INTRODUCTION

Uremic syndrome is characterized by the accumulation of uremic toxins due to inadequate kidney function. In literature, more than 90 compounds are identified as uremic toxins ***(Vanholder et al., 2003)***. The European Uremic Toxin (EUTox) Work Group proposed a practical classification based on physico– chemical characteristics that influence their dialytic removal. Such classification defines 'small solutes' as those less than 500 Da, with urea as a prototypal compound, 'middle molecules' are those more than 500 Da such as beta-2-microglobulin, and 'large solutes'; which include α heterogeneous class of molecules such as small and middle protein-bound molecules that are bound to plasma proteins ***(Yavuz et al., 2005; Galli, 2007 and Jourde-Chiche et al., 2009).***

An ideal dialytic therapy should remove all of these compounds. However, only small toxins are easily removed by all dialytic techniques; inspite of introducing some improvements to those techniques ***(Galli et al., 2005; De Smet et al., 2007 and Krieter et al., 2010).***

Despite this, the hemodialysis adequacy and dosing are usually discussed only in terms of Kt/V_{urea}; which is a mathematical model that takes into account the urea clearance in a single hemodialysis session ***(Gotch and Sargent, 1985 and Noce et al., 2012).*** Recent studies showed that Kt/V_{urea} in

dialysis cannot represent correctly the removal of other solutes, indicating that this parameter alone should not be used as the sole indicator of dialysis adequacy ***(Meyer et al., 2011 and Basile and Lomonte, 2012).***

Erythrocyte glutathione transferase (e-GST), an enzyme compartmentalized in the red cells and then non-dialyzable, could be ideal for this role. GSTs represent a super-family of ubiquitous enzymes devoted to cell protection by promoting the conjugation of glutathione with toxins of very different shape ***(Armstrong, 1997; Jakoby and Keen, 1997 and Noce et al., 2012).***

In healthy subjects, the intra- cellular level of e-GST remains virtually constant during childhood and adult life**.** It was found that e-GST increases only in two pathological conditions; that is hyperbilirubinemia and uremia ***(Galli et al., 1999)***. No other pathologies have been reported to induce e-GST hyper-activity ***(Dessi et al., 2012).***

AIM OF THE WORK

The Aim of the present study is to assess the clinical utility of Erythrocyte Glutathione Transferase (e- GST) assay as a biomarker of uremic toxicity in chronic kidney disease patients on conservative therapy. In addition, to investigate if e- GST has a role in assessment of adequacy of hemodialysis comparable to the usual methods used.

Chapter (1)
CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD) is defined as the presence of objective kidney damage and/or the presence of glomerular filtration rate(GFR) of 60 mL/min/1.73 m^2 body surface area or less for at least three months irrespective of the underlying etiology of the kidney damage ***(Levey et al., 2011 and KDIGO, 2013).***

Evidence of kidney damage may be either structural or functional in nature and may be derived from renal histology or from the results of appropriate urine, blood or renal imaging studies. The presence of abnormal sediment on urine microscopy or the demonstration of multiple cysts on renal imaging in a patient with a family history of polycystic kidney disease would meet the requirement for objective kidney damage ***(Levey et al., 2011 and KDIGO, 2013).***

A. Incidence & Prevalence of Chronic Kidney Disease:

CKD is a major medical and economic problem worldwide. Both incidence and prevalence of treated end stage renal disease (ESRD) are increasing in the developing world. Reasons for this include an actual increase in incidence, improved survival from other diseases, and wider acceptance criteria for renal replacement therapy (RRT). The global

incidence estimated to be about 100 new patients per million population ***(Naicker, 2003).***

According to the most recent Egyptian renal registry in 2008, the prevalence of ESRD is 483 per million population as shown in **Figure (1)** and the total recorded number of ESRD patients on dialysis is 40000. Ninety-eight percent of these patients are on hemodialysis (HD) ***(Finkelstein et al., 2007).***

The mean age of ESRD patients in Egypt and most developing countries is much lower, 32 to 42 years, than that in the developed world, 60 to 63 years ***(Rao et al., 2008).***

Males constitute 55.2% of Egyptian ESRD patients. More than half the patients are between 40 and 59 years of age (mean 49.8 ± 19 years). Hypertension is responsible for 36.6% of ESRD cases in Egypt. The other significant percentages are ESRD of unknown etiology (15.2%), diabetic nephropathy (13.5%), and chronic glomerulonephritis (7.8%). Diabetes and hypertension together followed by hypertension alone are the most common causes of CKD at the age of 37-56 years. Diabetes and hypertension together followed by hypertension alone are the most common causes of CKD at the age of 57-76 years. Hypertension is the most common cause of CKD in males and that diabetes and hypertension together are the most common causes of CKD in female ***(Correa-Rotter, 2001& Mahmoud et al., 2009).***

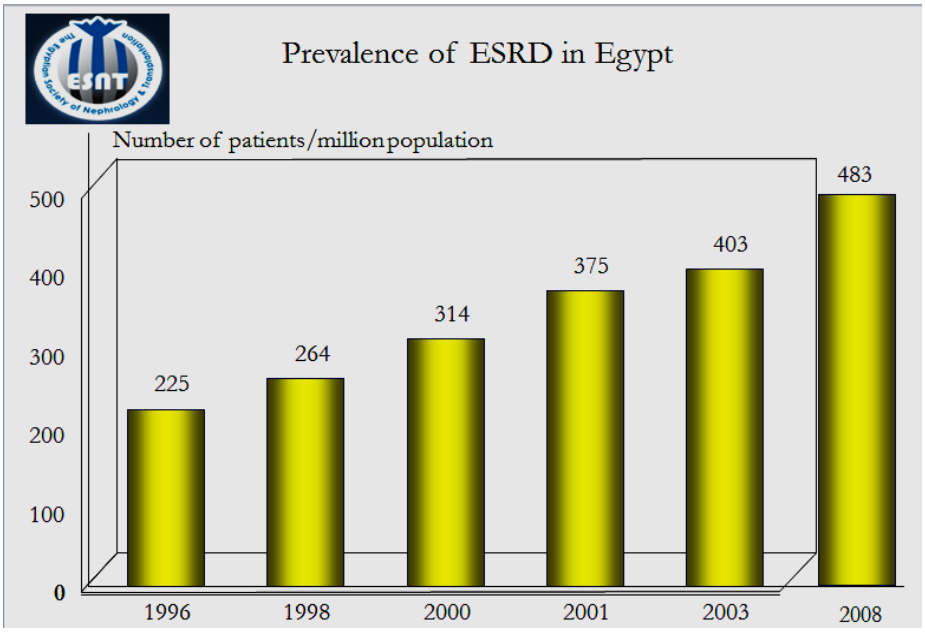

Figure (1): Prevalence of ESRD in Egypt ***(Afifi, 2008).***

B. Pathophysiology of Chronic Kidney Disease:

Approximately one million nephrons are present in each kidney, each contributing to the total GFR. Regardless of the etiology of renal injury, with progressive destruction of nephrons, the kidney has an innate ability to maintain GFR by hyperfiltration and compensatory hypertrophy of the remaining healthy nephrons. This nephron adaptability allows for continued normal clearance of plasma solutes so that substances such as urea and creatinine start to show significant increases in plasma levels only after total GFR has decreased to 50%. When the renal reserve has been exhausted, the plasma creatinine value will approximately double with a 50% reduction in GFR. A rise in plasma creatinine from a baseline

value of 0.6 mg/dL to 1.2 mg/dL in a patient, although still within the reference range, actually represents a loss of 50% of functioning nephron mass. The residual nephron hyperfiltration and hypertrophy, although beneficial for the reasons noted, has been hypothesized to represent a major cause of progressive renal dysfunction. This is believed to occur because of increased glomerular capillary pressure, which damages the capillaries and leads initially to focal and segmental glomerulosclerosis and eventually to global glomerulosclerosis ***(Matyus et al., 2008).***

Factors that may cause progressive renal injury include the following; systemic hypertension, acute insults from nephrotoxins or decreased perfusion, proteinuria, increased renal ammoniagenesis with interstitial injury, hyperlipidemia and hyperphosphatemia with calcium phosphate deposition ***(Polzien, 2007).***

C. Etiology of Chronic Kidney Disease:

1- Diabetic Nephropathy:

Diabetes mellitus is a state of chronic hyperglycemia sufficient to cause long-term damage to specific tissues, notably the retina, kidneys, nerves and arteries. Diabetic nephropathy (DN) is the leading cause of CKD. DN is characterized by progressive expansion of the mesangial matrix and thickening of the glomerular basement membrane, resulting in the obliteration of glomerular capillaries. Advanced glycation end

products (AGEs) produced as the result of hyperglycemia are known to stimulate the production of extracellular matrix (ECM) proteins, resulting in glomerulosclerosis ***(Hideharu et al., 2011).***

Diabetic nephropathy is a clinical diagnosis based on the finding of proteinuria in a patient with diabetes and in whom there is no evidence of urinary tract infection. Overt nephropathy is characterized by protein excretion greater than 0.5 g/day. This is equivalent to albumin excretion of around 300 mg/day. It is preferable to assess proteinuria as albuminuria because it is a more sensitive marker for CKD in diabetic patients. Patients are considered to have microalbuminuria when the urinary albumin excretion is between 30 and 300 mg/day ***(Zerbini et al., 2006).***

In patients with type 1 diabetes, the microalbuminuria will progress to overt nephropathy at an average rate of 20% over 5 years ***(Rigalleau et al., 2008).*** Since the onset of type 2 diabetes is difficult to define, it is difficult to estimate the incidence of microalbuminuria. As albuminuria worsens and blood pressure increases, there is relentless decline in GFR. In some patients with microalbuminuria, renal lesions are already quite advanced and therefore, it may be a marker of nephropathy rather than a predictor of renal structural changes ***(Zerbini et al., 2006).***

There may be a genetic predisposition to develop diabetic nephropathy. Genetic determinants and their impact on the initiation and progression of diabetic nephropathy continue to be actively investigated. Numerous metabolic pathways and associated groups of genes have been proposed as candidates to play a role in the genetic susceptibility to nephropathy ***(Murphy et al., 2008).***

2- Hypertension/Ischemic Kidney Disease:

Hypertension is the second most common attributed etiology of CKD in the world. There is abundant evidence that hypertension, especially systolic hypertension, is a powerful promoter of kidney damage. It may exacerbate the renal injury and rate of decline that occurs from a given disease ***(Hausberg et al., 2008).*** The relationship between hypertension and CKD is difficult to establish because hypertension is a frequent consequence of CKD and thus is likely to be present in a large proportion of subjects with CKD regardless of their initial etiology ***(Padwal et al., 2008).*** However, there is also clear evidence that hypertension predates an increased risk of ESRD. In addition, control of blood pressure clearly decreases the risk of CKD progression ***(Rao et al., 2008).***

Renal artery stenosis is one of the important causes of renal vascular hypertension, which commonly occurs in *women* under the age of 50. The remainder of renal vascular disease is due to atherosclerotic stenosis of the proximal renal arteries

(Lee et al., 2006). The mechanism of hypertension is excessive renin release due to reduction in renal blood flow and perfusion pressure. Renal vascular hypertension may occur when a single branch of the renal artery is stenotic, but in as many as 25% of patients both arteries are obstructed ***(Hausberg et al., 2008).***

3- Post-Infectious Glomerulonephritis:

Post-infectious glomerulonephritis is often following post-streptococcal infections caused by nephritogenic group A beta-hemolytic Streptococci, especially type 2. It commonly appears after pharyngitis within one week after infection. Other causes of post-infectious glomerulonephritis include bacteremic states such as systemic Staphylococcus aureus infection, infective endocarditis and shunt infections ***(Biyth et al., 2007).***

Patients with post-infectious glomerulonephritis complain of oliguria, generalized oedema and variable hypertension. Serum complement levels are low, antistreptolysin O titres (ASOT) can be high unless the immune response had been blunted with previous antibiotic treatment. Classically, the urine is described as cola-colored. Urinary red blood cells, red cell casts, and proteinuria under 3.5 g/day is present. Immunofluorescence shows IgG and complement (C3) in granular basement membrane. Electron microscopy shows large, dense sub-epithelial deposits ***(Srisawat et al., 2006).***

4- Berger's Disease (lgA Nephropathy):

Berger's disease (lgA nephropathy) is a primary renal disease of IgA deposition in the glomerular mesangium. The inciting cause is unknown, but the same lesion is seen in Henoch-Schonlein purpura. IgA nephropathy is also associated with hepatic cirrhosis, celiac disease, and infections such as human immunodeficiency virus (HIV) and cytomegalovirus (CMV)**.**This disease is characterized by hypertension, persistent microscopic hematuria, glomerulosclerosis, abnormal renal function and the most unfavorable prognostic indicator is proteinuria > 1 g/day. Serum IgA level is increased in up to 50% of patients, and for that reason a normal serum IgA does not rule out the disease. Serum complement levels are usually normal. Renal biopsy is the standard for diagnosis. It shows a focal glomerulonephritis with diffuse mesangial IgA deposits and proliferation of mesangial cells in the glomeruli. IgG and C3 can also be seen in the mesangium of all glomeruli ***(Lau et al., 2005).***

5- Nephrotic Disease:

a. Minimal-change disease:

Minimal-change disease is most commonly seen in children but is occasionally present in adults. In patients over 40 years, the incidence of minimal-change disease is 20-25%, with equal distribution between men and women. In younger patients, there is a male predominance. Minimal-change disease

can be idiopathic. However, it can occur following viral upper respiratory tract infections ***(Fujinaga et al., 2006).***

b. Membranous nephropathy:

Membranous nephropathy is the most common cause of primary nephrotic syndrome in adults. It is a disease characterized by immune complex deposition in the sub-epithelial portion of glomerular capillary walls. The antigens in primary disease are not known. Secondary disease is associated with infections such as hepatitis B, endocarditis, and syphilis, autoimmune disease such as systemic lupus erythematosus, mixed connective tissue disease, and thyroiditis, carcinoma and certain drugs such as gold, penicillamine, and captopril. Membranous nephropathy occurs most commonly in adults at their fifth and sixth decades, and almost always after the age of 30 years ***(Lee et al., 2006).***

c. Focal segmental glomerular sclerosis:

This lesion can present as idiopathic disease or secondary to conditions such as heroin use, obesity, and HIV infection. Clinically, patients show evidence of nephrotic syndrome, but they also have more nephritic features than membranous nephropathy or minimal-change disease ***(Appel, 2014).***

Eighty percent of patients have microscopic hematuria at presentation, and many are hypertensive. Decreased renal function is present in 25-50% of cases at time of diagnosis. The

diagnosis requires renal biopsy. Light microscopy shows the lesions of focal segmental glomerular sclerosis. It is thought that these lesions occur first in the juxta-medullary glomeruli and are then seen in the superficial renal cortex. IgM and C3 are seen in the sclerotic lesions on immunofluorescence. Electron microscopy shows fusion of epithelial foot processes as seen in minimal-change disease. Patients with focal segmental glomerular sclerosis typically progress to end-stage renal disease in 6-8 years ***(Barisoni et al., 2009).***

D. Staging of Chronic Kidney Disease

The purpose of CKD staging is to guide management, including stratification of risk for progression and complications of CKD. Risk stratification is used as a guide to inform appropriate treatments and the intensity of monitoring and patient education ***(Levey et al., 2009).***

In patients who are diagnosed with CKD using the criteria described above, staging of the CKD is done according to : cause of the disease, GFR and albuminuria ***(KDIGO, 2013).***

1. Cause of kidney disease:

Identifying the cause of kidney disease (eg, diabetes, drug toxicity, auto-immune diseases, urinary tract obstruction, kidney transplantation, etc.) enables specific therapy directed preventing further injury. In addition, the cause of kidney disease has implications for the rate of progression and the risk

of complications. It can be difficult to ascertain the cause of kidney disease. In clinical practice, CKD is most often discovered as decreased eGFR during the evaluation and management of other medical conditions ***(Seliger et al., 2008 and Levey et al., 2011).***

2. GFR:

The GFR (G-stages) follow the original CKD classification scheme:

Table (1): Stages of Chronic Kidney Disease

Stage	Description	GFR(mL/min/1.73 m2)
G1	Kidney damage with normal or increasing GFR	≥ 90
G2	Kidney damage with mild decreasing GFR	60-89
G3a	Moderate decreasing GFR	45-59
G3b	Severe decreasing GFR	30-44
G4	Severe decreasing GFR	15-29
G5	Kidney failure	<15 (or dialysis)

GFR: glomerular filtration rate ***(National Kidney Foundation, 2002).***

Since the original Kidney Disease Improving Global Outcomes (KDIGO) classification was published, stage 3 CKD (a GFR of 30 to 59 mL/min per 1.73 m2) has been subdivided into GFR stages 3a and 3b to be more accurately reflect the continuous association between lower GFR and risk for mortality and adverse kidney outcomes. Patients receiving treatment with dialysis are subclassified as GFR stage 5D to

highlight the specialized care that they require ***(NKF, 20O2 and Levey et al., 2011).***

3. Albuminuria:

The three albuminuria stages follow familiar definitions of “normal”, “high” (formerly microalbuminuria), and “very high” (formerly macroalbuminuria and nephrotic range) albuminuria:

Table (2): Stages of albuminuria

Albuminuria (A)	ACR
A1	<30mg/g (3.4mg/mmol)
A2	30-300mg/g (3.4-34.0mg/mmol)
A3	>300mg/g (>34.0mg/mmol)

ACR : Albumin Creatinine Ratio

(Levey and Coresh, 2012)

The addition of albuminuria staging to GFR staging was done in 2005 since the original KDIGO classification scheme was published ***(Levey et al., 2005).***

Albuminuria staging has been added because of the graded increase in risk for mortality, progression of CKD, and ESRD at higher levels of albuminuria, independent of eGFR, without an apparent threshold value ***(Levey et al., 2011).***