Original Research Article

Study of lipid profile and lipid peroxidation in chronic kidney disease patients on dialysis

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Abstract

Introduction: Chronic kidney Disease is defined as abnormalities of kidney structure or function, present for > 3 months, with implications for health.[1] The kidney disease out comes quality initiative defines chronic kidney disease as either kidney damage or a decreased kidney Glomerular filtration Rate (GFR) of less than 60 ml/min/1.7m^2 or more months.[2] The burden of chronic kidney disease in India cannot be assessed accurately. The approximate prevalence of CKD is 800 per million population (PMP), and the incidence of End-stage renal disease (ESRD) is 150 -200 per million population.**Materials and Methods:** The study was conducted during the period April 2017 to October 2018 in the department of Biochemistry, Prathima medical college, Karimnagar, Telangana. The individuals for study were selected from nephrology department of Prathima Hospital, Karimnagar. The individuals were with e GFR (90ml/min- 15ml/min). After 12 hrs fasting 5 ml. venous blood sample was collected in plain tubes, the samples were allowed to clot for half an hour following which a samples were centrifuged for 15 minutes at 3000 rpm. **Results:** The total number of cases is 50, Males 35 and Females 15, The total number of controls are 50, Males 35 and Females 15. P value <0.001 is statistically significant. The serum Creatinine is significantly higher (P<0.001) in Cases when compared to controls. Data is presented as Mean ± 95% confidence interval of blood urea levels for cases and controls. Blood Urea is significantly higher (P<0.001) Cases when compared to controls. Data is presented as Mean ± 95% confidence interval of Serum triglycerides levels for Cases and Controls. The mean serum total cholesterol is not significantly altered in Cases when compared with Controls. (P = 0.0535).**Conclusion:** Based on the findings in this study, it may be reasonable to propose that therapeutic regimens aimed at strengthening the antioxidant defences as well as normalizing lipids concentrations would be useful in protecting CKD patients against oxidative stress and any related complications.

Keywords: Chronic kidney Disease, GFR, blood urea, PMP, ESRD.

Introduction

Chronic kidney Disease is defined as abnormalities of kidney structure or function, present for >3 months, with implications for health[1]. The kidney disease out comes quality initiative defines chronic kidney disease as either kidney damage or a decreased kidney Glomerular filtration Rate (GFR) of less than 60 ml/ min/1.7m^2 or more months[2]. The burden of chronic kidney disease in India cannot be assessed accurately. The approximate prevalence of CKD is 800 per million population (PMP), and the incidence of End-stage renal disease (ESRD) is 150 -200 per million population [3]. Complications may include heart disease, high blood pressure, bone disease of anaemia The most common cause of CKD is diabetes mellitus followed by high blood pressure and glomerulonephritis[4].

Diseases attributable to CKD

Polycystic kidney disease
Diabetic nephropathy
Renal artery stenosis
Hemolytic – uremic syndrome
IgA nephropathy
Lupus Nephritis
Reflux Nephropathy
Focal segmental Glomerulosclerosis
Benign prostatic hyperplasia
Glomerulonephritis

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Chronic kidney Disease (CKD) is characterized by specific metabolic abnormalities of plasma lipids both qualitatively and quantitatively most common lipid abnormalities encountered are increased serum triglycerides and decreased Serum HDL cholesterol with small alteration of other lipoprotein fraction in serum and in dialysis patients there is more of lipidemia rather than hyperlipidemia this may be a significant risk factor for vascular complications leading to increased morbidity and mortality in C K D patients[5].

Aims and objectivesof the study: The overall aim of this study is to evaluate the use of renal function equations in the assessment of renal function in CKD and to identify specific oxidative and metabolic risk factors in CKD.

The specific objectives are as follows:-

- To evaluate the levels of lipid profile markers in chronic kidney disease patients under dialysis treatment.
- To evaluate the oxidative stress as evidenced by serum malondialdehyde and serum superoxide dismutase activity with emphasis on patients under dialysis treatment

Materials and methods

Setting: The study was conducted during the period April 2017 to October 2018 in the department of Biochemistry, Prathima Medical College, Karimnagar, Telangana.

The individuals for study were selected from nephrology department of Prathima Hospital. The individuals were with e GFR (90ml/min-15ml/min).

Inclusion criteria

- Males and females 25-70yrs,
- Clinical signs & symptoms of kidney disease along
- With raised blood urea & serum Creatinine levels.

- Lowered e GFR [90ml/min- 15m/min]

Exclusion criteria

- Individuals suffering from disease likely to modify the lipid profile, oxidative & anti – oxidative status.
- Persons with history of drugs which are likely to modify lipid profile, oxidative & anti- oxidative status.

Sample collection: After 12 hrs fasting 5 ml. venous blood sample was collected in plain tubes, the samples were allowed to clot for half an hour following which a samples were centrifuged for 15 minutes at 3000 rpm.

The following methods were applied to the samples to obtain the required bio marker levels;

1. Creatinine - Semiautoanalyzer- Jaffe's Method
2. Urea-Semiautoanalyzer - Gldh – Urease Method
3. Total Cholesterol-Semiautoanalyzer-Cholesterol Oxidase Enzymatic Method
3. HDL-C- Semiautoanalyzer - Phosphotungstic Acid Method
4. Triglycerides- Semiautoanalyzer - Gpo - Pap Enzymatic Method
5. LDL- C- Friedwalds Equation
6. VLDL- C- Tg/5
7. MDA-Enzyme Linked Immuno Sorbent Assay Method
8. SOD-Enzyme Linked Immuno Sorbent Assay Method

Serum creatinine

Method: Jaffe's method (2 point fixed kinetic)

Reference: Bartels. H, et al. Clin Chem Acto 1971; 32:81

Principle: Creatinine forms orange – red coloured complex with picric acid in alkaline medium the rate of formation of the colored complex is measured

Reagents:

Reagent 1 (R1) = Creatinine standard 2 mg/ dl
Reagent 2 (R2) = Picric acid
Reagent 3 (R3) = Sodium hydroxide
Reagent mixture:
Prepare 1+1 mixture of R2 and R3. Stable for 5 days

Precautions:

Avoid contamination, reagent caps should always be tightly closed, fresh pipette tips should be used Reagents are stable until expiry date on the label.

Procedure:

Wavelength: 490- 510nm
Method : 2 point fixed kinetic
Incubation time : 5 minutes
Temperature : 37°C
Standard : 2 mg/ dl
Take 3 clean test tubes and label 'S' for standard, 'T' for test and 'B' for blank

Table1: Reagents

Reagent	Blank	Standard	Test
Reagent mixture	1000 µl	1000µl	1000µl
Deionised water	---	100µl	---
Sample	---	---	100µl
Standard	---	100µl	----

Incubate for 5 minutes at RT read absorbance of standard (S)and (T) against blank (B)at 490- 510nm
As it is a 2 point fixed kinetic method, the semi auto analyser automatically takes 2 readings and calculates the result.

Calculations:

$$\text{Creatinine conc. in mg/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times 2$$

UREA (BUN)

METHOD: GLDH- UREASE METHOD

PRINCIPLE: The estimation of urea in serum involves the following enzyme catalyzed reactions:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \alpha\text{-KG} + \text{NADH} \xrightarrow{\text{GLDH}} \text{Glutamate} + \text{NAD}$$

α - KG: α- Ketoglutarate

The rate of decrease in absorbance is monitored at 340 nm and is directly proportional to urea concentration in the sample.

Reagent composition (when reconstituted as directed)

Reagent 1: urea reagent

Table 2: Urea reagent composition

α – Ketoglutarate	7.5 mmol/L
NADH	0.32 mmol/L
Urease	≥ 8.000 IU/L
GLDH	≥ 1.000 IU/L
ADP	1.2 mmol/ L
Tris Buffer pH 7. 9 ± 0.1 at 25°C	100 mmol/L

Also contains non- reactive fillers stabilizers

UREA STANDARD

Table 3: Urea standard

Urea (bun)	50 mg/dl (23.4mg/dl)

REAGENT RECONSTITUTION

Allow the reagent and Aqua -4 (supplied in the kit) to attain room temperature (15-30°C). Add the amount of Aqua- 4

SPECIMEN COLLECTION AND HANDLING

Use unheamolytic serum, plasma or urine.

ASSAY PARAMETERS

Table 4: Assay Parameters

Mode	Fixed time
Wavelength 1 (nm)	340
Sample Volume (µl)	10/20
Reagent Volume (µl)	500/1000
Leg Time (Sec)	20
Reaction Temperature (^oC)	37
Reaction direction	Decreasing
No. Of readings	1
Normal low (mg/ dl)	13
Normal high (mg/dl)	45
Linearity low (mg/dl)	0
Linearity high (mg/dl)	250
Concentration of Standard (mg/dl)	50
Blank with	Dl Water
Absorbance Limit (Min.)	0.800
Units	mg/dl

Calculation: Determine absorbance change (A) for the standard and unknown samples by using the formula

$\Delta A = A_1 - A_2$

$$\text{Urea (mg/dl)} = \frac{\Delta A \text{ of Test}}{\Delta A \text{ of standard}} \text{ X Concentration of standard (mg/dl)}$$

ASSAY PROCEDURE

Table 5: Assay Procedure

Pipette into tubes marked	Standard	Test
Working reagent	1000µl	1000µl
Standard	20µl	----
Test	---	20 µl

Mix well, and aspirate standard followed by samples

Quality control

For quality control ERBA NORM ANDERBA PATH are recommended

Expected values:In serum / Plasma

Table 6: Expected values in serum/plasma

Adults	(mg/dl)	(mmol/l)
Women < 50 years	13 - 40	2.6 - 6.7
Women > 50 years	21 - 43	3.5-7.2
Men < 50 years	19 - 45	3.2-7.3
Men>50years	18-55	3.0 - 9.2

eGFR (GLOMERULAR FILTRATION RATE)

Cockcroft - Gault formula for estimating Creatinine clearance

$$\text{MALE(Cr Cl mL/min)} = \frac{\text{(140- age) X Lean Body Weight (kg)}}{\text{Serum Creatinine (mg/dl)X72}}$$

$$\text{FEMALE(Cr Cl mL/min)} = \frac{\text{(140- age) X Lean Body Weight (kg)}}{\text{Serum Creatinine (mg/dl)X72}} \text{ X (0.85)}$$

TOTAL CHOLESTEROL

Method: This method is based on the Trinders methodology

Principle:

1. Cholesterol ester + H_2o $\xrightarrow{CHE}$ Cholesterol + fatty acids
2. Cholesterol O_2 $\xrightarrow{CHO}$ Cholest – 40 en – 3- one + H_2O_2
3. $2H_2O_2$+ 4AAP + phenol $\xrightarrow{POD}$ Quinoneimne dye + 4 H_2O

Absorbance of Quinoneimine so formed is directly proportional to cholesterol concentration

Reagent Composition:

Table 7: Reagents Composition

Goods buffer (pH- 6.4)	100mmol/L
Cholesterol oxidase	>100u/L
Cholesterol esterase	>200U/L
Peroxidase	>3000U/L
4- Amino Antipyrine	0.3mmol/L
Phenol	5mmol/L

Non reactive stabilizers & surfactants

Cholesterol standard - 200mg/dl
Reagent preparation the reagent supplied is ready for use Sample
Serum were used cholesterol in serum is stable for 3 days at - 8°C

Table 8: Assay parameters

Mode	End point
Wave length 1 (nm)	505
Wave length 2 (nm)	670
Working reagent volume (µl)	5/10
Incubation time (min)	5
Incubation temperature	37
Normal low (mg/ dl)	50
Normal high (mg/dl)	230
Linearity low (mg/dl)	0
Linearity high (mg/ dl)	750
Concentration of standard (mg/dl)	200
Absorbance limit	0.4
Blank with	Reagent
Units	Mg/ dl

Assay procedure

Table 9: Assay blank, standard, test

Pipette into tubes	Blank	Standard	Test
Marked working	1000µl	1000µl	1000µl
Distilled water	10µl	----	--
Standard	----	10µl	--
Sample	---	----	10µl

Mixed well and incubated for 5min at 37°C or 10min at 20-25°C. The absorbance of the test and standard are read at 505nm against reagent blank
Calculations:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Abs of test}}{\text{Abs of standard}} \times \text{concentration of std (mg/dl)}$$

Reference values

Table 10: Reference Values

Mode: serum/ plasma	End point: mg/dl
Children ≤4 week	50-170
2-12months	60-190
≥1year`	110-230
Adults	< 200

Linearity: Up to 750 for higher values it is recommended to dilute the samples with normal saline and repeat the assay multiply the results with the dilution factor.

Pack presentation

Table 11: Pack Presenatation

Product code	Pack size	Reagent –Cholesterol Reagent	Cholesterol standard
120168	5X30ml	5x30ml	1x5ml

HDL CHOLESTETROL

Method: Phosphopotungstic Acid Method

Principle: Chylomicrons, LDL and VLDL (low and very low density lipoproteins) are precipitated from serum by phosphotungstate in the presence of divalent cations such as magnesium. The HDL cholesterol remains unaffected in the supernatant and is estimated using ERBA cholesterol reagent

$$\text{Serum} \xrightarrow{\text{Phosphotungstate}} \text{HDL (supernatant)} + \text{(LDL+VLDL+ chylomicrons) (precipitate)} \quad Mg^{2+}$$

Reagent composition:

Reagent 1: precipitating reagent

Table 12: precipitating reagent composition

Phosphotungstic	2.4mmo/L
Magnesium chloride	40mmol/L

HDL cholesterol standard 25mg/dl

Reagent preparation: The reagent is ready for use

Sample: Un haemolysed serum or EDPA plasma was used citrate and heparin were not used as anticoagulants samples were stable for seven days at 2-8°C.

1. Precipitation :Precipitation of LDL, VLDL and chylomicrons

Table 13: Tests and precipitating reagents

Pipette	Volumes
Test	250µL
Precipitating Reagent	250µL

Mix well and allow the reaction mixture to stand for 10 minutes at room temperature (15-30°C), centrifuged at 4000 rpm (1800xg) for 10 minutes to Obtain a clear supernatant. Supernatant was used to determine the concentration of HDL cholesterol in the samp

Assay Parameters

Table 14: Assay parameters

Mode	End point
Wavelength 1 (nm)	505
Wavelength 2	670
Sample volume (µl)	25/50
Reagent volume	500/1000
Incubation time (min)	10
Incubation temperature (°C)	37
Normal low (mg/ dl)	30
Normal high (mg/dl)	80
Linearity low (mg/dl)	
Linearity high (mg/ dl)	125
Concentration of standard (mg/ dl)	
Blank with	Reagent
Absorbance limit (max)	0.3
Units	Mg/ dl

Assay procedure

Table 15: Assay procedure

Pipette into tubes	Blank	Standard	Test
Cholesterol working reagent	1000 µl	1000µl	1000 µl
Distilled water	50 µl	---	---
HDL standard	---	50 µl	--
Supernatant	---	----	50 µl

Mixed well and incubated for 10min, at 37°C. Absorbance of the standard and each test were reach at 505nm against reagent blank

Linearity The assay is linear up to 125 mg/dl or mm01/l of HDL. For higher values the samples are diluted with normal saline and the assay is repeated. The results are multiplied with the dilution factor

Normal Values reference of guidelines

In males : 30-65 mg/dl

In females : 35-80 mg/dl

Pack presentation

Table 16: Pack presentation

Product code	Pack size	Reagents -1 precipitation reagent	HDL Cholesterol standard
120227	2x50ml	2x50ml	1x5ml

TRIGLYCERIDES

Method: GPO- PAP enzymatic method

Principle:

Triglycerides + H_20 —LPL→ glycerol + free fatty acids

GK

Glycerol + ATP → Glycerol -3- phosphate +ADP

Mg +2

Glycerol – 3- phosphate + 0_2 —GPO→ DAP + H_20_2

H_20_2 + 4AAP + 3.5-DHBS —peroxidase→ Quinoneimine dye + $2H_20$

LPL - lipoprotein lipase

GK -Glycerol Kinase

GPO - Glycerol phosphate oxidase

DAP - Dihydroxy acetone phosphate

ATP – Adenosine Triphosphate

4- AAP – 4 Aminoatipyrine

DHBS – 3, 5 – Dicholro -2- hydroxybene sulfonate

The intensity of chromogen [Quinoneimine] formed is proportional to the triglycerides concentration in the sample when measured at 505nm(500-540nm).

Reagent Composition (when reconstituted as directed)

Reagent 1: Triglycerides reagent

Table 17: Triglycerides reagent composition

Active Ingredient	Concentration
ATP	1.5mmol/L
Mg +2	2.5 mmol/L
4 – Aminoantipyrine	0.8 mmol/L

3-5DHBS	1mmoI/L
Peroxidase	>200 U/L
Glycerol kinase	>550U/L
GPO	8000 U/L
Lipoprotein lipase	>3500U/L
Buffer(PH 7.0+0.1 at 20°C)	53mmol/L

Also contains non – reactive fillers, stabilizers and surfactants

Reagent Reconstitution: The reagent bottle and AQUA- A (Supplied in the kit) were allowed to attain room temperature (15-30ºC) The amount of AQIUA- 4 indicated on the label were added to the contents of each vial. Swirled to dissolve and allowed to stand for 10minutes at room temperature

Sample: Serum samples were collected after 12-16 hours fast. Triglycerides in the serum is stable for 3 days at 4^0C.

Assay parameters

Table 18: Assay parameters

MODE	END POINT
Wavelength 1(nm)	505
Wavelength 2 (nm)	670
Sample volume (µl)	5/10
Reagent volume (µl)	500/1000
Incubation time (min)	10
Incubation temperature (ºC)	37
Normal low (mg/dl)	25
Normal high (mg/dl)	160
Linearity high (mg/dl)	900
Concentration of standard	200
Blank with	Reagent
Absorbance limit	0.5
Units	mg/dl

Assay procedure

Table 19: Assay Procedure

Pipette into tubes marked	Blank	Standard	Test
Working Reagent	1000µl	1000µl	1000µl
Distilled water	10µL	--	--
Standard	---	10µl	--
Test	--	---	10µl

Mixed well and incubated for 10min, at 37ºC. The absorbance of the standard and each test were read at 505nm on bichromatic analysers against reagent blank

Linearity: The assay is linear up to 900mg/ dl (10.2mmol/l) for higher values the sample is diluted with normal saline and the assay is repeated. Results are multiplied with the dilution factor.

Normal values (reference for guidelines): Normal fasting levels – 25-160 mg/dl.

Pack presentation

Table 20: Pack presentation

Product code	Pack size	Reagents -1 triglycerides DES reagent	Triglycerides standard	Aqua- 4
120211	2x20ml	2x20ml	1x5ml	1x100ml
120212	5X6.5ml	5x6.5ml	1x5ml	1x35ml
120213	10x50ml	10x50ml	2x5ml	2x300ml

MALONDIALDEHYDE (MDA)

Intended use: The kit uses a double – antibody sandwich enzyme – linked immunosorbent one – step process assay (ELISA) to assay the level of Malondialdehyde (MDA) in samples.

Standard, test sample and HRP – labelled Malondialdehyde (MDA) Antibodies to enzyme wells which are Pre- coated with Malondialdehyde (MDA) antibody, then carry out incubation and wash to remove the uncombined enzyme. Upon adding Chromogen Solution A and B, the colour of the liquid will change into blue, and the reaction with the acid will cause the colour to become yellow. The depth of colour and the concentration of the Malondialdehyde (MDA) sample are positively correlated.

Performance Kit

1. Assay range: 9.3ng/ml- 300ng/ml
2. Assay method: The kit uses a double – antibody sandwich enzyme- linked immunosorbent one-step process assay (ELISA).
3. Characteristics: For content determination. In serum, plasma, cell culture supernatant, tissue homogenate and any other biological fluid.
4. Accuracy: standard linear regression correlation coefficient R with the expected value of the concentration, greater than or equal to 0.9900.
5. Specificity: This kit has no cross – reaction with other soluble structural similar object.
6. Repeatability: The plate coefficient of variation is less than 15%
7. Plate type: Pre-coated, 8*12strips, 96wells
8. Reliability: Can be preformed within two hours. Contrary to traditional Elisa methods, only a single incubation and wash step is required, resulting in fewer handling steps, which reduce errors and deliver more consistent results. Thorough and regular tests of the system guarantee the stability and reliability of the kit.

Sample Collection and Storage

1. Samples that contain NaN_3 cannot be detected, because NaN_3 inhibits HRP.
2. Serum: During the operation, use non- pyrogenic and endotoxin tubes, to avoid any cell stimulation, collect blood: centrifuge 3000 rpm for 10 minutes. Carefully separate the

serum and red blood cells as quickly as possible. If precipitation appears, centrifuge again.

3. Plasma: Use suited EDTA citrate or heparin as an anticoagulant, mix 20 minutes, centrifuge 30 minutes at the speed of 3000rpm, collect supernatant. If precipitation appears, centrifuge again.

Materials Supplied

Table 21: MDA Materials supplied

Reagents (store at 2-8ºC)	1x96 Wells	Pre- coated
96 wells	8*12strip	Ready to use
Standard (3300ng/ml)	0.6ml	Dilute according to instructions
Standard diluents	6.0 ml	Could be used as blank controls
Special diluents	6.0 ml	Ready – to –use
HRP- Conjugate reagent	6.0ml	Ready - to -use
20 X Wash solution	25 ml	Dilute according to instructions
Chromogen Solution A	6.0 ml	Ready – to –use
Chromogen Solution B	6.0 ml	Ready – to –use
Stop solution	6.0 ml	Ready – to –use
Microplate	2	Ready – to –use
User manual	1	Ready – to –use
Sealed bags	1	Ready – to –use

Note: Dilute the standard with Standard diluents in the method of Multiple proportion dilution, and the concentrations are as follows: 300, 150, 75, 37.5, 18.7, 0 ng/ml

Reagent preparation

20X dilution of washing buffer: distilled water, diluted by 1:20, or 1 copy of the 20x washing buffer plus 19 copies of the distilled water

SUPER OXIDE DISMUTASE (SOD)

METHOD: ELISA

Pre- assay preparation

Reagent preparation

1. Assay buffer (10x) :Dilute 3 ml of assay buffer concentrate with 27 ml of hplc grade water for assaying 96 wells. Prepare additional assay buffer as needed. This final assay buffer (50 mm tris- hcl, ph 8.0, containing 0.1 mm diethylene triaminepentaacetic acid (dtpa) and 0.1 mm hypoxanthine should be used to dilute the radical detector. When stored at 4ºc, this diluted assay buffer is stable for at least two months

2. Sample buffer (10x):Dilute 2ml of sample buffer concentrate with 18 ml of hplc – grade water for assaying 96 wells. Prepare additional sample buffer as needed. This final sample buffer (50 mm tris – hci,ph 8.0) should e used to prepare to assaying. When stored at 4ºc, this diluted sample buffer is stable for at least six months.

3. Radical detector :The vials contain 250 µl of a tetrazolium salt solution. Priorto use, transfer 50µl of the supplied solution to another vial and dilute with 19.95 ml of diluted assay buffer. Cover with tin foil. The diluted radical detector is stable for to hours. This is enough radical detector for 96 wells, prepare additional detector as needed. Store unused radical detector at-20^0c.

4. SOD standard :The vials contain 100 µl of bovine erythrocyte sod (cu/zn). Store the thawed enzyme on ice and see standard preparation on page 14 for preparing the standard curve. Store unused enzyme at -20^0c. The enzyme is stable for at least two freeze/thaw cycles.

5. Xanthine oxidase

These vials contain 150 µl of xanthine oxidase. Prior to use, thaw one vial and transfer 50 µl of the supplied enzyme to another vial and dilute with 1.95 ml of sample buffer (dilute). Store the thawed and diluted xanthine oxidase on ice. The diluted enzyme is stable for one hour. This is enough xanthine oxidase for 96 wells. Prepare additional xanthine oxidase as needed. Do not refreeze the thawed enzyme. Any unused enzymes should be thrown away.

Results

Statistics analysis: The parameters were analyzed in 100 individuals who were divided into 2 categories

1. Normal individuals
2. CKD patients

The results were compared between 2 groups.

1) Total cases vs Total controls

The results were expressed as mean SD and p value. The results data were statistically analyzed using univariate analysis of variance for descriptive statistics. Unpaired T test was used for comparison between the groups. All the analyses were carried out using statistical software, SPSS- 15 and MS-Excel in windows 7.

Table 22: Gender wise Distribution of controls and cases

Group	Gender	No.of subjects
Controls (n=50)	Male	35
	Female	15
Cases (n=50)	Male	35
	Female	15

1. The total number of cases are 50, Males 35 and Females 15.
2. The total number of controls are 50, Males 35 and Females 15.

Data is presented as Mean ± 95% confidence interval of serum Creatinine levels for cases and controls

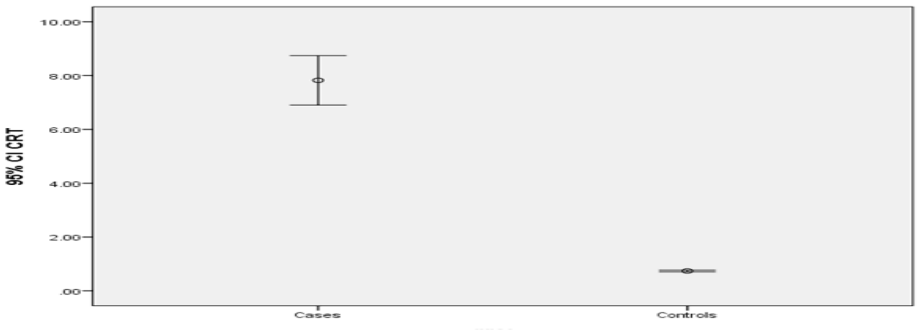

Fig 1:Serum creatinine

Table 23: Serum creatinine (mg/dl)

	Cases (n=50)(mean ± S D)	Control (n=50)(mean ± S D)	P value
Serum creatinine (mg/dl)	7.82 ±3.23	0.73±0.11	<0.001

P value <0.001 is statistically significant
The serum Creatinine is significantly higher (P<0.001) in Cases when compared to controls
Data is presented as Mean ± 95% confidence interval of blood urea levels for cases and controls

Table 24: Blood urea (mg/dl)

	Cases (n=50)(mean±SD)	Control (n=50)(mean±SD)	P Value
Blood urea (mg/dl)	110.9±35.02	21.92±3.60	<0.001

P value <0.001 is significant .Blood Urea is significantly higher (P<0.001) Cases when compared to controls
Data is presented as Mean ± 95% confidence interval of Serum triglycerideslevels for Cases and Controls

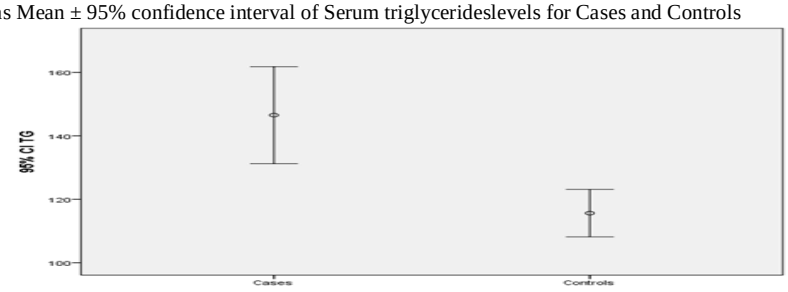

Fig 2: Serum triglycerides levels

Table 25: Serum triglycerides (mg/dl)

	Cases (n=50)(mean±SD)	Control (n=50) (mean±SD)	P Value
Serum triglycerides (mg/dl)	153.16±47.93	114.90±26.04	<0.001

The mean Serum Triglycerides in Cases is increased when compared to Controls The increase is statistically significant (P<0.001)
Data is presented as Mean ± 95% confidence interval of Total Cholesterol levels for Cases and Controls.

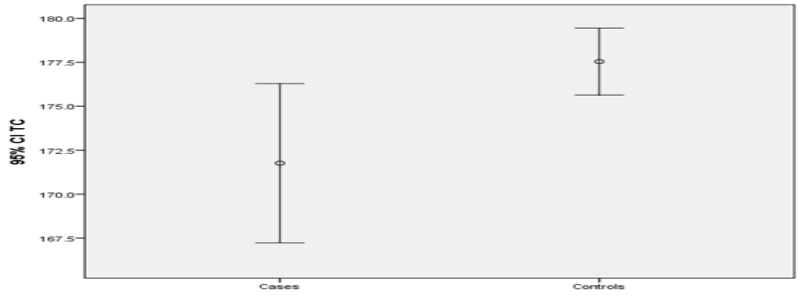

Fig 3: Total Cholesterol levels

Table 26: Serum total cholesterol

	Cases (n=50)(mean ±SD)	Control(n=50)(mean ±SD)	P-Value

Serum total cholesterol	174.14±12.66	178.12±6.87	0.0525

The mean serum total cholesterol is not significantly altered in Cases when compared with Controls. (P = 0.0535)Data is presented as Mean ± 95% confidence interval of LDL levels for Cases and Controls

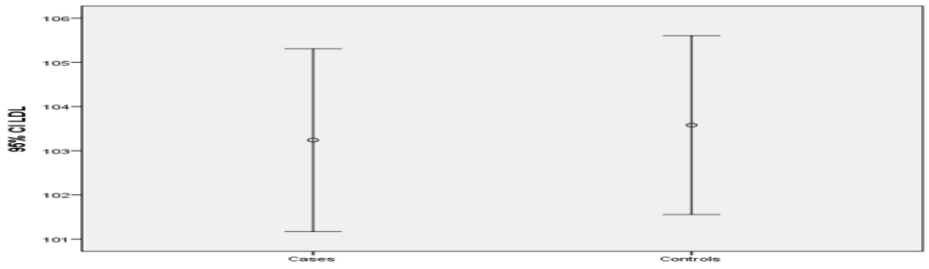

Fig 4: LDL levels

Table 27: Serum LDL (mg/dl)

	Cases (n=50) (mean±SD)	Control (n=50) (mean±SD)	P Value
Serum LDL (mg/dl)	102.84±7.17	103.54±6.85	0.6189

The mean LDL cholesterol is not significantly altered in Cases when compared with controls. (P= 0.6189)Data is presented as Mean ± 95% confidence interval of HDL levels for Cases and Controls

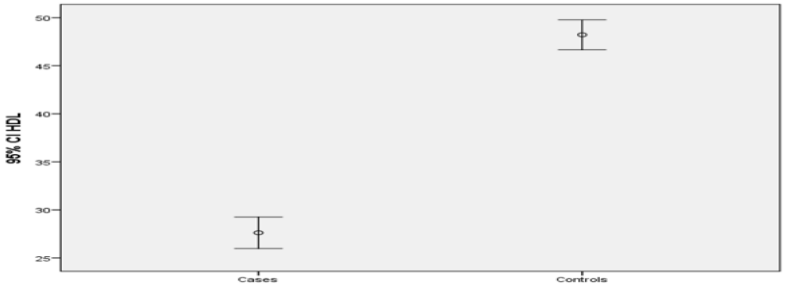

Fig 5: HDL Levels

Table 28: Serum HDL-C (mg/dl)

	Cases (n=50) (mean ±SD)	Control (n=50) (mean ±SD)	P value
Serum HDL-C (mg/dl)	27.65±5.79	48.22±27.65	<0.001

P values <0.001 statistically significant

The mean Serum HDL cholesterol decreased in Cases when compared to controls. (P<0.001).Data is presented as Mean ± 95% confidence interval of VLDL levels for Cases and Controls

Fig 6: VLDL Levels

Table 29: VLDL

	Cases (n=50) (mean ±SD)	Control (n=50) (mean ±SD)	P value
Serum VLDL	43.34±7.13	25.42±5.98	<0.001

The mean VLDL in Cases is increased when compared to control the increase is statistically significant (P<0.001)
Data is presented as Mean ± 95% confidence interval of MDA levels for Cases and Controls

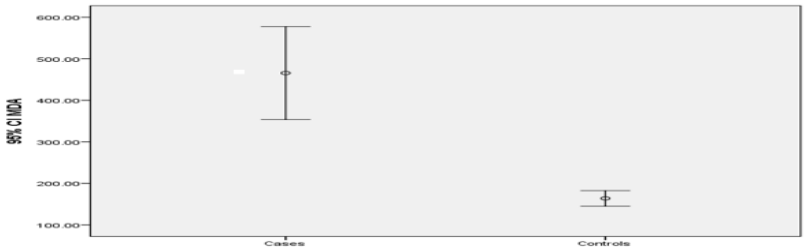

Fig 7: MDA Levels

Table 30: MDA ng/ml

	Cases (n=50) (mean ±SD)	Control (n=50) (mean ±SD)	P value
MDA ng/ml	451.136±403.053	158.016±69.785	<0.001

P value <0.001 statistically significantThe concentrations of serum MDA is significantly higher in Cases when compared to controls.
Data is presented as Mean ± 95% confidence interval of SOD levels for Cases and Controls.

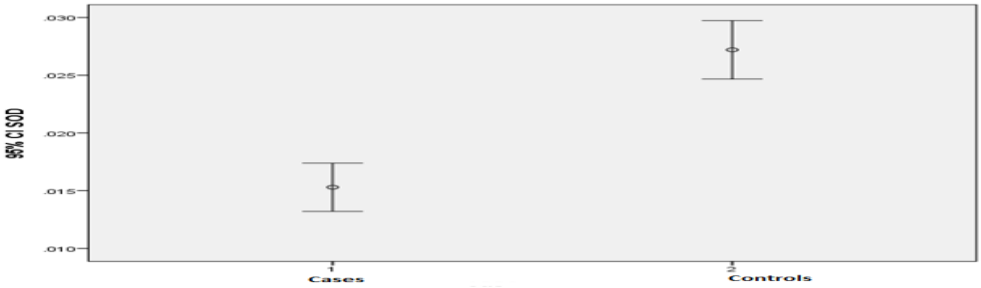

Fig 8: SOD Levels

Table 31: SOD U/ml

	Cases (n=50) (mean ±SD)	**Control (n=50) (mean ±SD)**	**P value**
SOD U/ml	0.014±0.007	0.027±0.008	<0.001

P value <0.001 statistically significant.The concentrations of serum SOD is significantly decreased in Cases when compared to controls.

Discussion

CRF is a worldwide health problem and is the leading cause of morbidity and mortality in the developed world. Patients with CRF are at high risk for CVD and cerebrovascular disease (CBVD), and they are more likely to die of CVD than to develop ESRD. CRF is associated with premature atherosclerosis and increased incidence of cardiovascular morbidity and mortality. Several factors contribute to atherogenesis and cardiovascular disease in patients with CRF, the notably among all is dyslipidemias. Chronic renal failure primarily affects the metabolism of high-density lipoprotein (HDL) and triglyceride (TG)-rich lipoproteins[5].In the present study, Triglycerides were elevated in Cases when compared to Controls which is statistically significant (p-values <0.001).Maheshwari N et al. Reported hypertriglyceridemia and low HDLc and elevated lipoprotein-a, which could contribute to atherosclerosis and cardiovascular disease that may increase the morbidity and mortality in patients on maintenance haemodialysis[6,7].Serum triglyceride is one of the most valuable lipid types altered in kidney diseases. Hypertriglyceridemia mostly occurs in early stages of CKD. The most dramatic increase in triglyceride levels usually occurs in patients with nephrotic syndrome, but it also increases in other kidney diseases due to both abnormal production and reduced catabolism of triglycerides[8]. Hypertriglyceridemia leads to alteration of size and composition of HDL and LDL. Hypertriglyceridemia leads to increased VLDL secretion from liver which activates Cholesteryl Ester Transfer Protein (CETP). CETP transfers triglycerides to LDL and HDL leading to formation of triglyceride rich LDL and HDL. Hepatic triglyceride lipase hydrolyses the triglyceride content of the HDL and LDL particles leading to formation of a sub fraction called small dense LDL and HDL. Small dense LDL (sdLDL) has low affinity for LDL receptor, can penetrate arterial wall easily and are more susceptible to oxidation. Oxidized sdLDL is highly atherogenic which increases the risk of cardiovascular diseases. The activity of hepatic lipase and VLDL receptor and of hepatic LRP is also diminished in CKD[9]

In the present study, total cholesterol levels were lower in Cases as compared to controls, (p-value 0.0535) which is statistically not significant.In the present study the values attained were not statistically significant. The similar results were forwarded by Saland et al and Jain et al[10]In the present study, it was observed that the MDA activity was significantly higher in Cases when compared to controls and serum SOD values in Cases is significantly decreased (p<0.001) when compared with Cont

Conclusion

Based on the findings in this study, it may be reasonable to propose that therapeutic regimens aimed at strengthening the antioxidant defences as well as normalizing lipids concentrations would be useful in protecting CKD patients against oxidative stress and any related complications.

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