

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Challenges in methods of urine proteins measurement

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Lecturer Introduction

- **Mohammad Reza Bakhtiari**

- 1993: Doctorate in Clinical Laboratory Sciences (DCLS)
- 2002: PhD in Medical Biotechnology
- Academic: Biotech Dept. of IROST
- Member to IFCC Committee on Standardization of Thyroid Function Tests (C-STFT)



Chronic Kidney Disease (CKD)

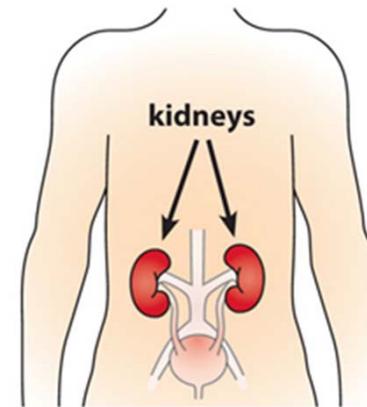
CKD means the kidneys are damaged and can't filter blood the way they should.

The main risk factors:

- diabetes,
- high blood pressure,
- heart disease,
- family history of kidney failure.

Symptoms:

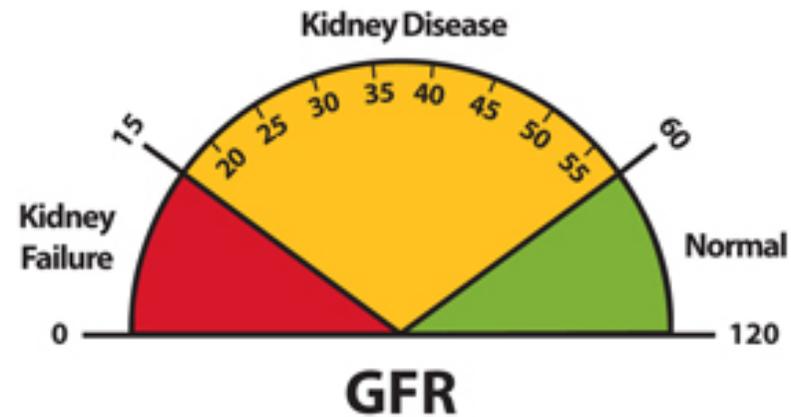
- Early Stages: Nothing
- Later Stages:
 - Edema
 - chest pain
 - dry skin
 - itching or numbness
 - feeling tired
 - headaches
 - increased or decreased urination
 - loss of appetite
 - muscle cramps
 - nausea
 - shortness of breath
 - sleep problems
 - trouble concentrating
 - vomiting
 - weight loss



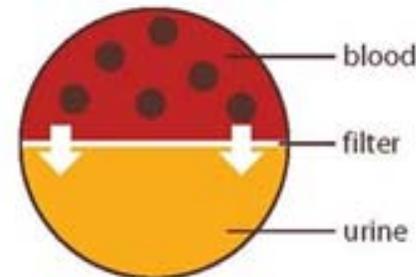
Laboratory Diagnosis of CKD

Lab Diagnosis:

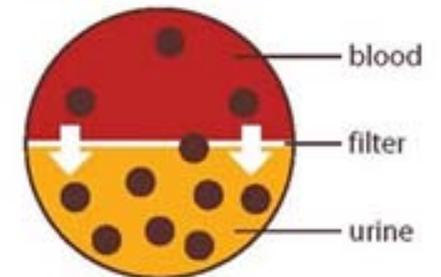
1. GFR Estimation
2. Urine Albumin-to-Creatinine Ratio (UACR)
 - 30 mg/g or less = normal
 - >30 mg/g may be a sign of CKD
3. Urine Test for Albumin
4. Blood Urea
5. Protein Excretion Rate (PER)



Inside a *healthy* kidney



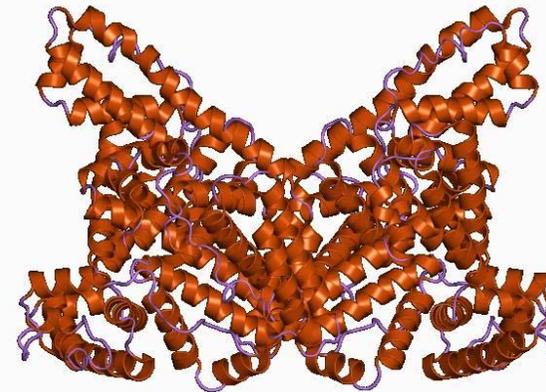
Inside a *damaged* kidney



● albumin

Urine Proteins

1. Urine Total Protein
2. Native Albumin
 - Native Alb: 585 aa, 66473 Da
3. Modified Albumin (Alb Species)
 - Ligands: FAs, bilirubin, Ca, Mg,
 - Truncated
 - Glycated
4. Exposure to Δ pH, ions, urea, Glu, ascorbate,
5. Δ Conformation Changes \rightarrow Δ Filtration Rate



17 disulfide bonds, 4 globular domains

Principles of Test Standardization

Goal: Measurement results be comparable between laboratories and methods, over time, with common reference ranges.

Requires a primary reference system comprising:

- A **Defined** measurand
- Metrology Principles
 - ✓ Reference Measurement Procedure (RMP)
 - ✓ Certified Standard Reference Material (CRM)
 - ✓ Full Calibration Traceability
- Commutability
- Broad Range Coverage

Methods for Measurement of Urine Albumin

- Immunoassay-based Methods (94%)
- Immunturbidimetric (85%)
- Not all immunoassays perform equally
- whether modified albumin is measured appears to depend on assay design,
- in particular whether polyclonal or monoclonal antibodies are used
- While standardization is clearly important, the major cause of variation in urine albumin measurement occurs outside the analytical process
- much can be done to improve practice in both the pre- and post-analytical phases.

Patient	Sample
Hydration status	Collection type
Exercise	Adsorption to plastic
Fever	Storage temperature
Posture	Sample clarity

Specimen Handling for Measurement of Urine Albumin

- Albumin is stable in untreated urine for at least one week when stored at either 4 or 20 °C.
- Freezing at -20 °C causes fragmentation,
- a single freeze-thaw cycle may cause apparent albumin loss of 40%
- Samples should ideally be analyzed fresh
- freezing at -80 °C appears to preserve albumin integrity when measured by immunoassay.
- Creatinine is also stable in urine for at least one week at either 4 or 20 °C and is unaffected by freezing at either -20 or -80 °C.
- Prior to analysis, urine should be inspected for clarity and centrifuged if visibly cloudy.
- There is some evidence that albumin binds to the surface of some plastics which may lead to under-recovery of albumin at low concentrations.
- In the meantime, it seems prudent to collect an adequate volume to minimize potential losses.

Key recommendations for urine albumin sample collection and handling (NKDEP/IFCC)

- 'urine albumin' rather than 'micro-albumin'.
- Patients should be well at baseline. No urinary tract infection, no acute febrile illness, no intense exercise within the previous 24 hours and not be menstruating.
- A fresh, first morning void. A minimum of 5 mL should be collected.
- If a first morning void is not practicable, random spot samples are acceptable.
- Urine creatinine must also be measured.
- Samples not able to be delivered to the laboratory within 8 hours, should be refrigerated.
- Analysis should be performed on the day of receipt but samples can be stored for up to 7 days at 2-8 °C if necessary.
- Cloudy or particulate samples should be centrifuged prior to analysis.
- Positive ACR results must be confirmed, ideally on a fresh, first morning void, by repeat measurement on 1-2 occasions within 3 months.
- Prolonged storage should be at -70 °C; samples should not be stored at -20 °C.

Thank you for your kind attention

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