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Chronic kidney disease (CKD) is a clinical syndrome characterized by a gradual loss of kidney function that persists for >3 months with health implications and affects nearly 500 million people worldwide. Diabetes and hypertension are the 2 most common causes of CKD, which account for up to two-thirds of the cases.1-3 Accumulating evidence has shown that hyperuricemia is tightly associated with the pathogenesis of CKD.1-4 Hyperuricemic nephropathy is a condition characterized by glomerular hypertension, arteriolosclerosis, and tubule interstitial fibrosis. Prior studies demonstrate that decreasing uric acid levels delays the development of CKD and slows its progression and uric acid is an independent predictor of future development of CKD.1-4 The mechanistic processes by which hyperuricemia induces chronic renal injury involve deposition of uric acid crystals in the collecting duct of the kidney, renal angiotension system activation, oxidative stress, tubular epithelial cell transition and inflammation.4-7

The epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptors. EGFR is activated by binding of its specific ligands such as epidermal growth factor (EGF) and transforming growth factor α (TGFα). Additionally, EGFR can be transactivated by cytokines, oxidants as well as indirectly via activation of G protein-coupled receptors such as angiotension II receptor and G protein-coupled estrogen receptor-1 (GPER-1).8-10 Upon activation, EGFR undergoes dimerization, a transition from an inactive monomeric form to an active homodimer. EGFR dimerization promotes its intrinsic intracellular protein-tyrosine kinase activity, which results in autophosphorylation of several tyrosine residues in the C-terminal domain of EGFR. This autophosphorylation initiates several signal transduction cascades such as the mitogen-activated protein kinases (MAPK), protein kinase B (PKB) also known as Akt and Jun amino-terminal kinases (JNK) pathways, regulating cell migration, adhesion, and proliferation.8,9

Numerous studies have shown that EGFR activation contributes to chronic renal injury and glomerular sclerosis.4,8 Intriguingly, a recent study by Liu et al4 reported that EGFR activation was critically involved in uric acid-induced chronic renal injury in a rat model of hyperuricemic nephropathy. Using this model, they demonstrated that blockage of EGFR by gefitinib, a drug that specially inhibits EGFR activation and is clinically used for treating various cancers, alleviated renal fibrosis and renal tubular injury and inhibited activation of renal interstitial fibroblast, a pathological process that initiates the development of renal fibrosis and promotes its progression.7 Mechanistically, inhibition of EGFR abrogated the expression of TGF-β and phosphorylation of Smad3 (a downstream molecule of TGF-β), and blocked NF-κB pathway activation and macrophage infiltration in the kidney of hyperuricemic rat.4 In addition, gefitinib treatment decreased release of various proinflammatory cytokines/chemokines including TGFα, interleukin 1 beta (IL-1β), monocyte chemoattractant protein-1 (MCP-1) and RANTES.4 Furthermore, blockage of EGFR reduced serum uric acid levels through inhibiting the activity of the enzyme xanthine oxidase, a key enzyme for the production of uric acid, as well as through preventing the downregulation of urate transporters, organic anion transporter
1 (OAT1) and OAT3. Collectively, Liu and colleagues have demonstrated for the first time that EGFR activity contributes to the pathogenesis of hyperuricemic nephropathy and that EGFR blockage alleviates development of hyperuricemia-induced nephropathy through blocking TGF-β signaling pathway, inhibiting inflammatory response, and reducing uric acid production. Thus, EGFR may serve as a therapeutic target for treating uric acid-associated CKD.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES


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Obesity and Kidney Disease: Hidden Consequences of the Epidemic

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ABSTRACT

Obesity has become a worldwide epidemic, and its prevalence has been projected to grow by 40% in the next decade. This increasing prevalence has implications for the risk of diabetes, cardiovascular disease and also for chronic kidney disease (CKD). A high body mass index is one of the strongest risk factors for new-onset CKD. In individuals affected by obesity, a compensatory hyperfiltration occurs to meet the heightened metabolic demands of the increased body weight. The increase in intraglomerular pressure can damage the kidneys and raise the risk of developing CKD in the long-term. The incidence of obesity-related glomerulopathy has increased ten-fold in recent years. Obesity has also been shown to be a risk factor for nephrolithiasis, and for a number of malignancies including kidney cancer. This year the “World Kidney Day” promotes education on the harmful consequences of obesity and its association with kidney disease, advocating healthy lifestyle and health policy measures that makes preventive behaviors an affordable option.

KEYWORDS: Obesity; Chronic kidney disease; Nephrolithiasis; Kidney cancer; Prevention.

ABBREVIATIONS: WHO: World Health Organization; CKD: Chronic Kidney Disease; ESRD: End-Stage Renal Disease; CVD: Cardiovascular disease; WC: Waist Circumference; WHR: Waist Hip Ratio; GFR: Glomerular Filtration Rate; IGF-1: Insulin-like growth factor 1; ISN: International Society of Nephrology; IFKF: International Federation of the Kidney Foundation.

INTRODUCTION

In 2014, over 600 million adults worldwide, 18 years and older, were obese. Obesity is a potent risk factor for the development of kidney disease. It increases the risk of developing major risk factors for chronic kidney disease (CKD), like diabetes and hypertension, and it has a direct impact on the development of CKD and end-stage renal disease (ESRD). In individuals affected by obesity, a (likely) compensatory mechanism of hyperfiltration occurs to meet the heightened metabolic demands of the increased body weight. The increase in intraglomerular pressure can damage the kidney structure and raise the risk of developing CKD in the long-term.

The good news is that obesity, as well as the related CKD, are largely preventable. Education and awareness of the risks of obesity and a healthy lifestyle, including proper nutrition and exercise, can dramatically help in preventing obesity and kidney disease. This
article reviews the association of obesity with kidney disease on the occasion of the 2017 World Kidney Day.

**EPIDEMIOLOGY OF OBESITY IN ADULTS AND CHILDREN**

Over the last 3 decades, the prevalence of overweight and obese adults (body mass index (BMI)≥25 kg/m²) worldwide has increased substantially.¹ In the US, the age-adjusted prevalence of obesity in 2013-2014 was 35% among men and 40.4% among women.² The problem of obesity also affects children. In the US in 2011-2014, the prevalence of obesity was 17% and extreme obesity 5.8% among youth 2-19 years of age. The rise in obesity prevalence is also a worldwide concern,³,⁴ as it is projected to grow by 40% across the globe in the next decade. Low- and middle-income countries are now showing evidence of transitioning from normal weight to overweight and obesity as parts of Europe and the United States did decades ago.³ This increasing prevalence of obesity has implications for cardiovascular disease (CVD) and also for CKD. A high BMI is one of the strongest risk factors for new-onset CKD.⁵,⁶

Definitions of obesity are most often based on BMI (i.e. weight [kilograms] divided by the square of his or her height [meters]). A BMI between 18.5 and 25 kg/m² is considered by the World Health Organization (WHO) to be normal weight, a BMI between 25 and 30 kg/m² as overweight, and a BMI of >30 kg/m² as obese. Although BMI is easy to calculate, it is a poor estimate of fat mass distribution, as muscular individuals or those with more subcutaneous fat may have a BMI as high as individuals with larger intra abdominal (visceral) fat. The latter type of high BMI is associated with substantially higher risk of metabolic and cardiovascular disease. Alternative parameters to more accurately capture visceral fat include waist circumference (WC) and a waist hip ratio (WHR) of >102 cm and 0.9, respectively, for men and >88 cm and >0.8, respectively, for women. WHR has been shown to be superior to BMI for the correct classification of obesity in CKD.

**ASSOCIATION OF OBESITY WITH CKD AND OTHER RENAL COMPLICATIONS**

Numerous population based studies have shown an association between measures of obesity and both the development and the progression of CKD (Table 1). Higher BMI is associated with the presence⁶ of proteinuria in individuals without kidney disease. Furthermore, in numerous large population-based studies, higher BMI appears associated with the presence⁴ and development of low estimated glomerular filtration rate (GFR),⁸,¹⁰,¹³ with more rapid loss of estimated GFR over time,¹⁴ and with the incidence of ESRD.¹⁵,¹⁶ Elevated BMI levels, class II obesity and above, have been associated with more rapid progression of CKD in patients with pre-existing CKD.¹⁹ A few studies examining the association of abdominal obesity using WHR or WC with CKD, describe an association between higher girth and albuminuria,¹⁹ decreased GFR⁶ or incident ESRD⁷ independent of BMI level.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Exposure</th>
<th>Outcomes</th>
<th>Results</th>
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<tr>
<td>Prevention of Renal and Vascular End-Stage Disease (PREVEND) Study⁵</td>
<td>7,676 Dutch individuals without diabetes</td>
<td>Elevated BMI (overweight and obese*), and central fat distribution (waist-hip ratio)</td>
<td>-Presence of urine albumin 30-300 mg/24h</td>
<td>-Obese + central fat: higher risk of albuminuria</td>
<td>Cross sectional analysis</td>
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<td>Multinational study of hypertensive outpatients¹⁰</td>
<td>20,828 patients from 26 countries</td>
<td>BMI and waist circumference</td>
<td>Prevalence of albuminuria by dip stick</td>
<td>Higher waist circumference associated with albuminuria independent of BMI</td>
<td>Cross sectional analysis</td>
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<td>Framingham Multi-Detector Computed Tomography (MDCT) cohort¹¹</td>
<td>3,099 individuals</td>
<td>Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT)</td>
<td>Prevalence of UACR &gt;25 mg/g in women and &gt;17 mg/g in men</td>
<td>VAT associated with albuminuria in men, but not in women</td>
<td>Cross sectional analysis</td>
</tr>
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<td>CARDIA (Coronary Artery Risk Development in Young Adults) study¹²</td>
<td>2,354 community-dwelling individuals with normal kidney function aged 28-40 years</td>
<td>Obesity (BMI &gt;30 kg/m²), Diet and lifestyle-related factors</td>
<td>Incident microalbuminuria</td>
<td>Obesity (OR 1.9) and unhealthy diet (OR 2.0) associated with incident albuminuria</td>
<td>Low number of events</td>
</tr>
<tr>
<td>Hypertension Detection and Follow-Up Program¹³</td>
<td>5,897 hypertensive adults</td>
<td>Overweight and obese BMI* vs. normal BMI</td>
<td>Incident CKD (1+ or greater proteinuria on urinalysis and/or an eGFR &lt;60 mL/min/1.73 m²)</td>
<td>Both overweight (OR 1.21) and obesity (OR 1.40) associated with incident CKD</td>
<td>Results unchanged after excluding diabetics</td>
</tr>
</tbody>
</table>
Higher visceral adipose tissue measured by computed tomography has been associated with a higher prevalence of albuminuria in men. The observation of a BMI-independent association between abdominal obesity and poorer renal outcomes is also described in relationship with mortality in patients with ESRD and kidney transplant, and suggests a direct role of visceral adiposity. In general, the associations between obesity and poorer renal outcomes persist even after adjustments for possible mediators of obesity’s cardiovascular and metabolic effects, such as high blood pressure and diabetes mellitus, suggesting that obesity may affect kidney function through mechanisms in part unrelated to these complications (vide infra).

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<tr>
<th>Study Description</th>
<th>Participants</th>
<th>BMI Categories</th>
<th>Incident Proteinuria</th>
<th>Higher BMI not associated with CKD3 after adjustments</th>
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<tr>
<td>Framingham Offspring Study&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,676 individuals free of CKD stage 3</td>
<td>High vs. normal BMI&lt;sup&gt;*&lt;/sup&gt;</td>
<td>-Incident CKD stage 3</td>
<td>-Higher BMI not associated with CKD3 after adjustments</td>
<td>-Higher BMI associated with increased odds of incident proteinuria</td>
<td>Predominantly white, limited geography</td>
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<td>Physicians’ Health Study&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11,104 initially healthy men in US</td>
<td>-BMI quintiles</td>
<td>Incident eGFR &lt;60 mL/min/1.73 m²</td>
<td>-Higher baseline BMI and increase in BMI over time both associated with higher risk of incident CKD</td>
<td>Predominantly white, limited geography</td>
<td>Exclusively men</td>
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<td>Nation-wide US Veterans Administration cohort&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3,376,187 US veterans with baseline eGFR ≥60 mL/min/1.73 m²</td>
<td>BMI categories from &lt;20 to &gt;50 kg/m²</td>
<td>Rapid decline in kidney function (negative eGFR slope of &gt;5 mL/min/1.73 m²)</td>
<td>-Higher BMI associated with 3x higher risk of CKD</td>
<td>Associations more accentuated in older individuals</td>
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<td>Nation-wide population-based study from Sweden&lt;sup&gt;d&lt;/sup&gt;</td>
<td>926 Swedes with moderate/advanced CKD compared to 998 controls</td>
<td>BMI ≥25 vs. &lt;25 kg/m²</td>
<td>CKD vs. no CKD</td>
<td>Higher BMI associated with 3x higher risk of CKD</td>
<td>-Risk strongest in diabetics, but also significantly higher in non-diabetics</td>
<td>-Cross-sectional analysis</td>
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<td>Nation-wide population-based study in Israel&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1,194,704 adolescent males and females examined for military service</td>
<td>Elevated BMI (overweight and obesity) vs. normal BMI&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Incident ESRD</td>
<td>Overweight (HR 3.0) and obesity (HR 6.89) associated with higher risk of ESRD</td>
<td>Associations strongest for diabetic ESRD, but also significantly higher for non-diabetic ESRD</td>
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<td>The Nord-Trøndelag Health Study (HUNT-1)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>74,986 Norwegian adults</td>
<td>BMI categories*</td>
<td>Incidence of ESRD or renal death</td>
<td>BMI &gt;30 kg/m² associated with rapid loss of kidney function</td>
<td>Associations strong in men, but not in women</td>
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<td>Community-based screening in Okinawa, Japan&lt;sup&gt;g&lt;/sup&gt;</td>
<td>100,753 individuals &gt;20 years old</td>
<td>BMI quartiles</td>
<td>Incidence of ESRD</td>
<td>Higher BMI associated with increased risk of ESRD in men, but not in women</td>
<td>Average BMI lower in Japan compared to Western countries</td>
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<tr>
<td>Nation-wide US Veterans Administration cohort&lt;sup&gt;h&lt;/sup&gt;</td>
<td>453,946 US veterans with baseline eGFR ≥60 mL/min per 1.73 m²</td>
<td>BMI categories from &lt;20 to &gt;50 kg/m²</td>
<td>Incident ESRD</td>
<td>-Incidence of ESRD</td>
<td>Associations present but weaker in patients with more advanced CKD</td>
<td></td>
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<tr>
<td>Kaiser Permanente Northern California&lt;sup&gt;i&lt;/sup&gt;</td>
<td>320,252 adults with and without baseline CKD</td>
<td>Overweight, class I, II and extreme obesity; vs. normal BMI&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Incident of ESRD</td>
<td>Linearly higher risk of ESRD with higher BMI categories</td>
<td>Associations remained present after adjustment for DM, hypertension and baseline CKD</td>
<td></td>
</tr>
<tr>
<td>REGARDS (Reasons for Geographic and Racial Differences in Stroke) Study&lt;sup&gt;j&lt;/sup&gt;</td>
<td>30,239 individuals</td>
<td>Elevated waist circumference or BMI</td>
<td>Incident of ESRD</td>
<td>BMI above normal not associated with ESRD after adjustment for waist circumference</td>
<td>Association of waist circumference with ESRD became on-significant after adjustment for comorbidities and baseline eGFR and proteinuria</td>
<td></td>
</tr>
</tbody>
</table>

<sup>*Normal weight: BMI 18.5 to 24.9 kg/m²; overweight: BMI 25.0 to 29.9 kg/m²; class I obesity: BMI 30.0 to 34.9 kg/m²; class II obesity: BMI 35.0 to 39.9 kg/m²; class III obesity: BMI ≥40 kg/m²; BMI: Body Mass Index; CKD: Chronic Kidney Disease; DM: Diabetes Mellitus; eGFR: estimated Glomerular Filtration Rate; ESRD: End Stage Renal Disease; HR: Hazard Ratio; OR: Odds Ratio; UACR: Urine Albumin-Creatinine Ratio.</sup>

Table 1: Studies examining the association of obesity with various measures of CKD.
The deleterious effect of obesity on the kidneys extends to other complications such as nephrolithiasis and kidney malignancies. Higher BMI is associated with an increased prevalence and incidence of nephrolithiasis. Furthermore, weight gain over time, and higher baseline WC were also associated with higher incidence of nephrolithiasis. Obesity is associated with various types of malignancies, particularly cancers of the kidneys. In a population-based study of 5.24 million individuals from the UK, a 5 kg/m² higher BMI was associated with a 25% higher risk of kidney cancers, with 10% of all kidney cancers attributable to excess weight. Another large analysis examining the global burden of obesity on malignancies estimated that 17% and 26% of all kidney cancers in men and women, respectively, were attributable to excess weight. The association between obesity and kidney cancers was consistent in both men and women, and across populations from different parts of the world in a meta-analysis that included data from 221 studies (of which 17 examined kidney cancers). Among the cancers examined in this meta-analysis, kidney cancers had the 3rd highest risk associated with obesity (relative risk per 5 kg/m² higher BMI: 1.24, 95% CI 1.20-1.28, p<0.0001).

MECHANISMS OF ACTION UNDERLYING THE RENAL EFFECTS OF OBESITY

Obesity results in complex metabolic abnormalities which have wide-ranging effects on diseases affecting the kidneys. The exact mechanisms whereby obesity may worsen or cause CKD remain unclear. The fact that most obese individuals never develop CKD, and the distinction of up to as many as 25% of obese individuals as “metabolically healthy” suggests that increased weight alone is not sufficient to induce kidney damage. Some of the deleterious renal consequences of obesity may be mediated by downstream comorbid conditions such as diabetes mellitus or hypertension, but there are also effects of adiposity which could impact the kidneys directly, induced by the endocrine activity of the adipose tissue via production of (among others) adiponectin, leptin, and resistin.

(Figure 1). These include the development of inflammation, oxidative stress, abnormal lipid metabolism, activation of the renin-angiotensin-aldosterone system, and increased production of insulin and insulin resistance.

These various effects result in specific pathologic changes in the kidneys which could underlie the higher risk of CKD seen in observational studies. These include ectopic lipid accumulation and increased deposition of renal sinus fat, the development of glomerular hypertension and increased glomerular permeability caused by hyperfiltration-related glomerular filtration barrier injury, and ultimately the development of glomerulomegaly and focal or segmental glomerulosclerosis (Figure 2). The incidence of the so-called obesity-related glomerulopathy (ORG) has increased ten-fold between 1986 and 2000. Importantly, ORG often presents along with pathophysiologic processes related to other conditions or advanced age, conspiring to result in more accentuated kidney damage in patients with high blood pressure or in the elderly.

Obesity is associated with a number of risk factors contributing to the higher incidence and prevalence of nephrolithiasis. Higher body weight is associated with lower urine pH and increased urinary oxalate, uric acid, sodium and phosphate excretion diets richer in protein and sodium may lead to a more acidic urine and decrease in urinary citrate, also contributing to kidney stone
risk. The insulin resistance characteristic of obesity may also predispose to nephrolithiasis through its impact on tubular Na-H exchanger and ammoniagenesis, and the promotion of an acidic milieu. Complicating the picture is the fact that some weight loss therapies result in a worsening, rather than an improvement in the risk for kidney stone formation; e.g. gastric surgery can lead to a substantial increase in enteral oxalate absorption and enhanced risk of nephrolithiasis.

The mechanisms behind the increased risk of kidney cancers observed in obese individuals are less well characterized. Insulin resistance, and the consequent chronic hyperinsulinemia and increased production of insulin-like growth factor 1 (IGF-1) and numerous complex secondary humoral effects may exert stimulating effects on the growth of various types of tumor cells. More recently, the endocrine functions of adipose tissue, its effects on immunity, and the generation of an inflammatory milieu with complex effects on cancers have emerged as additional explanations.

OBESITY IN PATIENTS WITH ADVANCED KIDNEY DISEASE: THE NEED FOR A NUANCED APPROACH

Considering the above evidence about the overwhelmingly deleterious effects of obesity on various disease processes, it is seemingly counterintuitive that obesity has been consistently associated with lower mortality rates in patients with advanced CKD and ESRD. Similar “paradoxical” associations have also been described in other populations, such as in patients with congestive heart failure, chronic obstructive pulmonary disease, rheumatoid arthritis, and even in old individuals. It is possible that the seemingly protective effect of a high BMI is the result of the imperfection of BMI as a measure of obesity, as it does not differentiate the effects of adiposity from those of higher non-adipose tissue. Indeed, studies that separated the effects of a higher waist circumference from those of higher BMI showed a reversal of the inverse association with mortality. Higher muscle mass has also been shown to explain at least some of the positive effects attributed to elevated BMI. However, there is also evidence to suggest that higher adiposity, especially subcutaneous (non-visceral) fat, may also be associated with better outcomes in ESRD patients. Such benefits may indeed be present in patients who have very low short term life expectancy, such as most ESRD patients. Indeed, some studies that examined the association of BMI with time-dependent survival in ESRD have shown a marked contrast between protective short term effects vs. deleterious longer term effects of higher BMI. There are several putative short term benefits that higher body mass could portend, especially to sicker individuals. These include a benefit from the better nutritional status typically seen in obese individuals, and which provides better protein and energy reserves in the face of acute illness, and a higher muscle mass with enhanced antioxidant capacity and lower circulating actin and higher plasma gelsolin levels, which are associated with better outcomes. Other hypothetically beneficial characteristics of obesity include a more stable hemodynamic status with mitigation of stress responses and heightened sympathetic and renin-angiotensin activity increased production of adiponectines and soluble tumor necrosis factor alfa receptors by adipose tissue neutralizing the adverse effects of tumor necrosis factor alfa; enhanced binding of circulating endotoxins by the characteristically higher cholesterol levels seen in obesity; and sequestration of uremic toxins by adipose tissue.

POTENTIAL INTERVENTIONS FOR MANAGEMENT OF OBESITY

Obesity engenders kidney injury via direct mechanisms through deranged synthesis of various adipose tissue cytokines with nephrotoxic potential, as well as indirectly by triggering diabetes and hypertension, i.e. two conditions that rank among the strongest risk factors for CKD. Perhaps due to the survival advantage of obesity in CKD, the prevalence of end stage kidney disease is on...
the rise both in the USA\textsuperscript{77} and in Europe.\textsuperscript{78} Strategies for controlling the obesity related CKD epidemic at population level and for countering the evolution of CKD toward kidney failure in obese patients represent the most tantalizing task that today’s health planners, health managers and nephrologists face.

**COUNTERING CKD AT POPULATION LEVEL**

Calls for public health interventions in the community to prevent and treat CKD at an early stage have been made by major renal associations, including the International Society of Nephrology (ISN), International Federation of the Kidney Foundation (IFKF), the European renal association (ERA-EDTA) and various national societies. In the USA, Healthy People 2020, a program that sets 10-year health targets for health promotion and prevention goals, focuses both on CKD and obesity. Surveys to detect obese patients, particularly those with a high risk of CKD (e.g., hypertensive and/or diabetic obese people) and those receiving suboptimal care to inform these patients of the potential risk for CKD they are exposed to, is the 1\textsuperscript{st} step towards developing public health interventions. Acquiring evidence that current interventions to reduce CKD risk in the obese are efficacious and deployable, is an urgent priority to set goals and means for risk modification. Appropriate documentation of existing knowledge distilling the risk and the benefits of primary and secondary prevention interventions in obese people, and new trials in this population to fill knowledge gaps (see below) are needed. Finally, surveillance programs that monitor progress on the detection of at-risk individuals and the effectiveness of prevention programs being deployed\textsuperscript{79} constitute the third, fundamental element for establishing efficacious CKD prevention plans at population level.

A successful surveillance system for CKD has already been implemented in some places such as the United Kingdom (UK).\textsuperscript{80} A campaign to disseminate and apply K-DOQI CKD guidelines in primary care within the UK National Health Service was launched. This progressively increased the adoption of K-DOQI guidelines and, also thanks to specific incentives for UK general physicians to detect CKD, led to an impressive improvement in the detection and care of CKD, i.e., better control of hypertension and increased use of angiotensin-converting enzyme and angiotensin receptor blockers.\textsuperscript{80} This system may serve as a platform to improve the prevention of obesity-related CKD. Campaigns aiming at reducing the obesity burden are now at center stage worldwide and are strongly recommended by the WHO and it is expected that these campaigns will reduce the incidence of obesity-related complications, including CKD. However obesity-related goals in obese CKD patients remain vaguely formulated, largely because of the paucity of high-level evidence intervention studies to modify obesity in CKD patients.\textsuperscript{81}

**PREVENTION OF CKD PROGRESSION IN OBESE PEOPLE WITH CKD**

Observational studies in metabolically healthy obese subjects show that the obese phenotype unassociated with metabolic abnormalities per se predicts a higher risk for incident CKD\textsuperscript{82} suggesting that obesity per se may engender renal dysfunction and kidney damage even without diabetes or hypertension (vide supra). In overweight or obese diabetic patients, a lifestyle intervention including caloric restriction and increased physical activity compared with a standard follow-up based on education and support to sustain diabetes treatment reduced the risk for incident CKD by 30\%, although it did not affect the incidence of cardiovascular events.\textsuperscript{83} Such a protective effect was partly due to reductions in body weight, HbA1c, and systolic BP. No safety concerns regarding kidney-related adverse events were seen.\textsuperscript{83} In a recent meta-analysis collating experimental studies in obese CKD patients, interventions aimed at reducing body weight showed coherent reductions in blood pressure, glomerular hyper-filtration and proteinuria.\textsuperscript{81} A thorough post-hoc analysis of the REIN study showed that the nephron-protective effect of ACE inhibition in proteinuric CKD patients was maximal in obese CKD patients, but minimal in CKD patients with normal or low BMI.\textsuperscript{84} Of note, bariatric surgical intervention have been suggested for selected CKD and ESRD patients including dialysis patients who are waitlisted for kidney transplantation.\textsuperscript{85-87}

Globally, these experimental findings provide a proof of concept for the usefulness of weight reduction and ACE inhibition interventions in the treatment of CKD in the obese. Studies showing a survival benefit of increased BMI in CKD patients, however, remain to be explained.\textsuperscript{88} These findings limit our ability to make strong recommendations about the usefulness and the safety of weight reduction among individuals with more advanced stages of CKD. Lifestyle recommendations to reduce body weight in obese people at risk for CKD and in those with early CKD appear justified, particularly recommendations for the control of diabetes and hypertension. As the independent effect of obesity control on the incidence and progression of CKD is difficult to disentangle from the effects of hypertension and type 2 diabetes (T2D), recommendation of weight loss in the minority of metabolically healthy, non-hypertensive obese patients remains unwarranted. These considerations suggest that a therapeutic approach to overweight and obesity in patients with advanced CKD or other significant comorbid conditions has to be pursued carefully, with proper considerations of the expected benefits and potential complications of weight loss over the life span of the individual patient.
CONCLUSIONS

The worldwide epidemic of obesity affects the Earth’s population in many ways. Diseases of the kidneys, including CKD, nephrolithiasis and kidney cancers are among the more insidious effects of obesity, but which nonetheless have wide ranging deleterious consequences, ultimately leading to significant excess morbidity and mortality and excess costs to individuals and the entire society. Population-wide interventions to control obesity could have beneficial effects in preventing the development, or delaying the progression of CKD. It is incumbent upon the entire healthcare community to devise long-ranging strategies towards improving the understanding of the links between obesity and kidney diseases, and to determine optimal strategies to stem the tide. The 2017 World Kidney Day is an important opportunity to increase education and awareness to that end.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES


68. Noori N, Kopple JD, Kovesdy CP, et al. Mid-arm muscle circumference and quality of life and survival in maintenance hemo-


The Unfolded Protein Response: A Novel Insight into Chronic Kidney Disease (CKD)

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Chronic kidney disease (CKD) is a clinical syndrome, characterized by a progressive decline of renal function. CKD is associated with a wide range of metabolic abnormalities including hypertension, anemia, acidosis, and mineral bone diseases. The progression of CKD may lead to end-stage renal diseases. CKD affects nearly 500 million people worldwide and thus has become a global public health concern. While the pathogenesis of CKD remains poorly understood, accumulating studies have revealed that the endoplasmic reticulum (ER) stress contributes to the onset and progression of CKD including renal fibrosis, which can lead to end-stage renal disease, independent of the original cause of CKD.

ER is an organelle responsible for protein synthesis, folding, assembly, modification and secretion. Numerous adverse factors, such as oxidative stress, energy depletion, Ca²⁺ depletion, overproduction of misfolded or unassembled proteins, altered glycosylation, ischemia/hypoxia and inflammation, can disturb ER homeostasis leading to accumulation of misfolded proteins in the ER lumen, a condition termed ‘ER stress’. To clear misfolded and aggregated proteins, cells have evolved a series of evolutionarily conserved signal machineries for orchestrating adaptive responses to maintain the ER homeostasis. One of these machineries is the unfolded protein response (UPR) which is executed by three ER transmembrane protein sensors (Figure 1): ER kinase (PKR)-like ER kinase (PERK), inositol-requiring kinase 1 (IRE1), and activating transcription factor 6 (ATF6). Upon ER stress, an ER lumen resident protein,
BiP (binding immunoglobulin protein chaperon, or glucose-regulated protein 78) dissociates from these protein sensors and binds to misfolded proteins, the first step that triggers the UPR pathways. PERK activation results in the phosphorylation and inhibition of eIF2α, a component of the translation initiation complex, thereby attenuating protein translation to decrease protein load on the ER. Activated IRE1 splices XBP1 mRNA, which in turn induces transcription of different genes involved in the ER-associated degradation pathway. This IRE1-mediated arm targets misfolded or unassembled proteins to ubiquitin-proteasomal and/or autophagy-lysosomal machineries for degradation. Additionally, UPR activation induces translocation of ATF6 to the Golgi and its cleavage by site 1 and site 2 proteases in the Golgi.\(^1,2\) The cytosolic, DNA-binding fragment of ATF6 then traffics to the nucleus, where it activates the transcription of ER chaperones and enzymes that promote protein folding. Consequently, activated UPR reduces global protein synthesis, enhances degradation of misfolded proteins, and increases the ER protein-folding capacity. Activation of the UPR may alleviate ER stress and restore ER homeostasis, thereby promoting cell survival. However, if pathogenic stimuli are severe and persistent and ER stress cannot be rescued, excessive activation of the UPR will initiate pro-apoptotic pathways to eliminate the stressed cells.\(^9\) In this case, the PERK arm of the UPR may also trigger activation of ATF4, a transcription factor that increases the transcription of pro-apoptotic genes such as CHOP (CCAAT-enhancer-binding protein homologous protein) and decreases anti-apoptotic genes such as B-cell lymphoma 2 (Bcl-2).\(^1,3,8\) Furthermore, the IRE1α arm may also activate Caspase 12 (rodents)/Caspase 4 (humans) and c-Jun N-terminal kinase (JNK) phosphorylation, leading to initiation of pro-apoptotic pathways.\(^2\) Thus, the UPR is a double-edged sword acting as either a cell-protective machinery during mild ER stress or a cell-destructive terminator during severe or long-term ER stress. Interestingly, accumulating evidence has shown the association of ER stress and dysregulated UPR with the pathogenesis of CDK.\(^1,4\)

Several lines of evidence from clinical and experimental studies have revealed that the accumulation of misfolded proteins of the slit diaphragm elicits ER stress-associated renal injury and proteinuria.\(^1,5\) It has been demonstrated that various pathogenic factors, such as proteinuria, hyperglycemia, and uremic toxins, induce UPR-mediated cell apoptosis, compromise the repair capacity of tubular epithelial cells, and accelerate the progression of CKD.\(^1,2\) Chiang et al\(^1\) reported that ER stress-induced excessive activation of UPR leads to tubular cell death and promotes tubulointerstitial fibrosis in a rat model, which can be alleviated through the optimization of UPR activation by an angiotension receptor blocker, Candesartan. Proteinuria can induce severe tubulointerstitial injury, leading to end-stage renal disease in CKD.\(^5,7\) El Karoui et al\(^8\) demonstrated that albumin activates the UPR via increasing cytosolic calcium in tubular cells, resulting in tubular apoptosis through ATF4-mediated Lipocalin 2 (LCN2) modulation. LCN2, a small, secreted iron-transporting protein, has been considered as a biomarker for endothelial damage, inflammatory processes, and kidney injury.\(^10,11\) Induction of ER stress upregulates LCN2 expression in human prostate cancer cells, while inhibition of the UPR by 4-phenylbutyric acid remarkably down regulates LCN2 production.\(^11\) Intriguingly, a high level of LCN2 was found in proteinuric patients with CKD.\(^8\) Animal experiments showed that LCN2 transcription and translation progressively increase in proteinuric mice.\(^3\) Downregulation of LCN2 attenuates ER stress-induced tubular apoptosis, tubulointerstitial lesions and mortality in proteinuric mice. Furthermore, the inhibition of ER stress by 4-phenylbutyric acid protects kidneys from morphological and functional injury in proteinuric mice.\(^8\)

Additionally, ER stress has been reported to contribute to diabetic-hypertensive kidney diseases. The coexistence of diabetes mellitus and hypertension increases the risk for onset and progression of chronic renal diseases.\(^12\) Wang et al showed that diabetes mellitus and hypertension interact synergistically to promote oxidative stress and ER stress, thereby leading to progressive renal injury in a rat model of mild type 2 diabetes mellitus combined with hypertension.\(^12\) Treatment of hypertensive-diabetic rat with ER stress inhibitor, tauroursodeoxycholic acid (TUDCA) for 6 weeks decreases blood pressure, proteinuria, glomerular injury, and oxidative stress, while increasing glomerular filtration rate in the kidneys, suggesting that diabetes mellitus and hypertension synergistically cause renal dysfunction and injury via induction of ER stress.\(^12\)

Autophagy functions as a pivotal protein degradation machinery that degrades and removes misfolded and aggregated proteins and damaged organelles under both physiological and pathological conditions.\(^9\) Activated UPR targets ubiquitinated misfolded and aggregated proteins to autophagosome for degradation under ER stress.\(^9\) It has been shown that induction of autophagy protected acute kidney injury\(^13\) and suppressed the progression of kidney fibrosis by increasing mature TGF-β1 degradation.\(^14\) Recently, Du et al\(^14\) demonstrated that Sphingosine kinase 1 protects renal tubular epithelia cells from fibrosis via activation of autophagy.

Collectively, ER stress and dysregulated UPR are closely associated with the pathogenesis of CKD. Targeting the components of the UPR machinery to optimize UPR activity and alleviating ER stress have been considered to be promising strategies for treating or delaying the progression of CKD.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.
REFERENCES


Hypertension-Chronic Kidney Disease Relationships

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A large number of epidemiologic, clinical and experimental studies indicate a strong association between hypertension (HT) and chronic kidney disease (CKD). Further, this association portends an ominous prognosis. Patients with HT and CKD are at a much greater risk for cardiovascular disease as compared with those without chronic kidney disease.

Hypertension defined as office systolic and/or diastolic blood pressures (SBP/DBP) equal to or greater than 140/90 mmHg, and involving about 30-40% of the world population, is a major contributor to heart disease, stroke and renal disease.

Chronic kidney disease, defined as a persistent kidney damage, as reflected either by reduced glomerular filtration rate (an estimated GFR <60/ml/min/1.73 m²) and/or increased urinary albumin excretion, is emerging as a major public health problem.

The hypertension-chronic kidney disease relationship is complex and appears to be dependent on several factors which include i) etiology of the hypertensive disorder; ii) level of BP; iii) type of the CKD; iv) BP phenotype.

ETIOLOGY OF THE HYPERTENSIVE DISORDER

Hypertension is the second cause of end stage renal disease (ESRD) after diabetes mellitus. However, discrepant opinion exists regarding whether non-malignant essential (primary) hypertension can cause ESRD. Several epidemiologic studies reported low prevalence and incidence rates of renal failure among hypertension subjects.

In one study, creatinine clearance, as a determinant of glomerular filtration rate (GFR), fell only by 0.92 ml/min per year in hypertensive subjects as compared to 0.75 ml/min per year in normotensive subjects. The prevalence of CKD varies between 8-16% of the adult population. Although, severe kidney damage is associated with an increased risk of endstage renal disease (ESRD), a milder degree of kidney damage is a marker of cardiovascular disease (CVD).

The hypertension-chronic kidney disease relationship is complex and appears to be dependent on several factors which include i) etiology of the hypertensive disorder; ii) level of BP; iii) type of the CKD; iv) BP phenotype.

The diagnosis of benign hypertensive nephrosclerosis is a diagnosis by exclusion. It is a clinical diagnosis based on history, physical examination, urinalysis and laboratory evaluation. Since kidney biopsy is rarely performed, the diagnosis is typically made in patients with chronic kidney disease (CKD) who have had a long-standing hypertension, subnephrotic range proteinuria without evidence of other kidney disease. Histopathologic lesions in benign hypertensive nephrosclerosis are characterized by vascular, glomerular and tubular changes. These changes have been attributed to loss of renal autoregulation and exposure of the intrarenal...
vasculature to long-standing elevated BP levels.4

LEVEL OF BLOOD PRESSURE

Several studies have also reported low incidence rates of ESRD in hypertensive patients with no evidence of underlying primary intrinsic renal disease.10,11 In both Multiple Risk Factor Intervention Trial (MRFIT) and Kaiser Permanente of Northern California, two large population studies, the incidence of nephropathy in hypertensive subjects with no evidence of primary renal disease was low at 15.6 and 14.3 cases per 100,000 persons-years respectively.10,11 Further, in both studies, the risk of ESRD was associated with increasing BP levels throughout the BP readings above the optimal level.10,11 The higher the BP levels the higher the risk of renal failure. In the Kaiser Permanente study, compared with subjects with a BP <120/80 mmHg, the adjusted relative risks for developing ESRD were 1.62 for BP=120-129/80-84 mmHg, 1.98 for BP=130-139/85-89 mmHg, 2.59 for BP=140-159/90-99 mmHg, 3.86 for BP=160-179/100-109 mmHg, 3.88 for BP=180-209/110-119 mmHg, and 4.25 for BP ≥210/120 mmHg.11 The systolic BP component appears to be the stronger predictor of CKD.10 In the MRFIT, a systolic BP higher by 1 standard deviation (SD) was associated with doubling of risk of ESRD.10 In contrast, increasing diastolic blood pressure (DBP) levels may act as initiators of CKD when associated with systolic blood pressure (SBP) within the normotensive range.10 In the MRFIT, in subjects with stage 1 diastolic hypertension (DBP=90-99 mmHg), the incidence of ESRD rose sharply from 9.8 to 16.4 per 100,000 persons-years across categories of SBP within the normotensive range.10

Other risk factors have been reported to increase the susceptibility to renal failure in benign nephrosclerosis, including Afro-American race, obesity, glucose intolerance, high serum uric acid levels, albuminuria, and genetic factors.8

Several studies have indicated that genetic factors especially D allele of the ACE gene may be associated with increased susceptibility to CKD and ESRD in patients with primary hypertension.12

In contrast, if the BP becomes markedly elevated and exceeds a certain threshold, malignant hypertension and malignant nephrosclerosis, characterized by disruption of the intrarenal microvascular and glomerular structures, renal functional impairment, proteinuria and hematuria develop.9

TYPE OF THE CHRONIC KIDNEY DISEASE (CKD)

Hypertension is very frequent in patients with CKD with an estimated prevalence of about 60%, reaching about 95% in stages 3-5 CKD.8 This prevalence rate depends on the type of nephropathy and the degree of renal functional impairment.13

The prevalence of hypertension is highest in renal vascular disease (93%), in established diabetic nephropathy (87%), and in polycystic kidney disease (74%) compared to a lower prevalence in glomerulonephritis and tubular interstitial disease.13

The prevalence of hypertension increases with worsening renal function. An inverse relationship between renal functional impairment and prevalence of hypertension has been reported in the Chronic Renal Insufficiency Cohort (CRIC) study.14 About 92% of patients with an estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m² were hypertensive while 67% of those with eGFR >60 ml/min/1.73m² had elevated BP.14

Other factors increase the risk of hypertension in CKD such as urinary albumin excretion, obesity, and race.15 Pooled data from the National Health and Nutrition Examination Survey (NHANES) III and NHANES 1999-2005 revealed that albuminuria was an independent risk for hypertension in CKD.16 Urine albumin/creatinine ratio greater than 6.67 mg/g in men and greater than 15.27 mg/g in women was associated with doubling the risk of developing hypertension.17

Obesity is an important risk factor of hypertension in CKD. In the modification of diet in renal disease (MDRD) study, body mass index (BMI) was a strong predictor for hypertension in patients with eGFR= 25-55 ml/min/1.73m².18

The prevalence of hypertension in CKD reveals racial disparities, being higher among non Hispanic blacks than whites or Mexican Americans.18

BLOOD PRESSURE PHENOTYPE

Hypertension related nephropathy is dependent, not only on the level of BP, but also on BP patterns.19,20 Several recent studies indicate that among the various BP patterns elucidated by ambulatory BP monitoring, masked and nocturnal hypertension are associated with a greater risk of hypertensive nephropathy than diurnal and white-coat hypertension.19,20 In CKD hypertensive...
patients, non-dipping and reverse dipping patterns were also associated with a greater risk of ESRD.\textsuperscript{19} Further, resistant hypertension is frequently observed in renal patients, at least partly due to inadequate treatment of the elevated BP states.\textsuperscript{21}

In the light of these recent observations, recent guidelines recommend ambulatory BP monitoring in the management of patients with CKD.\textsuperscript{19}

Inadequate BP control is a major cause for the development and progression to CKD and ESRD.\textsuperscript{22}

BP control in renal patients requires lifestyle modifications especially strict salt restriction, avoidance of weight gain, treatment of associated comorbid conditions and abnormal metabolic factors, and administration of antihypertensive medications. Combination therapy is usually required.\textsuperscript{4} Inhibitors of the renin-angiotensin system are recommended as primary agents, to reduce both BP and albuminuria.\textsuperscript{4,22,23} Changes in serum creatinine and potassium, possible complications of renin-angiotensin blockade should be carefully monitored.\textsuperscript{23} Addition of a thiazide diuretic is advisable.\textsuperscript{23}

BP goals of <130/80 have been generally recommended in renal patients.\textsuperscript{23} Intensive BP reduction may provide protection against progression to renal failure in patients with CKD.\textsuperscript{4}

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES


Post-Transplant Diabetes: New Insights Beyond Calcineurin Inhibition

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Despite impressive improvements in the 1-year graft survival for kidney transplantation, which has risen from 60% to 95% over the last few decades, little progress has been made in long-term graft survival. Half of the grafted and functional kidneys are lost within 10-12 years after transplantation because of premature recipient mortality; cardiovascular disease being a leading cause of both death and graft loss in this population. Many dialysis patients have acquired into end-stage kidney diseases because of diabetes, but among those non-diabetic patients, the majority suffer from a constellation of cardiovascular risk factors, including overweight, hypertension, dyslipidemia, and insulin resistance. After kidney transplantation, recipients often experience about 10-15% of weight gain, owing to increased appetite by steroids, as well as the loss of uremia and dietary restrictions from the dialysis period. Additionally, many recipients experience difficulties with exercise due to comorbid conditions like sarcopenia, amputations, or heart failure. All these circumstances make a perfect breeding ground for the development of diabetes after transplantation, or post-transplant diabetes (PTDM). PTDM develops from the interplay of transplant-specific factors such as immunosuppressive drugs on top of all these traditional risk factors. PTDM is a relatively common complication after kidney transplantation that influences graft and patient survival, and is defined as a type of diabetes that occurs after a transplant procedure in patients who did not present any remarkable feature of diabetes before the transplantation. The timing for diagnosis has not been clearly established but most PTDM happen during the first three months after transplantation, which is the period with the greatest exposure to the immunosuppressive cocktail. Therefore, the disease seems to develop because of the effect of immunosuppressive drugs in both insulin sensitivity (in peripheral tissues) and insulin secretion (by pancreatic beta-cells). However, minimising or withdrawing immune suppression is often unwanted because of the fear of rejection, and it remains difficult to lose weight after transplantation. Since no established therapy exists to prevent or revert PTDM, understanding the mechanisms involved in the effects of the immunosuppressive therapies in pancreatic beta-cells will be essential to improve preventive and therapeutic strategies for transplant recipients.

Calcineurin inhibition is one of the corner stones for current immunosuppressive regimens, it is based on two major drugs: tacrolimus and cyclosporine-A; tacrolimus being the most widely used now-a-days. The traditional paradigm is that calcineurin inhibition acts not only in lymphocytes, but also subsequently the main action of the drug itself induces failures in insulin production/secretion by inhibiting the calcineurin/nuclear factor of activated T-cells (NFAT) pathway in pancreatic beta-cells. However, trial and registry data have demonstrated that the use of tacrolimus increases the risk of PTDM when compared with cyclosporine-A, while both drugs share calcineurin as target. The general thought is that tacrolimus is more diabetogenic because it is a more potent calcineurin inhibitor; but taking into account that the trough levels used in clinical practise for cyclosporine-A are usually 20-times higher than those used for tacrolimus, the inhibition of the phosphatase calcineurin is not different between drugs. Additionally, when those clinical trough levels were used to treat beta-cells in culture, the same grade of calcineurin activity and NFAT activation was observed. So the higher diabetogenicity of tacrolimus must be due to some other factors in addition to the inhibition of calcineurin.
Porriñet al.9 analysed separately, kidney recipients with normal levels of triglycerides in blood before the transplantation from those with hypertriglyceridaemia (hypertriglyceridaemia is one of the characteristics of the metabolic syndrome). Their results showed that the use of tacrolimus as main immunosuppressive drug significantly increased the risk of developing PTDM compared with patients on cyclosporine-A, only in the hypertriglyceridaemic group. In the absence of hypertriglyceridaemia the risk for developing PTDM was the same for both calcineurin inhibitors. Additionally, in a study developed in obese Zucker rats, an animal model of insulin resistance that can compensate the increased demand of insulin not developing diabetes by itself, tacrolimus caused more diabetes than cyclosporin (100% vs. 40%), whereas neither tacrolimus nor cyclosporine-A caused diabetes in lean (insulin sensitive) Zucker rats.10 In these animals, tacrolimus reduced beta-cell proliferation compared with cyclosporine-A, together with a reduction in insulin levels and in islet area, but apoptosis was not identified as a reason for beta-cell failure or islet area reduction in this model. Consequently, the toxic effect of tacrolimus on pancreatic beta-cells depends on the pre-existence of metabolic alterations induced by increased levels of lipids, increased oxidative stress or an increased demand of insulin due to insulin resistance. Furthermore, both cessation of tacrolimus and conversion to cyclosporine-A, led to partial recovery of beta-cell function and proliferation. Importantly, this improvement in glucose metabolism after switching from tacrolimus to cyclosporine-A has been also observed in clinical studies,11 and it indicates that beta-cells are resilient enough to overcome the negative effect of the drug when it is not present anymore. The fact that cyclosporine-A has no such effects on the glucose metabolism indicates that there must be something else beyond calcineurin inhibition. However, the pathways involved in the mechanisms of tacrolimus-induced beta-cell failure are still unknown, but further research has evaluated similarities between beta-cell failure induced by tacrolimus with the “normal” beta-cell failure that happens in the progression towards type-2 diabetes.8

Now-a-days we know that, during development, when a progenitor cell differentiates into a beta-cell it does not acquire a locked state, but the maintenance of that differentiated new state (the functional beta-cell) needs an active and continuous production process of the pieces that form the machinery for glucose sensing, insulin synthesis and secretion. In other words, maintenance of the beta-cell identity requires the continuous activation of beta-cell-specific transcription factors.12 We have several evidences indicating that the alterations in these mechanisms of maintenance may conduce to a dedifferentiated state, a state in which the cell is not producing beta-cell identity markers anymore, and therefore it cannot be identified as beta-cell with the traditional immunological techniques. This may explain the contradictory absence of apoptosis observed together with a reduction in beta-cell mass in some experimental models.8 Importantly, whether this dedifferentiation in the absence of apoptosis is confirmed, it may bring some hope for the recovery of our patients with PTDM, and it opens a door for future treatments based on the reconstitution of beta-cell mass.

Among the transcription factors that are essential to maintain a functional beta-cell, it is worth to highlight the pancreatic and duodenal homeobox 1 (PDX1), v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MAFA), and neuronal differentiation 1 (NEUROD). The beta-cell transcription factors work together to induce the expression of those genes that give a beta-cell its fingerprint.13 Interestingly, the loss of some of them has been observed in patients with type-2 diabetes.14 Triñanes et al has shown how the addition of tacrolimus in metabolically stressed beta-cells induce the loss of some of these factors, while these changes cannot be induced neither by the treatment with cyclosporine-A nor in metabolically non-stressed beta-cells.8 Accordingly, with the fact that tacrolimus needs some metabolic alterations to induce beta-cell failure, we have examined what could be the molecular link between metabolic stress and tacrolimus-induced beta-cell failure. This synergy, or combined mechanism, does not have a molecular translation yet, but the fork-head box protein O1 (FOXO1) might be a relevant player in the process of PTDM.15 We have found that the nuclear translocation of FOXO1 in beta-cells is a characteristic that happens when these cells are more susceptible to tacrolimus-induced damage. This important transcription factor mediates proliferation in rodents’ beta-cells, being its nuclear exclusion necessary for beta-cell expansion in insulin resistant states.16 It is also known that FOXO1 plays an early role in beta-cell dysfunction, and normally its nuclear presence precedes the loss of the essential beta-cell transcription factor MAFA,17 being also related with the loss of other important beta-cell transcription factor like PDX1.18 Additionally, analyses of pancreata from type-2 diabetic patients have shown that nuclear levels of FOXO1 are higher in diabetic patients than in normal population.14

The loss of these beta-cell essential factors in our experimental set-up,8 together with the necessity of this metabolic stress (maybe mediated by FOXO1) for the induction of beta-cell failure by tacrolimus, have pushed us to think that beyond calcineurin inhibition, tacrolimus may induce an accelerated progression towards a beta-cell failure, resembling at a pancreatic level the progression towards type-2 diabetes. It is important to highlight that despite the higher incidence of PTDM in tacrolimus-treated patients, this drug produces better graft function and less nephrotoxicity than other immunosuppressive regimens,19 therefore become the standard therapy for kidney transplantation. On the other hand, calcineurin and NFAT, common targets of tacrolimus and cyclosporine-A, may have a relevant role in beta-cell function and integrity,9 and the inhibition of this pathway may also promote beta-cell dysfunction. However, tacrolimus definitely has additional effects in beta-cells and these effects need to be further studied. Likewise, it is also important to have a better knowledge about the diabetogenic mechanisms induced by other family of immunosuppressive drugs, the inhibitors of the mammalian target of rapamycin (mTOR), being sirolimus the most representative among them. These drugs act inhibiting the mTOR kinase, which is a downstream effector of the insulin signalling pathway that integrates different anabolic signals and enhances protein synthesis. The existence of this pathway in beta-cells indicates that sirolimus might
affect mechanisms of insulin synthesis and secretion, but further studies are needed to clarify the precise effects. The importance of organ transplantation now-a-days in medicine and the essential use of immunosuppressive drugs to achieve good results makes it crucial to obtain a better knowledge about these mechanisms. This approach will drive us to better immunosuppressive therapies and better long-term graft survival in kidney transplantation.

REFERENCES


Effect of Diethylenetriaminepentaacetic Acid (DTPA) on Crystal Growth and Morphology of Calcium Oxalate

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ABSTRACT

Introduction: One of the most common and painful diseases observed in the tropical regions is kidney stone formation. This can be due to various factors such as the amount of water consumption, climatic conditions, lifestyle and diet. Although, kidney stones are composed of calcium phosphate were only found to be 10%, it has been observed that the deposition of such stones have been increasing steadily for the past 30 years in females.

Aim: To study the effects of diethylenetriaminepentaacetic acid (DTPA) on crystal growth and morphology of calcium oxalate.

Materials and Methods: Calcium oxalate was synthesized from calcium chloride solution using oxalic acid in the presence of DTPA at different concentrations. The experiments were carried out at 60 °C. The samples were characterized using powder X-ray diffraction analysis (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM) techniques.

Results: The study revealed that in the absence of DTPA, samples exhibited flower like morphology by agglomeration of rod like basic blocks and the tendency for agglomeration decreases with increase in DTPA concentration.

Conclusion: Interpretation of XRD pattern confirmed that whewellite with a monoclinic structure is the most favoured structure in the absence of DTPA where as calcium oxalate hydrate having orthorhombic structure is most favoured structure in the presence of DTPA.

KEY WORDS: Calcium oxalate; Kidney stone; Chelation therapy; Crystal growth; Fourier transform infrared spectroscopy; Scanning electron microscope; X-ray diffraction; DTPA.

INTRODUCTION

Deposition of stones in kidney is one of the most common and painful diseases particularly in tropical regions. Various factors such as consumption of water, its quality, climate, lifestyle and diet can affect the formation and type of kidney stone. Majority of the kidney stones are composed of calcium, which may be in the form of calcium oxalate (70-80%) or calcium phosphate (10%) or mixture of both (40-50%). However, calcium phosphate stone composition has been increasing steadily for the past 30 years with female predominance. Apart from inorganic composition, kidney stones may also contain organic matrix accounting for 2-5% of the total stone weight.

Even though calcium oxalate exists in both monohydrate (COM) and dihydrate (COD) crystal phases, several studies have reported that the precipitate in the urinary tract consists of COM, having a greater stone forming tendency than COD and that all papillary stones are...
COM stones.\(^8,9\) The stone forming process is highly complex involving nucleation, crystal growth, and aggregation of crystals in an environment containing supersaturated crystal-forming ions (e.g., \(\text{Ca}^{2+}, \text{C}_2\text{O}_4^{2-}, \text{PO}_4^{3-}, \text{Mg}^{2+}, \text{SO}_4^{2-}\)) in the presence of promoters and inhibitors.\(^8\) The acid-rich urinary proteins suppress the crystallization of calcium oxalate even under supersaturated conditions,\(^14\) thus preventing the formation of stones. Chelation therapy is one of the recent techniques for the treatment of many diseases including kidney stones.\(^12\) Preferential crystallization of different forms of calcium oxalate has been reported by many researchers by using certain synthetic and natural molecules, such as polypeptides,\(^13,14\) sodium diisooctylsulfosuccinate,\(^15\) poly (ethylene glycol)-block-poly (methacrylic acid),\(^16\) renal epithelial cells,\(^17,18\) poly (sodium 4-styrene-sulfonate),\(^19\) biopolymeric additives,\(^20\) and protein isolated from bean seed coats.\(^21\)

Reports by Shinichi et al suggest that the conventional homogeneous precipitation results into monohydrate and granular calcium oxalate. However, hydrolysis of oxamic acid catalyzed by enzyme (hydrolase) generates trihydrate and fibrous calcium oxalate.\(^22\) Recent studies carried out on synthesis of \(\text{CaCO}_3\) revealed that the crystalline structure of calcium carbonate particles mainly depend on the precipitation condition, such as \(\text{pH}\), temperature and presence of sequestrants, etc.\(^25-30\)

The above survey revealed that effect of the presence of calcium ions in water on the formation of calcium oxalate while using oxalic acid has not been reported so far. In this paper we describe the effects of diethylenetriaminepentaacetic acid (DTPA), a chelating agent, on the morphology and structure of calcium oxalate precipitated from calcium chloride solution using oxalic acid. The results indicate that morphology and structure of the resulted calcium oxalate vary with the conditions. The architecture of the samples shows that DTPA has significant influence on the morphology and crystalline structure of calcium oxalate.

**EXPERIMENTAL DETAILS**

**Reagents and Materials**

Analytical grade \(\text{CaCl}_2\), DPTA and \(\text{Na}_2\text{C}_2\text{O}_4\) were obtained from Sigma-Aldrich chemical company, Bengaluru, KA, India. The reagents were used as such. Double distilled water was used for the preparation of aqueous solutions. Analytical grade 1:1 ammonia and acetic acid were used to adjust the \(\text{pH}\) whenever necessary.

**Methodology**

Experiments were carried out in a similar fashion as explained by Vijaya et al.\(^31\) Precipitation was carried out from a 0.1 M \(\text{CaCl}_2\) solution using 0.1 M oxalic acid at 60 °C. In a typical synthesis, 50 ml of \(\text{CaCl}_2\) solution was taken in a round bottom flask and 50 ml DTPA was added. The \(\text{pH}\) was adjusted to 7. The solution was heated to 60 °C and 50 ml oxalic acid was added from a burette. The mixture was kept at 60 °C for a period of 12 h and the precipitate obtained was filtered using Watman No. 40 filter paper. It was dried at 40 °C and then kept in a desiccator.

The \(\text{pH}\) measurements were made using Elico \(\text{pH}\) meter, model \(\text{LI}-120\). FT-IR spectra were taken in the range 500-4000 cm\(^{-1}\) using Avatar-330 FTIR spectroscopy after KBr pelletization. Microscopic morphological structure measurements were performed with Jeol JSM 5610 LV scanning electron microscope (SEM). The samples were coated with Au prior to examination. The powder X-ray diffraction (XRD) patterns were recorded on an INEL Equinox 1000 Advanced XRD diffractometer with Cu Ka radiation at \(\lambda=1.5406\) Å.

**RESULTS AND DISCUSSIONS**

**Interpretation of Powder XRD Data**

Figures 1a, 1b, 1c, 1d and 1e represents the XRD pattern of the samples. Figure 1a depicts the XRD pattern of the blank samples; i.e. prepared in the absence of DTPA. The data confirms the presence of calcium oxalate in the form of whewellite (calcium oxalate hydrate) vide JCPDS card 78-6695. The cell parameters \(a\), \(b\) and \(c\) are 9.9780, 7.295 and 6.292 respectively which confirmed that the samples exhibit monoclinic structure.

The XRD pattern of sample prepared in the presence of 10, 20, 30 and 40 ml DTPA are presented in Figure 1b, 1c, 1d and 1e respectively. The powder XRD pattern of samples showed the existence of calcium oxalate in the form of monoclinic. The samples with 10 ml and 20 ml DTPA had same cell parameters; \(a\), \(b\) and \(c\) as 9.794, 14.74 and 6.306 respectively and were matching with joint committee on powder diffraction standards (JCPDS) card 20-0231. The sample synthesized in presence of 30 ml DTPA had cell parameters; \(a\), \(b\) and \(c\) as 9.796, 7.294 and 6.291 respectively and were in good agreement with JCPDS card 20-0231. The cell parameters of the sample synthesized in presence of 40 ml DTPA were different from the above and were 9.976, 7.294 and 6.291 for \(a\), \(b\) and \(c\) respectively and were in good agreement with JCPDS card 20-0231.

Although, the positions of the peaks (°2θ) in the XRD pattern of all the samples correspond to whewellite and match very well with each other, the ratio of their intensities of the samples prepared in the presence of DTPA varied much from the blank sample. In the absence of DTPA, the two peaks around °2θ~15 and 24.5 showed similar intensities. With 10 ml and 20 ml DTPA, the peak at °2θ~24.5 was more intense than the peak around °2θ~15. With higher concentrations of DTPA (30 and 40 ml), the peak at °2θ~1.5 was more intense than the peak around °2θ~24.5. This indicates that the crystalline nature of the samples varied with the environment and the degree of crystallinity was affected much by the presence of DTPA.

**Interpretation of FTIR**

Aslin et al\(^12\) have reported the physicochemical analysis of urinary stones from Dharmapuri district in India. They have char-
Figure 1a: XRD Pattern of Calcium Oxalate Sample Synthesized in the Absence of DTPA.

Figure 1b: XRD Pattern of Calcium Oxalate Sample Synthesized in the Presence of 10 ml DTPA.

Figure 1c: XRD Pattern of Calcium Oxalate Sample Synthesized in the Presence of 20 ml DTPA.

Figure 1d: XRD Pattern of Calcium Oxalate Sample Synthesized in the Presence of 30 ml DTPA.

Figure 1e: XRD Pattern of Calcium Oxalate Sample Synthesized in the Presence of 40 ml DTPA.
characterized functional groups and phases of the stones using X-ray diffraction (XRD), Fourier transform Raman spectroscopy and Fourier transform infrared spectroscopy (FT-IR). Their study revealed that the majority of the stones were found to be calcium oxalate monohydrate (COM) and mixed stones had a minor existence of struvite and uric acid.

The FTIR spectrum of the blank sample as well as with 10, 20, 30 and 40 ml DTPA are shown in Figure 2a, 2b, 2c, 2d and 2e respectively. The characteristic bands assigned for sample without DTPA (Figure 2a) are 511 cm\(^{-1}\) to O-C-O in-plane bending, 660 cm\(^{-1}\) to weak band bending and wagging modes, 774 cm\(^{-1}\) (O=C=O), 954 cm\(^{-1}\) sym C-O stretch, 1319 sym C=O stretch, 1603 C-O stretch, 1784 cm\(^{-1}\), 3023 cm\(^{-1}\) symmetric and asymmetric O-H stretching.

The sample prepared in the presence of 10 ml DTPA exhibited frequencies at 511 cm\(^{-1}\) corresponding to O-C-O in-plane bending, 660 cm\(^{-1}\) weak band bending and wagging modes, 774 cm\(^{-1}\) (O=C=O), 888 cm\(^{-1}\) C-C stretch (rocking mode of water), 954 cm\(^{-1}\) sym C-O stretch, 1307 sym C=O stretch, 1603 C-O stretch, 3056 cm\(^{-1}\), 3250 cm\(^{-1}\), 3329 cm\(^{-1}\), 3433 cm\(^{-1}\), 3490 cm\(^{-1}\) symmetric and asymmetric O-H stretching.

Figure 2d shows the FTIR of the sample synthesized in presence of 30 ml DTPA. Characteristic bands were observed at 511 cm\(^{-1}\) O-C-O in-plane bending, 660 cm\(^{-1}\) weak band bending and wagging modes, 772 cm\(^{-1}\) (O=C=O), 886 cm\(^{-1}\) C-C stretch (rocking mode of water), 954 cm\(^{-1}\) sym C-O stretch, 1307 sym C=O stretch, 1613 C-O stretch, 3045 cm\(^{-1}\), 3250 cm\(^{-1}\), 3329 cm\(^{-1}\), 3488 cm\(^{-1}\) symmetric and asymmetric O-H stretching.

The calcium oxalate synthesized in presence of 50 ml DTPA exhibited FTIR bands at 511 cm\(^{-1}\) O-C-O in-plane bending, 660 cm\(^{-1}\) weak band bending and wagging modes, 784 cm\(^{-1}\)
\( ^1\text{O}C=O \), 876 cm\(^{-1}\) C-C stretch (rocking mode of water), 954 cm\(^{-1}\) sym C-O stretch, 1307 sym C=O stretch, 1613 C-O stretch, 3011 cm\(^{-1}\) symmetric and asymmetric O-H stretching.

It is obvious from the above data that in the absence of DTPA, frequencies/bands corresponding to C-C stretch (rocking mode of water) and sym C-O stretch at around 886 cm\(^{-1}\) and 954 cm\(^{-1}\) are absent. Similarly, the band around 1478 cm\(^{-1}\) is absent in all samples prepared in the presence of DTPA except with 40 ml. This band can be attributed to in-plane bending of CO (\( \nu \)). Again the bands around 511 cm\(^{-1}\) and 660 cm\(^{-1}\) are distinct and clear in the case of samples with 10, 20 and 30 ml DTPA. The intensities of these peaks decreased in blank as well as in the 40 ml DTPA sample. Analysis of urinary stone constituents using powder X-ray diffraction and FT-IR has been reported by Pragnya Bhatt and Parimal Paul.\(^{33}\) They have reported the presence of the apatite phase with an uncertainty on whether it has formed independently or the calcium oxalate monohydrate phase has been transformed into these phases with time. In our study, in the case of 40 ml, there could be a possibility of the formation of an apatite phase with the transformation of calcium oxalate monohydrate phase with time.

**Interpretation of SEM Images**

Figure 3a and 3b show the SEM images of sample prepared in the absence of DTPA. Flower like morphology formed by the interlinking of petals ranging from about 5-10 μm length and width of about 1 μm were observed under these conditions. The images also showed indications of smaller units exhibiting agglomeration at the centre of this structure or at the junction of cross linking (Figure 3b). The morphology of sample prepared with 10 ml DTPA are depicted in Figure 3c and 3d. They showed similarity with that of samples without DTPA. However, the tendency of agglomeration or degree of cross linking was comparatively less. Unlike the blank sample (without DTPA), it could be noticed from the SEM image (Figure 3b) that all the crystallites did not show flower like morphology. Some of the crystallites had only 4/6 petal like blocks attached to form the flower. In the sample without DTPA, the numbers of blocks to form agglomeration were too many. Some loose blocks were also seen in this image.

The sample prepared with 20 ml DTPA exhibited marked difference in the morphology. The flower like morphology was quite fewer in number. Majority of the crystallites exhibited block like structures (Figure 3e).

It could be observed from the magnified image (Figure 3f) that these blocks resulted from rod like structures. On increasing the DTPA concentration to 30 ml, the flower like morphology reappeared again (Figure 3g) along with the rod like
crystallites. However, with 30 ml DTPA, the flower like morphology was equal in numbers with the rod like crystallites. The rod like morphology showed tendency for sharp ends leading to an ellipsoidal shape. The trend continued with increase in DTPA concentration. With 40 ml DTPA, the dual morphology continued to exist. The number of ellipsoidal particles increased and the flower like structures reduced in numbers.

**Mechanism of Formation of Flower Like and Block Like Structures**

On the basis of the above experimental results, the mechanism of formation of this flower structure of calcium oxalate can be explained as follows: The crystallization of calcium oxalate in the absence of DTPA forms these basic blocks with rod like structures. From the SEM images (Figure 3a, 3b) it could be observed that there are no free building blocks in the images. This shows that the agglomeration is uninhibited in the absence of DTPA. Usually, the crystal growth is governed by both kinetic and thermodynamic factors. The observed flower morphology reflects the relative rates of growth of the crystal to different directions in the absence of DTPA. It is expected that in the absence of DTPA, more sites are available for further agglomeration or joining (Figure 3b). However, the presence of DTPA, which forms a complex with calcium, inhibits this tendency and the sites for growth could not go beyond a certain number. This is clearly observable in Figure 3c, 3d and further images, where the number of petals are few when compared to that of the blank. The above mechanism of inhibition of agglomeration or joining is further inhibited with the rise in concentration of DTPA and is evident from the SEM images of calcium oxalate synthesized in the presence of 30 and 40 ml DTPA.

**CONCLUSION**

The above observations indicate that DTPA strongly influences the crystallization of calcium oxalate. The study revealed that the crystallization process of calcium oxalate is entirely different in presence of DTPA. Whewellite with monoclinic structure is the most favoured polymorph in the absence of DTPA, where as calcium oxalate hydrate with an orthorhombic structure is the most favoured structure in the presence of DTPA. With increase in DTPA concentrations, there is significant difference in the morphology and the dual morphology was predominant. Similarly, the unit structures showed an increased tendency of cross linking in the absence of DTPA. These results could be helpful to understand the effects of DTPA on crystal growth of calcium oxalate based kidney stone and form a basis for the further studies on chelation therapy using DTPA and other chelating agents.

**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interest.

**REFERENCES**


219-238.


24. Palanisamy K, Subramanian VK. CaCO3 scale deposition on copper metal surface; Effect of morphology, size and area of contact under the influence of EDTA. *Powder Technology.* 2016; 294: 221-225. doi: 10.1016/j.powtec.2016.02.036


27. Naka K, Tanaka Y, Chuo Y, Ito Y. The effect of an anionic starburst dendrimer on the crystallization of CaCO3 in aque-


Variation of Peripheral Th17/Treg Imbalance in Patients with Idiopathic Membranous Nephropathy after Cyclosporin A Treatment: A Prognostic Marker of Idiopathic Membranous Nephropathy

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ABSTRACT

Objective: To investigate the variation and effect of peripheral T-helper 17 (Th17) and regulatory T (Treg) cells upon the clinical prognosis of idiopathic membranous nephropathy (IMN) patients before and after cyclosporin A (CsA) treatment.

Methods: Twenty-four patients diagnosed with IMN and 12 healthy controls at the Yong Chuan Hospital of Chong Qing Medical University between September 2015 and October 2016, were recruited for this study. All enrolled IMN patients received prednisolone acetate in combination with CsA on the basis of supportive treatment. After CsA therapy for 6 months, patients were assigned into the responsive and unresponsive groups according to the serum levels of albumin (ALB) and 24-hour urinary protein. The serum levels of ALB and 24-hour urinary protein were measured by full automatic biochemical analyzer. The peripheral Th17% and Treg% were detected and calculated by flow cytometry. The expression levels of Interleukin-17 (IL-17), tumor necrosis factor-alpha (TNF-α) and transforming growth factor beta (TGF-β) in the peripheral blood were quantitatively measured by ELISA.

Results: Compared with the healthy controls, in the peripheral blood of IMN patients, Th17 percentage and the expression levels of IL-17 and TNF-α were upregulated, whereas Treg percentage and TGF-β level were downregulated. All patients were assigned into the high-, middle- and low-risk groups according to quantitative analysis of the 24-hour urinary protein. In the high-risk group, the expression levels of IL-17, TNF-α and TGF-β were significantly upregulated, whereas the Treg% and TGF-β level were dramatically downregulated compared with those in the middle and low-risk groups. The 24-hour urinary protein level was positively correlated with Th17% and Th17/Treg ratio, whereas negatively correlated with Treg%. After a 6 month combined therapy of CsA and prednisone, 18/24 IMN patients fell into the effective group. In these patients, the 24-hour urinary protein level, Th17%, IL-17 and TNF-α levels were significantly downregulated, whereas the peripheral Treg% and TGF-β level were dramatically upregulated in the effective group (all \( p<0.05 \)). 6/24 IMN patients fell into the ineffective group. No significant changes were noted in these parameters in the ineffective group.

Conclusion: In IMN patients, present peripheral Th17/Treg imbalance is correlated with the severity of IMN. CsA treatment is an effective approach to improve peripheral blood Th17/Treg imbalance in a sub-population of IMN patients, which is associated with the clinical efficacy of CsA treatment. Monitoring the variations in peripheral concentration of Treg and Th17 is of significance for evaluation of the severity of IMN and clinical efficacy.

KEY WORDS: Th17; Treg; Idiopathic membranous nephropathy; Cyclosporin A; Efficacy evaluation.
ABBREVIATIONS: IMN: Idiopathic Membranous Nephropathy; CsA: Cyclosporin A; ALB: Albumin; Treg: regulatory T; Scr: Serum creatinine; CYP: Cyclophosphamide; IL-17: Interleukin-17; TNF-α: Tumor Necrosis Factor-alpha; TGF-β: Transforming Growth Factor-beta; ELISA: Enzyme-Linked Immunosorbent Assay.

INTRODUCTION

Idiopathic membranous nephropathy (IMN) is a common cause of nephrotic syndrome in adults. Previous investigations reveal that IMN is the most common single cause of nephritic syndrome, accounting for approximately 1/3 of cases globally. A major fraction of these patients do not achieve remission with immune therapy, and eventually progress to end-stage renal disease. The exact pathogenesis of IMN remains to be fully elucidated. Recent investigations have demonstrated that cellular immunity disturbance probably plays a vital role in the pathogenesis of IMN.

In recent years, the T-helper 17 (Th17)/regulatory T (Treg) balance, which is different from the CD4+ T-helper lymphocyte subset of Th1 and Th2, has been identified to play an influential role in the regulation of host immune tolerance, resistance of rejection reaction, infection, malignant tumor, inflammation and alternative diseases. Multiple studies have suggested that Th17 and its primary secretion interleukin-17 (IL-17) are involved with the pathogenesis of lupus nephritis, crescentic glomerulonephritis and proliferative glomerulonephritis, etc. Previous investigations have demonstrated that the peripheral Treg% in IMN patients is significantly lower compared to that in the healthy controls. After rituximab monoclonal antibody therapy, peripheral Treg% is elevated, which is intimately correlated with clinical efficacy. Nevertheless, existence of Th17/Treg imbalance in IMN patients, its correlation with the progression and clinical prognosis of IMN patients has not yet been thoroughly investigated.

Cyclosporin A (CsA) is a highly selective potent immunosuppressive agent, which can effectively inhibit the proliferation and activation of T-lymphocytes. Recent studies have reported that use of CsA exerts significant effect upon the Th17/Treg ratio in patients with autoimmune diseases and after organ transplantation. In addition, the variation in Th17/Treg ratio is probably correlated with clinical efficacy. However, the effect of CsA therapy upon the changes in Th17/Treg ratio in IMN patients has been seldom studied. Consequently, this study was designed to investigate the variation in Th17/Treg ratio before and after CsA therapy and its correlation with the clinical prognosis of IMN patients.

MATERIALS AND METHODS

Baseline Data

Twenty-four IMN patients (48.83±4.92 years, range 20-77 years, 15 males, and 9 females) admitted to the Yong Chuan Hospital between September 2015 and October 2016 were recruited for the clinical trial, 12 healthy people (48.55±5.82 years, range 21-73 years, 7 males, and 5 females) were enrolled as controls (Table 1). All subjects were informed and signed informed consent. Membranous nephropathy was diagnosed via renal biopsy. The possibility of alternative secondary membranous nephropathy was excluded.

Inclusion criteria were as follows: (1) patients of both genders aged ≥18 years; (2) those pathologically diagnosed with membranous nephropathy via renal biopsy; (3) those diagnosed with IMN for the first time and had no medical history of cyclophosphamide (CYP), CsA or other immunosuppressive agent use. Exclusion criteria were as below: (1) patients with a complicated infection, malignant tumor, hypertension and diabetes mellitus, etc.; (2) pregnant women. Twelve, age and gender-matched healthy volunteers, of which 7 were male and 5 females were recruited as the control group. None of the enrolled individuals had any history of immune or infectious diseases. All patients had signed the informed consents and completely cooperated with the study procedures.

All enrolled patients were administered with prednisone (0.5 mg/kg*d) in combination with CsA (3-5 mg/kg*d). The blood drug concentration of CsA was monitored and maintained to the standard range of 100-180 ng/ml. According to the Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline, the patients were divided into three groups, viz., low- middle- and high-risk. In the low-risk group, the kidney function is normal; in the middle-risk group, the kidney function was normal or slightly reduced; in the high-risk group, the kidney function was severely reduced. In the study, the patients were divided into two groups, viz., low- middle- and high-risk. In the low-risk group, the kidney function is normal; in the middle-risk group, the kidney function was normal or slightly reduced; in the high-risk group, the kidney function was severely reduced.

![Table 1: Baseline Data of the Enrolled Study Subjects](image)

<table>
<thead>
<tr>
<th></th>
<th>IMN Patients (n=24)</th>
<th>Health Control (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male:female)</td>
<td>15/9</td>
<td>7/5</td>
</tr>
<tr>
<td>Age (year)</td>
<td>48.86±7.31</td>
<td>48.55±5.62</td>
</tr>
<tr>
<td>Process (month)</td>
<td>11.04±17.31</td>
<td>-</td>
</tr>
<tr>
<td>IL-17 (pg/ml)</td>
<td>56.02±9.54*</td>
<td>43.6±8.81</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>149.8±13.09*</td>
<td>36.51±7.79</td>
</tr>
<tr>
<td>TGF-β (pg/ml)</td>
<td>935.16±47.02*</td>
<td>264.30±66.23</td>
</tr>
<tr>
<td>Th17%</td>
<td>1.15±0.62*</td>
<td>0.77±0.22</td>
</tr>
<tr>
<td>Treg%</td>
<td>0.96±0.59*</td>
<td>1.67±0.52</td>
</tr>
<tr>
<td>Th17/Treg</td>
<td>1.15±0.33*</td>
<td>0.41±0.18</td>
</tr>
</tbody>
</table>

*p<0.05 vs. healthy control
ney function was normal and the albuminuria level <4 g/24 h within 6 months. In the middle-risk group, the renal function was normal and the albuminuria level ranged from 4 g/24 h to 8 g/24 h. In the high-risk group, renal insufficiency or kidney atrophy was noted, and the albuminuria level exceeded 8 g/24 h. Quantitative analysis of the 24-hour urinary protein was utilized as a parameter to evaluate the clinical efficacy.

After 6 months treatment, according to the KDIGO guidelines, the patients were divided into two groups: the effective group and the ineffective group, the effective group classification criteria were as follows: (1) Complete remission: 24UPro<0.3g/L (UPro/Scr<300 mg/g or <30 mg/mmol), the two determinations are at least one week apart, with normal ALB and serum creatinine (Scr), (2) Partial remission: 24 UPro<3.5 g/d (UPro/Scr <3500 mg/g or <350 mg/mmol), and urinary protein reduction to reach or exceed the peak of 50%, the two determinations are at least one week apart, accompanied by ALB and Scr improved or return to normal.

METHODS

8-20 ml of fasting venous blood samples were collected from both the IMN patients and healthy controls before and 6 months after corresponding treatment. The plasma was separated from the cells within 2 hours after blood sampling collection. The serum levels of ALB and 24-hour urinary protein were detected by full automatic biochemical analyzer. The peripheral blood levels of IL-17, tumor necrosis factor-alpha (TNF-α) and transforming growth factor-beta (TGF-β) were measured by enzyme-linked immunosorbent assay (ELISA) and the serum Th17% and Treg% were calculated by flow cytometry.

Statistical Analysis

SPSS 20.0 software package was utilized for the statistical analysis. Measurement data were expressed as mean±standard deviation and analyzed using t-test. Enumeration data were expressed in percentage and statistically processed by chi-square test. A value of less than 0.05 was considered as statistical significance.

RESULTS

Compared to Healthy Controls, IMN Patients Exhibited Significantly Higher Th17% and Significantly Lower Treg% in their Peripheral Blood

Baseline data of the IMN patients and healthy controls were illustrated in Table 1. No statistical significance was identified in gender and age between two groups (both p>0.05).

Compared to the healthy group, IMN group exhibited higher Th17% (1.15±0.62% vs. 0.77±0.22%), and lower Treg% (0.96±0.59% vs. 1.67±0.52%) (Table 2). The related cytokine concentrations IL-17 and TNF-a in IMN group were higher (56.02±9.54 vs. 43.6±8.81 and 149.8±13.09 vs. 36.51±7.79) while TGF-β was lower than the healthy group (935.16±417.02 vs. 264.30±66.23)

In IMN Patients, Th17/Treg Ratio Shows Positive Correlation with the 24-Hour Urinary Protein Levels

Correlation analysis between 24-hour urinary protein level analysis and Treg and Th17 levels of all 24 IMN patients revealed that there was a strong positive correlation between the 24-hour urinary protein level with peripheral Th17% as well as Th17/Treg ratio. However, these analyses also showed a negative correlation between the 24-hour urinary protein level and peripheral Treg% in IMN patients (Figure 1).

Th17/Treg Ratio Imbalance is more Pronounced in Middle-Risk and High-Risk Patients Compared to Low-Risk Patients

According to the 24-hour urinary protein level, all IMN patients were divided into the low-, middle- and high-risk groups. As illustrated in Table 3, the peripheral Th17% and Th17/Treg ratio in the middle and high-risk groups were significantly upregulated compared with those in the low-risk group (all p<0.05). In the middle and high-risk groups, the peripheral Treg% was considerably downregulated than that in the low-risk group (all p<0.05). Furthermore, compared with the middle-risk group, significant variations were observed in terms of the parameters above in the high-risk group (all p<0.05).

Cyclosporin A Treatment Effectively Corrected the Th17-Treg Imbalance and Significantly Decreased the 24-Hour Urinary Protein Levels in a Majority of Patients

As outlined in the methods section, flow cytometry was used to measure peripheral Th7 and Treg cell levels. Typical flow cytometry diagrams of peripheral Th7 and Treg cell levels in IMN patients before and after CsA treatment are depicted in Figure 2.

As illustrated in Table 3, the 24-hour urinary protein level, Th17%, IL-17 and TNF-α levels were significantly upregulated, whereas the peripheral Treg% and TGF-β level were...
remarkably downregulated in IMN patients compared with those in healthy controls (all $p<0.05$). After CsA therapy for 6 months, patients were assigned into the effective and ineffective groups according to the serum levels of Alb and 24-hour urinary protein. In the effective group, the 24-hour urinary protein level, peripheral Th17%, IL-17 and TNF-α levels were significantly downregulated, whereas the serum level of ALB, peripheral Treg% and TGF-β were considerably upregulated after CsA treatment (all $p<0.05$). In the unresponsive group, the 24-hour urinary protein level, serum level of ALB, peripheral Th17%, Treg%, IL-17, TNF-α and TGF-β levels did not significantly differ before and after the CsA treatment (all $p>0.05$).

**Cyclosporin A Treatment has a Significantly Increased impact on Low-Risk Patients Compared to the Middle-Risk and High-Risk Patients**

Stratification of data presented in Table 4 among various risk levels indicates that CsA treatment improved the clinical parameters of 8 out 9 patients in the low-risk group and 8 out of 11 patients in the middle-risk group but only 2/4 patients in the high-risk group.

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**Table 3: Changes in Clinical Parameters and Peripheral Th17, Treg and Relevant Cytokines in IMN Patients before and after CsA Treatment.**

<table>
<thead>
<tr>
<th></th>
<th>Effective group (n=18)</th>
<th>Ineffective group (n=6)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>24-hour urinary protein</td>
<td>6.82±3.89*</td>
<td>4.64±2.63</td>
</tr>
<tr>
<td>(g/24 h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alb (g/L)</td>
<td>29.44±4.91*</td>
<td>32.27±6.50</td>
</tr>
<tr>
<td>IL-17 (pg/mL)</td>
<td>52.90±10.4*</td>
<td>43.15±7.34</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>123.8±7.91*</td>
<td>81.5±5.5</td>
</tr>
<tr>
<td>TGF-β (pg/mL)</td>
<td>869.15±369.21*</td>
<td>654.21±158.23</td>
</tr>
<tr>
<td>Th17%</td>
<td>1.05±0.54*</td>
<td>0.81±0.43</td>
</tr>
<tr>
<td>Treg%</td>
<td>1.11±0.60*</td>
<td>1.64±0.39</td>
</tr>
<tr>
<td>Th17/Treg</td>
<td>0.99±0.48*</td>
<td>0.45±0.22</td>
</tr>
</tbody>
</table>

*p<0.05 vs after treatment.
DISCUSSION

At present, the pathogenesis of IMN is that the circulating autoantibody recognizes the target antigen of glomerular podocytes. After the antigen-antibody binding, an immune complex forms subepithelially, which activates the complement to form membrane-attack complex, leading to basement membrane and glomerular filtration barrier injury and generates albuminuria. Until now, no biomarker has been utilized to monitor the immune activity in IMN patients in clinical practice. Recent investigations have demonstrated that the pathogenesis of IMN is correlated with the immune disorder of T/B lymphocytes. Zhihong et al reported that the quantities of multiple immunologically competent cells were substantially altered in IMN patients, including a decrease in Treg, increase in B-cells, and an elevated CD4/CD8 ratio. Masutani et al employed flow cytometry to quantitatively analyze the ratio of each cell subset and found that the quantity of IL-4+ T-cells is significantly upregulated, whereas the Th1/Th2 ratio was considerably downregulated in the IMN patients compared to healthy controls. In addition, the serum level of IL-4 is intimately correlated with the quantity of urinary protein. The immune responses of T lymphocytes dominated by Th1 downregulation and Th2 polarization disrupt the host immune balance, which probably promotes the incidence of IMN. As the CD4+ T-cell subset, Treg and Th17 cells have been proven to differ from Th1 and Th2. Th17/Treg balance plays a pivotal role in maintaining immune homeostasis and preventing the occurrence of autoimmune diseases. Consequently, we investigated the association of Th17/Treg imbalance with IMN severity and treatment response.

As a common drug for membranous nephropathy, CsA can be utilized as the initial treatment of IMN or alternative therapy if other medications are ineffective. The primary mechanism underlying the decrease of albuminuria is to inhibit immune response, selectively suppress the activation of T-cells, repress the production of IL-2, inhibit the secretion of calcineurin, block the dephosphorylation of synaptopodin induced by calcineurin and stabilize the cytoskeleton of kidney podocyte, thereby reducing the generation of protein. Previous studies have reported that administration of CsA exerts a significant effect upon immune diseases and after organ transplantation, which is possibly associated with clinical efficacy. Our observation that CsA treatment has a significant impact on Th17/Treg imbalance offers a novel mechanism.

CONCLUSION

The prime objective of this study was to assess Treg/Th17 imbalance in patients with IMN. The study revealed Th17/Treg ratio was different in IMN vs. control, Th17% and related cytokines level was higher while Treg% and serum TGF-β levels...
were lower in IMN compared to healthy controls, suggesting that Th17/Treg immune imbalance (reduced Treg cells and increased Th17 cells) exists in IMN patients. Downregulation of Treg cells may activate the immune system and promote pathological reactions that contribute to the pathogenesis of IMN. In addition, it was also found that the Th17 cells and the levels of IL-17, TNF-α and IL-6 were upregulated in IMN patients. We suggest that IMN initiates the Th17-type immune response through the release of IL-17, TNF-α and other pro-inflammatory cytokines, thereby provoking regional kidney tissue inflammation and upregulating the expression of pro-inflammatory cytokines and chemokines.

The secondary objective of this study was to investigate the relationship between Treg/Th17 imbalance with IMN progression and severity. Treg/Th17 cell imbalance was found to correlate with 24 hours total urine protein, and the patients with a greater Treg/Th17 imbalance had more 24 hours total urine protein. Further subgroup analysis revealed that the Th17% and Th17/Treg ratio was higher while Treg% was lower in the middle-risk and high-risk group when compared to low-risk and healthy control group. The We suggest that Treg/Th17 imbalance is associated with severity and progression of IMN through immune-mediated injury. Therefore, immunotherapy with the goal of decreasing the inflammations caused by Treg/Th17 imbalances may have a protective effect in patients with IMN.

The third question addressed by this study was to observe the variations of peripheral Th17/Treg imbalance after CsA therapy and its correlation with the clinical prognosis of IMN patients. In this study, we observed that after 6 months of CsA, the 24-hour urinary protein level, peripheral Th17%, IL-17 and TNF-α levels were significantly downregulated, whereas the serum level of ALB, peripheral Treg% and TGF-β were considerably upregulated in 18/24 responsive patients, hinting that CsA probably affects clinical efficacy and prognosis of IMN patients by regulating the Th17/Treg immune imbalance. Moreover, Th17/Treg ratio did not significantly alter in 6/24 non-responsive group. However, we did not have further research on why CSA treatment of IMN was unresponsive. CsA does not prevent continuing autoantibody formation; production and deposition of IgG4 in the glomeruli may promote the development of MN, but still need larger samples, a long-term follow-up to confirm.

In conclusion, IMN patients present with peripheral Th17/Treg imbalance are correlated with the severity of IMN. CsA therapy is an efficacious approach to improve the peripheral Th17/Treg imbalance, which is linked to the clinical efficacy of CsA treatment. Dynamic monitoring of the variation in the peripheral levels of Treg and Th17 contributes to evaluate the severity of IMN and assess the clinical efficacy.

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CONFLICTS OF INTEREST

The authors have no potential conflicts of interest.

REFERENCES


