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# STUDY OF SERUM PCSK9 LEVELS IN CHRONIC KIDNEY DISEASE PATIENTS UNDERGOING HAEMODIALYSIS.

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**Abstract**

**Introduction:** Chronic Kidney Disease is defined as presence of kidney damage or an estimated glomerular filtration rate (eGFR) less than 60ml/min/1.73mt2 persisting for 3 months or more. PCSK-9 (Proprotein convertase subtilisin/kexin type 9) is a new biomarker which has been observed to be raised in CKD patients.

**Aims:** To compare serum PCSK9 levels in patients suffering from chronic kidney disease and normal individuals and to observe the effect of haemodialysis on the levels among patients.

**Materials and methods:** The study was conducted from 1st June 2018 to 31st February 2019. 60 patients suffering from CKD in between 16 years to 65 years of age of both genders were consecutively enrolled (Study Group). 60 normal age matched and gender matched controls were also included in study for comparison (Control Group). Serum PCSK9 was estimated using Ultra-Sensitive Human PCSK9 ELISA EK1147 PicoKine™ (BOSTER). Its levels were again estimated in study group after haemodialysis. Findings were compared using appropriate statistical instruments.

**Observation:** Serum PCSK9 levels were significantly higher in study group(1983.00±1243.17ng/ml) as compared to control group (23.48±8.71ng/ml). There was significant reductional effect of haemodialysis on serum PCSK9 levels ( Pre-dialysis value 1983.00±1243.17ng/ml and post-dialyses value 1889.88±1237.64 ng/ml.).

**Conclusion:** PCSK9 is a new biochemical diagnostic tool, which is elevated when person has chronic kidney disease but levels fall down when haemodialysis is performed.

**Keywords:** Chronic Kidney Disease (CKD), Proprotein convertase subtilisin/kexin type 9 (PCSK9) and Ultra-Sensitive Human PCSK9 ELISA EK1147 PicoKine™ (BOSTER).

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## 1. INTRODUCTION

There are two bean shaped kidneys present in our body, which are situated on the posterior wall of abdomen in extra-peritoneal cavity, each weighing about 150 gms and about 12 cms in length. They receive blood from the paired renal arteries, blood exists into the paired renal veins. Each kidney is attached to a ureter, that carries excreted urine to the bladder.[1]

The nephron is the structural and functional unit of the kidney. It is composed of a glomerulus and a complex tubular system. The first portion of the tubular system known as the proximal convoluted tubule (PCT), are located in the renal cortex, following the PCT, the loop of Henle, a hairpin- like structure, penetrates the medulla and returns to the cortex to connect with the distal convoluted tubule (DCT). Finally, the nephron drains into the collecting duct via connecting tubules. Each human adult kidney contains about 1 million nephrons.[2]

Chronic Kidney Disease (CKD) is defined as presence of kidney damage or an estimated glomerular filtration rate (eGFR) less than 60ml/min/1.73mt2 persisting for 3 months or more. It is also characterized by reduction in number of nephrons.[3]

Globally 1.2 million people died from CKD in 2017. Global all-age mortality rate increased 41.5% between 1990 and 2017. In 2017, 697.5 million cases of all stage CKD were recorded.[4] Whereas in India, reported prevalence of CKD in different regions ranges from 1% to 13%. According to recent data from International Society of Nephrology's Kidney Disease Data Centre Study , reported prevalence of CKD is 17.5%.[5]

During past few years proprotein convertase subtilisin kexin 9 (PCSK9) has emerged as new therapeutic and diagnostic modality. It has become the target for treating hypercholesterolemia and interest has been shown to it in academia, research and industry alike.[6] PCSK9 is produced predominantly from hepatocytes and other accessory sites are kidney and intestines.[7][8] It is found in plasma in primarily 2 monomeric forms: an active form representing the full-length plasma protein and an inactive/less-active, shorter fragment, which is a cleavage product of the full-length protein by the protease furin.[9] It is a 692 amino acid protein with a molecular weight of 72 kilo Dalton that consists of a prodomain (PD), a catalytic domain and a Cysteine- and Histidine-rich C-terminal Domain (CHRD).[10] It binds to Low Density Lipoprotein Receptors (LDLRs). It is regulated by the Sterol Regulatory Element Binding Protein (SREBP) through a sterol regulatory element motif in the promoter region. [11] Apart from just binding to LDLR, PCSK9 also is responsible for internalization and degradation of LDLRs, production of apolipoprotein B and expression, regulation and lysosomal degradation of Very Low-Density Lipoprotein (VLDL).[12]

Serum PCSK9 has been seen to be raised in different nephrotic syndromes and different proteinurias.[13] There is ample research on role of PCSK9 in cardiovascular disorders, but there is shortage of literature in its role in renal failure. Present study is an attempt to investigate its role in chronic kidney disease and to see the effect of haemodialysis on its serum levels.

## 2. MATERIAL AND METHODS

The study was conducted from 1st June 2018 to 31st February 2019. 60 participants suffering from CKD in between 16 years to 65 years of age of both genders were consecutively enrolled in Group A- Study Group and 60 normal age matched and gender matched controls were also enrolled in Group B- Control Group for comparison. Study participants were informed about the study in their local language and written informed consent was obtained from them before beginning the study. Sociodemographic data were recorded in sociodemographic Performa 1.

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2 ml blood was drawn from anti-cubital vein of all participants ensuring adequate antiseptic precautions. Blood was allowed to clot at room temperature. Clotted blood was centrifuged for 20 minutes to obtain serum, which was stored at 2–8-degree centigrade temperature. Estimation was done on following day.

Following method was used for estimation of serum PCSK9:

Ultra-Sensitive Human PCSK9 ELISA EK1147 PicoKine™ (BOSTER) was used to estimate serum PCSK9. Human PCSK9 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human PCSK9 that is pre-coated with antibody specific for PCSK9. The detection antibody is a biotinylated antibody specific for PCSK9. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Human PCSK9 with immunogen.

Working reagent was prepared in following steps: -

All reagents were brought at room temperature prior to use.

1. Wash buffer- given powder was dissolved in 1000ml of deionized water.
2. Biotinylated Anti-Human PCSK9 antibody -It is recommended to prepare this reagent immediately prior to use by diluting the Human PCSK9 Biotinylated antibody (100x) 1:100 with Antibody Diluent. 100 µl was prepared by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Then it was mixed gently and thoroughly and used within 2 hours of generation.
3. Avidin-Biotin-Peroxidase Complex -It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. 100 µl was prepared by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mixed gently and thoroughly and was used within 2 hours of generation.
4. Human PCSK9 Standard- It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. One 10ng of lyophilized Human PCSK9 standard has been used for each experiment. Gently the vial was spined prior to use. The standard was reconstituted to a stock concentration of 10ng/ml by using 1ml of sample diluent. Then the standard was allowed to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

Dilution of Human PCSK9 standard was done in following steps :-

1. First tubes were numbered from 1-8. Final Concentrations to be Tube # 1 –10000pg/ml, #2 –5000pg/ml, #3 – 2500pg/ml, #4-1250pg/ml, #5 – 625pg/ml, #6 – 312.5pg/ml, #7 – 156.25pg/ml, #8 – 0.0 (Blank).
2. For standard #1, 1000µl of undiluted standard stock solution was added to tube #1.
3. 300 µl of sample diluent was added to tubes # 2-7.
4. To generate standard #2, 300 µl of standard #1 from tube #1 was added to tube #2 to prepare a final volume of 600 µl. It was mixed properly.
5. To generate standard #3, 300 µl of standard #2 from tube #2 was added to tube #3 to prepare a final volume of 600 µl. Mixture was mixed properly.
6. Similar serial dilutions were continued from tube #4-7.
7. Tube #8 was set as a blank standard to be used with every experiment.

Standard curve for calibration was prepared as follows: -

1. All reagents and working standards were prepared as directed previously.

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2. Excess microplate strips were removed from the plate frame and sealed and stored in the original packaging.
3. 100 µl of the standard, samples, or control per well were added and 100 µl of the sample diluent buffer was added into the control well (Zero well). At least two replicates of each standard, sample, or control are usually recommended.
4. It was all covered with the plate sealer provided and incubated for 120 minutes at RT (or 90 min. at 37 °C).
5. Cover was removed and liquid was discarded in the wells into an appropriate waste receptacle. Then plate was inverted on the bench top onto a paper towel and tapped to gently blot any remaining liquid. It is usually recommended that the wells are not allowed to completely dry at any time.
6. 100 µl of the prepared 1x Biotinylated Anti-Human PCSK9 antibody was added to each well.
7. Plate sealer was covered and incubated for 90 minutes at RT (or 60 minutes at 37°C).
8. Plate was washed 3 times with the 1x wash buffer.
    a. Liquid was discarded in the wells into an appropriate waste receptacle. Then plate was inverted on the benchtop onto a paper towel and tapped to gently blot any remaining liquid. It is usually recommended that the wells are not allowed to completely dry at any time.
    b. 300 µl of the 1x wash buffer was added to each assay well. (For cleaner background incubate for 60 seconds between each wash). c. Then steps a-b 2 were repeated.
9. 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex were added into each well. Plate sealer provided was covered and incubated for 40 minutes at RT (or 30 minutes at 37°C).
10. Plate was washed 5 times with the 1x wash buffer.
    a. Liquid was discarded in the wells into an appropriate waste receptacle. Then, plate was inverted on the benchtop onto a paper towel and tapped to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
    b. 300 µl of the 1x wash buffer added to each assay well. (For cleaner background incubate for 60 seconds between each wash).
    c. Then steps a-b 4 were repeated.
11. 90 µl of Colour Developing Reagent added to each well. Plate sealer provided was covered and incubated in the dark for 30 minutes at RT (or 25-30 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. 100 µl of Stop Solution added to each well. The colour should immediately change to yellow. Reading of each well taken in ELISA reader at 450nm.
13. The O.D. absorbance has been readied with a microplate reader at 450nm.

| CONTROL VALUE (ng/ml) | ABSORBANCE (450 nm) |
|---|---|
| 0 | -0.016 |
| 156.2 | 0.2654 |
| 312.5 | 0.5801 |
| 625 | 0.9279 |
| 1250 | 1.3675 |
| 2900 | 1.8526 |
| 5000 | 2.498 |

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For assay procedure serum was taken in spite of calibrator and its procedure was same as done in case of calibration of standard curve and value was measured by calibration curve.
Relevant statistical instruments were applied where ever needed.

**Observations**

Following observations were made from participants of Group A and Group B,

Table 1: Comparison of serum PCSK9 levels in Group A and Group B participants.

| Group | Number of subjects | Range of PCSK9 (ng/ml) | Mean±SD | t-value | p-value |
|---|---|---|---|---|---|
| Group A (Study Group | 60 | 81-3395 | 1983.00±1243.17 | 9.025 | 0.001 |
| Group B (Control Group) | 60 | 89-1008 | 23.48±8.71 | | |

**p<0.05= significant (*); p<0.01= highly significant (**)**

Table 1 shows that serum PCSK9 levels are more in study participants having CKD (1983.00±1243.17ng/ml) as compared to age and gender matched healthy controls without CKD (23.48±8.71 ng/ml). This difference has high statistical significance (p=0.001)

Table 2: Effected of haemodialysis on serum PCSK9 levels in Group A participants.

| Haemodialysis | Number of participants in Group A undergoing dialysis | Range of PCSK9 (ng/ml) | Mean±SD | t-value | p-value |
|---|---|---|---|---|---|
| Pre-dialysis | 60 | 81-3395 | 1983.00±1243.17 | 11.782 | 0.001 |
| Post-dialysis | 60 | 45-3298 | 1889.88±1237.64 | | |

**p<0.05= significant (*); p<0.01= highly significant (**)**

Table 2 shows the effect of haemodialysis on study participants with CKD. It can be clearly seen that before dialysis serum PCSK9 levels are more (1983.00±1243.17 ng/ml) and after dialysis levels decreased (1889.88±1237.64 ng/ml). This difference had high statistical significance (p=0.001)

## 3. DISCUSSION

Present comparison study conducted on 60 participants with chronic kidney disease and 60 age and gender matched healthy subjects revealed that serum PCSK9 levels were significantly increased (p=0.001) in participants with chronic kidney disease (1983.00±1243.17ng/ml) compared to healthy controls (23.48±8.71 ng/ml). Our finding is concordant with Jin et al. (2014), who reported higher plasma PCSK9 levels (15.13±4.99ng/ml) compared to normal individuals (9.19±0.60ng/ml).[14] Similarly; in a multicentre, crossover, randomized, controlled trial conducted by Kwakernaak et al. (2013), it was reported that serum PCSK9 levels were almost 30% higher amongst 39 proteinuric subjects [213 (161–314)] ng/ mL] compared to normal controls [143 (113–190) ng/mL,$p < 0.001$].[15]

This study also revealed that serum PCSK9 levels were significantly higher in study participants before haemodialysis (1983.00±1243.17 ng/ml) and decreased after the procedure

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(1889.88±1237.64 ng/ml). This finding is similar to study conducted by H.Abujrad et al. (2014), they also found that serum PCSK9 level is decreased after hemodialysis.[16]
Possible reason behind increased levels among patients with CKD compared to non-CKD participants and subsequent fall in levels in former group after haemodialysis could be that PCSK9 is a serine protease which is produced and released into bloodstream primarily by hepatocytes but it is excreted by intestines and Kidneys. When renal glomerular function gets impaired, it is not filtered and levels increase but after performing haemodialysis levels fall down.[17]
However there are studies which refute any such association between renal dysfunction and serum PCSK9 levels.[18][19] There is a lot of confusion whether PCSK9 levels really increase in patients with renal failure or not. This confusion can be attributed to variety of confounding factors like different studies being conducted in different stages of renal failure. In this present era where PCSK9 inhibitors are emerging as new lipid lowering agents, large-scale studies are mandatory to establish PCSK9 as a new diagnostic tool in chronic kidney disease.

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