hyperglycemia forms PKC and via TGF-β, VEGF, ROS, AGLL activate mesengial expansion (32).

1.6.3 Oxidative Stress

Hyperglycemia causes overproduction of Reactive Oxygen species (ROS) and superoxide anions. ROS causes renal vasoconstrictors, DNA damage, membrane peroxidation, protein oxidation and activation of NF-κβ, PKC, AGEs and TGF-β (33). In mesengial cells oxidative stress also enhanced by AgII, oxidized LDL, Aldosterone, Amino Acids and serotonin (34).

1.6.4 Apoptosis/ Mesangiolysis

Hyperglycemia causes matrix overproduction by mesangial cells and some mesangial cell also undergoing apoptosis (35).

1.6.5 NADPH Activation

Diabetes causes to renal oxidative stress by enhancing the function of NADPH oxidase and also enhances the function of fibrinonectic and collagen- 1 that enhance the mesangial expansion (36).

1.6.6 JAK/ STAT Pathway (Janus kinase / signal transducers)

In glomerular mesangial cells hyperglycemia causes TGF-β and fibronectic synthesis and due to hyperglycemia JAK-2, STAT-1, 3, 5 activate (37)

1.6.7 Adenosine (A) and Adenosine receptors (AR)

In hyperglycemia enhanced A1-AR, A2a-AR protein levels via mRNA expression of A1-AR (38). In kidney A2a-AR gives protection during hyperglycemia and in renal glomerular VEGF expression induces via activation of A2b-AR (39, 40).

1.6.8 Peroxisome Proliferator Activated Receptors (PPARs)

PPARγ decrease the TGF-β but suppressing and also suppress the VEGF, PAI-1, Type-IV collagen and in glomerular finrorectic to prevent the development of diabetic nephropathy (41-43).

1.6.9 Dopamine

During diabetes level of dopamine increases in kidney, so it suggests dopamine play a role in pathogenesis of DN (44).

1.6.10 Activation of growth factors VEGF, CTGF, TGF-α and cytokines (33, 34)

1.7 Inflammation

Diabetes also causes inflammation. Diabetes cause's hemodynamic abnormalities (resulting from systemic and intraglomerular hypertension, altered shear stress and mechanical strain), metabolic dearrangement (hyperglycemia, hyperlipidemia, formation of advanced glycation end products) and more synthesis of hormone angiotensin II. Inflammation, oxidative stress and fibrosis are key links of DN. Increased synthesis of Angiotensin II activates a pathological pathway in kidney cells (endothelial, mesengial, epithelial, tubular cells, and podocytes and causes fibrosis and is major feature of DN and appearance of inflammation is there (45).

In diabetic patients proinflammatory cytokines are also involved (46-48). These cytokines are related to UAE (urinary albumin excretion). Inhibition of inflammatory cells shows protection in DN. Cytokines are low molecular weight and are polypeptides with autocrime, paracrime and juxtracrime effects. Cytokines regulate the inflammatory and immune responses and are produced by throughout the body. Cytokines are produced by cells of varied embryological origin.

Proinflammatory and fibrogenic cytokines synthesized and secreted by these cells in the local microenvironment directly damage kidney architecture (49-50).

1.8 Treatment

Treatment depends upon the patient with declining GFR or not and individual suffering from microalbuminuria or macroalbuminuria. Epidermiological data of diabetic nephropathy show a relation between glucose and blood pressure (51-52).

1.8.1 Multifactorial Treatment

Multifactorial treatment given to patient suffering from T2DM and micro albuminuria. In this treatment targets are HbA1c <6.5%, systolic blood pressure <130mmHg, diastolic pressure <80mmHg, fasting cholesterol <4.5 mmol/l and triglycerides <1.7 mmol/l. Drugs are used that are inhibitors of RAS, aspirin and that are to lower to glucose level. This follow up decrease 60% in proteinuria and decrease

50% cardiovascular events (53). This follow up for 5 years helps to decrease the risk of ESRD (54).

1.8.2 Intensive Glycaemic Control

Diabetic nephropathy prevented by glycemic control is good example of pancreatic transplantation. Pancreas transplantation shows normalization of glucose level and repairing of glomerular lesions (55). This shows clear results in 10 years not clear results in 5 years (56). This helps to reduce microvascular injury and glycemic control with HbA1c level <7% (57). Glycemic control reduces microalbuminuria by 39% and macrovascular by 56% (58). People treated with this, in UKPKS shows development of microalbuminuria reduction 30% (59). Kumamotes study also shows the reduction in microalbuminuria and macroalbuminuria and this is smaller than UKPDS (60-61). Intensive glycemic control also helpful to the complications of ESRD by 65%, microalbuminuria by 9%, macroalbuminuria by 30% and progression of albuminuria 10% (62). Glucose lowering agents also have a hypoglycaemic affect and also helpful to protect the kidneys. For ex- Peroxisome proliferator activated receptor ɤ (PPARɤ) also have hypoglycaemia affect and also renal protective affect (63, 64). These agents increase the risk of bone diseases, heart failure, ischemic heart disease and cancer of bladder (65-66). Metformin, glucagon like peptide I, sulfonylurea drugs are glucose lowering drugs and affects of these drugs are not clearly shown.

1.8.3 GLP-1(Gastric Inhibitory Polypeptide)

GLP hormone levels increased by Dipeptidyl peptidase-4(DPP4) inhibitors and are helpful to lowering the glucose level. DPP-4 also enhances the other polypeptides like β-type natriuretic peptide, CXC chemokines receptor type 4. Some studies show that DPP-4 inhibitors effects on kidney are linked with peptides (67, 68). Sodium glucose cotransporter-2(SGL-2) reduces the reabsorption of glucose in kidney and helps to decrease plasma levels of glucose (69).

1.8.4 Management of Micro albuminuria

ACE, ARBs are the inhibitors of RAS. RAS helps in the reduction of blood pressure and also good improvement in albuminuria and also have Reno protective effects.
Macroalbuminuria reduced 60% with the use of ACE inhibitors and also support to renal albuminuria (70). ARBs inhibitors helpful to reduce the microalbuminuria and macroalbuminuria ACE inhibitors helpful in T1DM and ARBs helpful in T2DM. ARB inhibitors helpful to reduce the nephropathy by 70% and 38% reduction in microalbuminuria (71). ARBs inhibitors also helpful to conversion of microalbuminuria to normal albuminuria about 30% (72).

1.8.5 Macro albuminuria

RAS blockers are helpful for the treatment of macroalbuminuria. Patients with T1DM also suffer from higher pressure are treated with captopril and is helpful to reduce dialysis, transplantation (73). ESRD in T2DM patients with macroalbuminuria reduced by the use of ARBs (74, 75). Treatment with RAS blockade prevents the albuminuria in T1DM and T2DM patients (76). Combined treatments of RAS blockade, ACE inhibitors, ARBs are helpful to treat the hypertension and albuminuria. Some new agents are used to treat hypertension and albuminurias are rennin inhibitors, antagonists of mineral corticoid receptor and non-pharmacological

approaches (77). Combined treatments with RAS bloackade, ACE inhibitors, ARBs are not helpful to decrease the risk of cardiovascular diseases (78).
Dual RAS blockade are not advisable because they increase the risk of acute renal failure and are advisable only patients who have diabetes mellitus with macroalbuminuria (79-80).

1.8.6 Aliskiren

Aliskiren is rennin inhibitor that blocks RAS. In AVOID study, aliskiren decrease the albumin/Creatinine ration in T2DM patients and overt nephropathy (81). In one another study aliskiren failed to reduce renal disease, cardiovascular disease and these study individuals already taking RAS blockade (82).

1.8.7 Mineralocorticoid Receptor Antagonists

By the use of aldosterone antagonists, it helpful to reduce the diabetic nephropathy, it helpful to reduce the diabetic nephropathy. In one study almost 1000 participants are involved shows the reduction in proteinurea but increased hyperkalemia, no improvement if GFR observed (83). Treatment with aldosterone antagonist eplerenome, spironolactone are not given to severe diabetic nephropathy individuals because they need carefully monitoring for proteinuria and hyperkalemia (84).

1.8.8 Endothelin Antagonists

Endothelin is vasoconstrictor that helps to reduce blood pressure and protect renal via hemodynamic pathway. Avosentan, atrensentan are endothelin antagonists that protect renal by reducing albuminuria and atransertan shows better results as compare to avosentan (85, 86).

1.8.9 Renal Denervation

Few studies show that deservation gives treatment of hypertension (87). This also provides glycemic control in diabetic patients (88).

1.8.10 Lipids

Alteration in lipids shows the development of albuminuria and glomerulosclerosis (89). According to FixnDiane Cohort of patients increased triglycerides level associated with albuminuria and increased cholesterol associated with renal failure (90). Generation of reactive oxygen species and macrophage infiltration pathways linked to lipid pathway. Statins/fibrates are using in diabetic nephropathy patients as lipid lowering therapy. As compare to statin therapy fibrate therapy is more complex (91). Fibrates effect is related to PPARα agonist and not directly linked to lipid effect (63-64).

CHAPTER 2

Review of literature

Review of Literature

Mi Kyung Son et al. (2015) studied the regression and progression of microalbuminuria in adolescents with childhood onset diabetes mellitus. Main purpose of this study was to determine the frequency of regression and prevalence of microalbuminuria in T1DM and T2DM patients with childhood onset. The results show the regression of microalbuminuria was 56.5% in T1DM and 37.5% in T2DM patients. Progression of albuminuria was 10.5% and 20% in T1DM and T2DM. Microalbuminuria prevalence at baseline was 21.1% in T1DM and 44.4% in T2DM.

Researcher takes 109 adolescent in that 109 individuals of T1DM and 18 individuals of T2DM. Follow up period was 0.5-7.0 years and diabetes duration was 7.8 to 14.0 years. Lipid profile HbA1c, GFR estimated as growth parameters. These results suggest that carefully monitoring in adolescents helpful to prevent End Stage Renal Disease.

Priyanka raina et al. (2015) studied the association of Transforming Growth Factor Beta-1 genetic variation with Type 2 diabetes and End Stage Renal Disease in 2 large population samples from North India. 1313 samples were taken in which 776 samples from Punjab and 507 samples from Jammu and Kashmir. Using PCR, samples were genotyped for rs1800469 and rs1800470. rs1800469 increased ESRD risk 5.5 fold only in Jammu and Kashmir population not to Punjab. Rs1800470 increases 3.1-4.5 folds ESRD to the both population. ESRD risk increases 2-3 folds by haplotype C-T and 4 fold ESRD increases by diplotype CC-CT. This study shows the TGF-β1 increases the risk of ESRD.

Dong Chan Jin (2015) et al. study the major changes and improvement of dialysis therapy in Korea. In 1985 ESRD patients were registered in Insan Prof. Byung –Suk Min Memorial ESRD patient's registery and is launched by Korean Society of Nephropathy. In 2000 about 15,853 registered in haemodialysis that increases to 52,378 in 2013. Patients of ESRD and diabetic nephropathy were increased also decrease in the no of patients of peritoneal dialysis. Treatment was successful to increase pulse pressure, decrease in mean blood pressure and also improvement in dialysis adequacy, anaemia treatment, dialysis patient's survival also improved.

Waden, J. et al. (2015) studied the effect of physical exercise on diabetic nephropathy. Main focus of this study was on exercise. In study 1390 individuals were included. Measure of diabetic nephropathy was done on the basis of albumin excretion in urine and follow up period was 6.5 years. Results of this study were renal progression with low intensity physical exercise was higher 24% and renal progression with high intensity physical exercise was lower (13.1%). In conclusion, more exercise more protection of kidneys.

Anu Shah et al. (2015) studied that the thioredoxin- interacting protein deficiency protects from diabetic nephropathy. Thioredoxin- interacting protein promotes oxidative stress. Role of TxNIP is in vivo and deficient TxNIP mesangial cells protect from collagen expression, protein kinase phosphorylation and HG induced oxygen species. In study TxNIP deficient mice shows no enhancement of Creatinine, proteinuria, albuminuria, cystatin C and thickness of glomerular basement membrane and these are not seen in TxNIP mice. This study shows that diabetic nephropathy can be prevented by TxNIP and TxNIP have a role in progression and promising therapeutic target.

CHAPTER 3

Aims and objectives

Objectives

- Collection of diabetic patients data and blood samples
- Determine blood glucose, blood urea, blood Creatinine tests and get the results
- To see how much patients have a diabetic nephropathy due to diabetes
- To see gender difference in diabetic nephropathy patients

CHAPTER 4

Plan of Work

Plan of Work

Literature survey	Completed
Collect diabetic patients blood samples	Completed
Determine Blood Glucose, Blood Urea, Blood Creatinine	Completed
Get results of Glucose, Urea Creatinine	Completed
Investigation of Diabetic nephropathy due to diabetes and get %	Completed
Gender difference in diabetic nephropathy patients	Completed

CHAPTER 5

Material and Methods

5. Material and Methods

5.1 Collection of Blood Sample

Blood samples were collected from the patients of Civil Hospital, Sangrur, during the month of January to April, 2015.

5.2 Requirements

Cotton swab
Tourniquet
Syringe
Needle
70% Alcohol
Test tubes
Test tube rack

5.3 Method of Venous Blood Collection

5.3.1 Site of puncture

Veins that present inside the bend of elbow (antecubital fossa) are used most commonly for vein puncture. Cephalic, median cubital, median basilica are main three veins. In some cases wrist or ankle may be used for blood collection.

5.3.2 Procedure

- Apply the tourniquet to the upper arm sufficiently tight to make the veins more clear and stand out.
- Ask the patient to keep arm straight.
- Feel the vein for blood collection so that most vein can be selected.

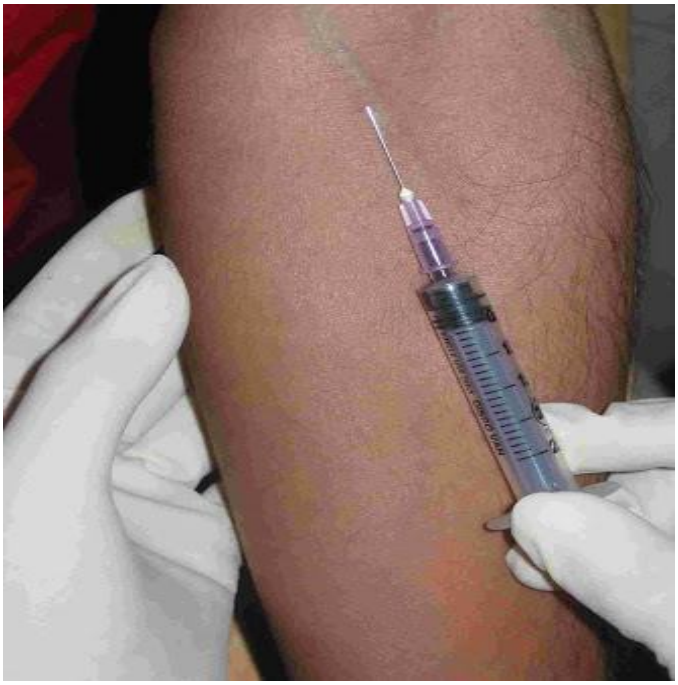


Fig 2. Collection of blood by vein puncture

- Gently massing the arm that helps in the dilation of vein.

- Clean the selected area with 70% alcohol for vein puncture and allow it for dry.
- Prepare the syringe and needle for vein puncture. Commonly 21 gauge needle is used for vein puncture. Always use fresh syringe and needle to avoid the cross infection.
- Use left thump to anchor the vein. Gently insert the needle into the vein at angle of 20 to 30 degree.
- When needle is entered into the needle blood is withdrawn in the needle hub.
- Collect the blood with the help of syringe. Release the tourniquet. Collect the blood sample in the syringe in required amount.
- Place cotton at the site of puncture and remove the needle from the vein.
- Press the puncture site with cotton to stop the blood flow.
- Remove the needle from the syringe and discard the needle in appropriate disposal.

Fig 3. Plan vial used for biochemical analysis

- Dispense the blood into the tube according to required amount.
- Apply strip dressing to the puncture site.

5.4 Separation of Serum

- After collecting the blood transfer into test tubes.
- Allow the samples to be clotted.
- Wait for 10 minutes
- Incubate the test tubes in hot air oven at 37* for 15 minutes
- Remove the tubes and centrifuge at 3000rpm for 10 minutes.
- After 10 minutes remove the test tubes from the centrifuge tube. Remove the tubes carefully after switch off the centrifuge to prevent test tubes damage. See the clear serum sample.
- Transfer the test tubes in a rack.

Fig. 4 and 5 shows the centrifugation process for the separation of serum for analysis of glucose, urea and Creatinine

5.5 Transfer of Serum Sample to the Cuvettes

- After centrifugation transfer the test tubes to the rack.
- In another rack labelled analyser cuvettes set in the rack.
- With the help of pipette or automated pipette transfer serum sample from test tubes to the cuvettes.
- Take care of proper labelling of tubes with cuvettes.

5.6 Erba 640x

Erba 40x is automated analyser that used to test various biochemical test ex. Blood Glucose, Urea, Creatinine, Bilirubin total and direct, SGOT, SGPT, ALP, ALB, Phosphorous, Cal, Na+ etc.

Fig 6. Shows the Erba 640x automated analyser

5.7 Determination of Glucose, Urea and Creatinine

In the analyser enter the details of the patients to perform the test enter the

- Serial no.

- Patient’s name.
- Age.
- Sex.
- UID no
- Then click on save.
- Click on the tests Glucose, Urea, Creatinine.
- Transfer the cuvettes at same serial no in the analyser.
- In the computer click on “RUN”.
- Tests are run.
- Wait for 20 to 30 minutes.
- In the computer tests circles appear green that are completed.
- Results list shown in the computer.
- Take the results print from the computer

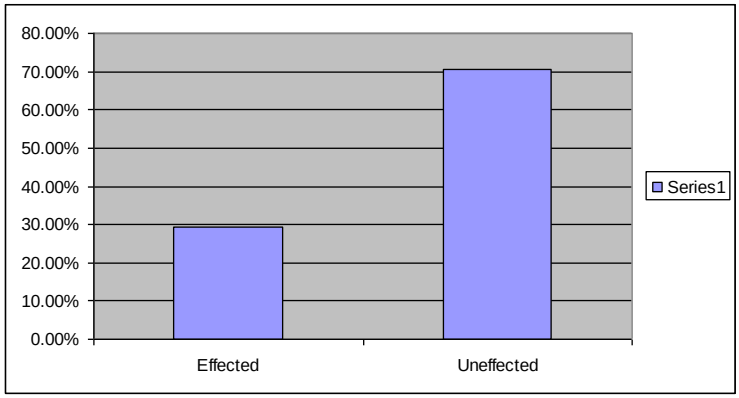
CHAPTER 6

Results

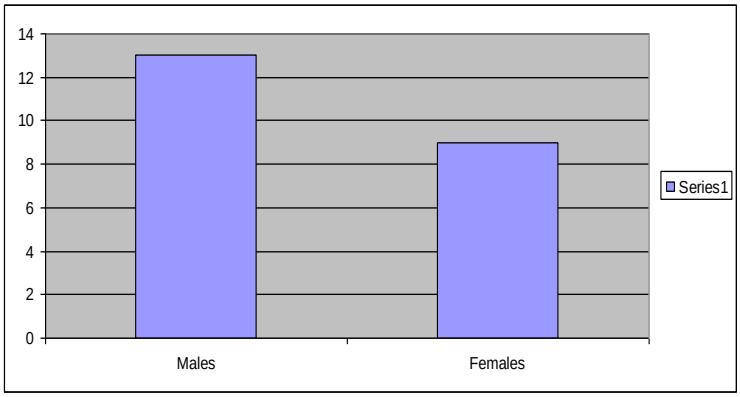
6.1Results

In study 75 Diabetic patients data and samples are collected in which 22 patients are affected with diabetic nephropathy.

Column	No. of patients	Percentage (%)
Total population	75	100.00
Total effected population	22	29.33
Total non effected population	53	70.66
Males effected population	13	17.33
Females effected population	9	12.00



Graff 1. Shows the effected and unaffected peoples with kidney disease due to diabetes

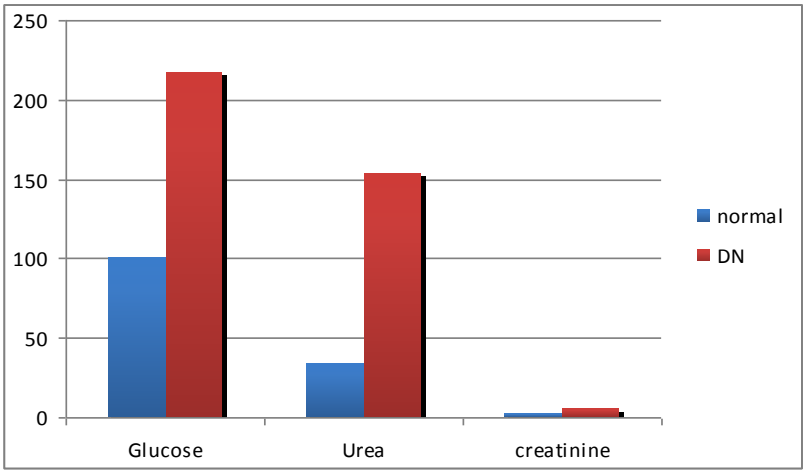


Graff 2. Shows the Gender difference between effected Male and Female patients with kidney disease due to diabetes.

6.2 Comparison of blood parameters of male patients with normal

Column 1	Normal	Diabetic Nephropathy	Mean with Std deviation
Glucose	**100**	**218**	**218.53±51.97**
Urea	**33**	**154**	**148.46±56.81**

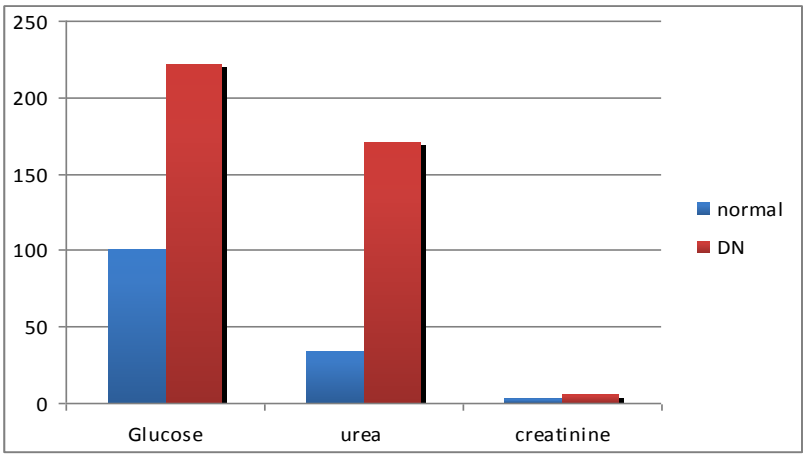
Creatinine	**2**	**5**	**5.26±2.23**



Graff 3. Shows the comparison of normal diabetic population with male diabetic nephropathy population

6.3 Comparison of Blood Parameters of Females with Normal

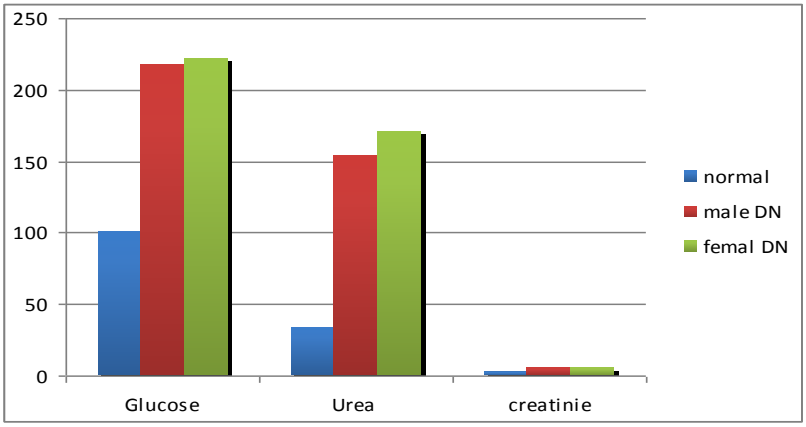
Column 1	Normal	Diabetic Nephropathy	Mean with Std deviation
Glucose	**100**	**222**	**222.44±59.14**
Urea	**33**	**171**	**171.66±21.98**
Creatinine	**2**	**5**	**5.35±1.56**



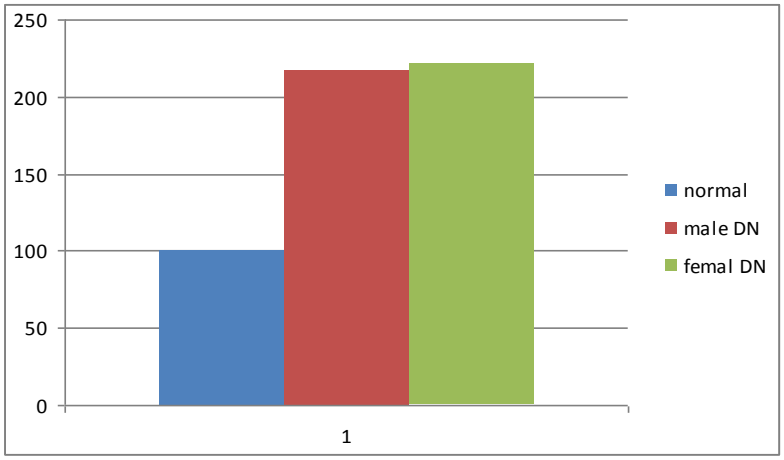
Graff 4. Shows the comparison of normal diabetic population with female diabetic nephropathy population

6.4 Comparison of normal population with male and female diabetic nephropathy population

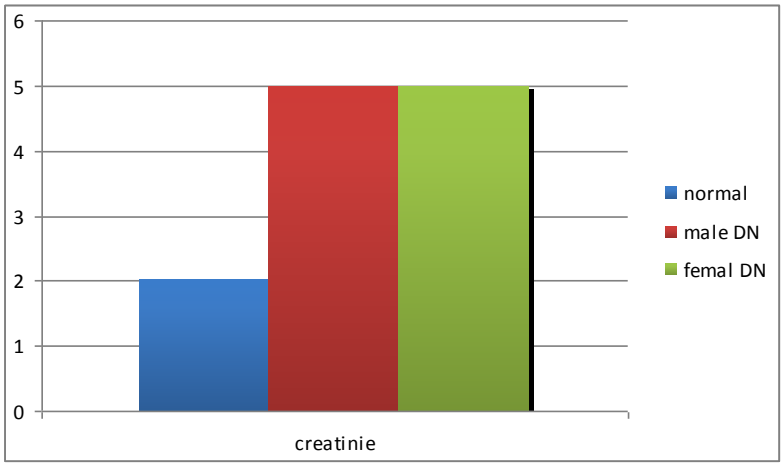
Column 1	Normal	Male DN	Female DN
Glucose	100	**218.53±51.97**	**222.44±59.14**
Urea	33	**148.46±56.81**	**171.66±21.98**
Creatinine	2	**5.26±2.23**	**5.35±1.56**



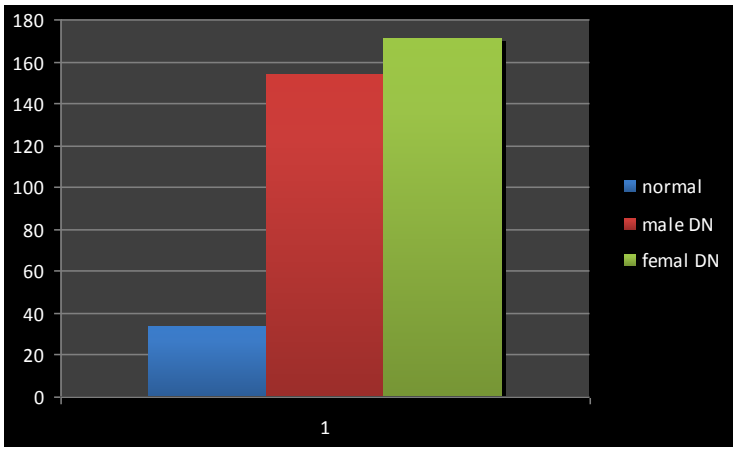
Graff 5. Shows comparison of normal, male DN, female DN population between Glucose, Urea and Creatinine levels.



Graff 6. Shows the comparison of Glucose levels between normal, male DN and female DN population.



Graff 7. shows the comparison of Creatinine levels between normal, male DN and female DN population.



Graff 8. Shows comparison of Urea levels between normal, male DN and female DN population

CHAPTER 7

Discussion

Discussion

According to our study 22 renal patients found out of 75 patients. These patients show the great relationship between diabetes and renal disease. As the glucose level higher, higher the urea and Creatinine level has been detected. Other studies also show that diabetes is major cause of renal failure. Level of renal tests urea and Creatinine increases when there any disfunctioning of the kidney. As according to studies urea, Creatinine level increases with increased glucose level. Increased value of renal tests (Urea, Creatinine) with increased glucose level indicates that hyperglycemia causes renal damage.

In our study findings gender difference of kidney disease shows 17.3% male diabetic patients having kidney disease and 12.0% female diabetic patients having kidney disease. Gender difference between male and females shows great variation. Male patients having more renal disease due to diabetes, as compare to females. Sugam Shrestha et al. show slightly Creatinine higher level than in male patients as compare females. Other studies also shows the variation in Creatinine level not in glucose and urea level. More kidney diseases in males due to storage of Creatinine in muscle mass. Males having more muscle mass as compare to females.

CHAPTER 7

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