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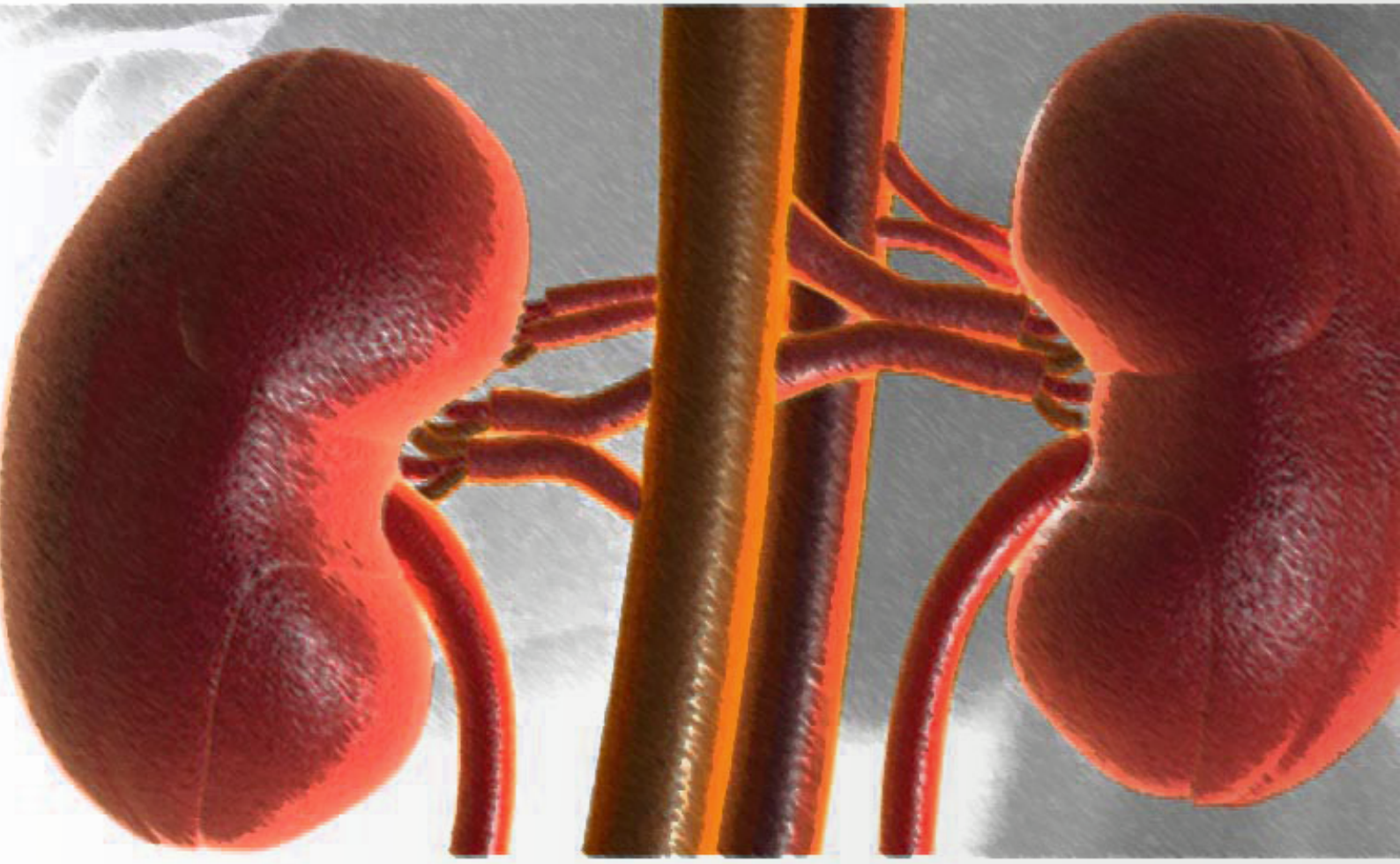

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Review

*Corresponding author
Toshiki Asao, PhD
Professor
Department of Agriculture
Faculty of Life and Environmental Science, Shimane University
2059 Kamihonjo, Matsue
Shimane 690-1102, Japan
Tel. +81 852341817
Fax: +81 852 34 1823
E-mail: asao@life.shimane-u.ac.jp

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Low Potassium Content Vegetables Research For Chronic Kidney Disease Patients in Japan

Md. Raihan Talukder, MS[1,2]; Md. Asaduzzaman, PhD[3]; Makoto Ueno, PhD[1]; Mikiko Kawaguchi, PhD[4]; Shozo Yano, MD, PhD[5]; Takuya Ban, PhD[6]; Hideyuki Tanaka, PhD[1]; Toshiki Asao, PhD[1*]

[1]Department of Agriculture, Faculty of Life and Environmental Science, Shimane University, 2059 Kamihonjo, Matsue, Shimane 690-1102, Japan
[2]Department of Environmental Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh
[3]Olericulture Division, Horticulture Research Center, Bangladesh Agricultural Research Institute, Gazipur 1701, Bangladesh
[4]Faculty of Home Economics, Otsuma Women's University, Chiyoda-ku, Tokyo 102-8357, Japan
[5]Faculty of Medicine, Shimane University, 89-1, Izumo 693-8501, Japan
[6]Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu 183-8509, Japan

ABSTRACT

Chronic Kidney Disease (CKD) is a common disorder to the elderly people, and its prevalence is increasing globally. We are turning into a super-aged society very rapidly and the number of CKD patients with dialysis treatment is now over 310,000 in Japan, which is the second largest population in the world. Japanese researchers have a pioneering status in addressing this issue through basic and clinical nephrology research. In addition, advanced research related to the dietary supplementation of CKD patients is essential for the management of this life threatening disease. This review aims to provide recent research advances of CKD on low-potassium vegetable production and dietary supplementation to dialysis patients in Japan.

KEYWORDS: Potassium; Vegetables; Hydroponics; Dietary supplementation; Dialysis patients.

INTRODUCTION

Potassium requirement is essential for normal function of the muscles, heart, and nerves in human body. It plays an important role in the contraction or relaxation of skeletal, smooth, and cardiac muscles, nerve impulse transmission, acid base equilibrium, enzymatic action, intracellular fluid tonicity, and renal function.[1,2] It is one of the most important electrolytes in the human body whose excess or deficiency may cause impairment of the body function and even result in death. It is also main electrolyte in the intracellular compartment, which accumulates more than 98% of total body potassium. The serum concentration of potassium is usually 3.5 to 5.0 mEq/L, while intracellular concentration is 115 to 150 mEq/L. Increase or decrease in these levels can negatively affect an individual's health. For example, high serum concentrations have adverse effect on heart muscle and may cause cardiac arrhythmias. Two-three times elevation of normal serum potassium level may result in cardiac arrest, which can be detected through the electrocardiogram.[3]

The kidneys normally excrete more than 90% of daily body potassium but patients with CKD can't completely excrete it, and thus, residual potassium accumulates in the body. In a study it is reported that a normal kidney has the capacity to excrete over 400 mmol potassium day[1,4] and it is unlikely that an individual will become chronically hyperkalemic without some degree of chronic renal impairment. The abnormally (2-3 times) elevated level of potassium in the blood may cause hyperkalemia, resulting in adverse effects on human body such as

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arrhythmias, muscle weakness, disturbed consciousness, heart failure, and even leading to sudden death.[5,6] The CKD population is increasing gradually and it is expected that the total number of patients will continue to increase progressively. According to Japanese Society of Nephrology, the number of CKD patients is estimated about 13.3 million (one in eight adults) in Japan.[7,8]

Dialysis, resin adsorbent, diuretic medication, intravenous Ca, glucose and insulin, and sodium bicarbonate are the common treatment methods for hyperkalemia but dietary intake most important. Therefore, CKD patients should pay attention to the diet. As a primary control measure, doctors restrict foods with high potassium content such as fresh vegetables, seaweed, beans and fruits including melon, strawberry, banana and kiwi.[9] Moreover, as potassium dissolves easily in water, CKD patients with dialysis are advised to cut these potassium-rich fruits and vegetables into small pieces and boil or soak them in a large volume of water prior to eating.[10] Although potassium content is partially reduced by these methods, the degree of reduction is limited through these food-preparation procedures.[11,12] In addition, these food preparation methods might result in wash out of other essential nutrients such as water-soluble vitamins and minerals and also the breakdown of desirable texture of raw lettuce.[11] In the above condition, to put it in an extreme way, dialysis patients do not eat the same dishes with other family members. For example, eating melon fruits is like a dream to them. As a result of such dietary restriction, their quality of life decreases greatly. Therefore, supplementation of vegetables containing less amount of potassium than usual would be a useful preventive method.

In Japan, researchers are giving effort to produce vegetables with low potassium content for example; low-potassium spinach, lettuce, melon, tomato, carrot, and strawberry.[13-19] Plants of these vegetables are grown in standard nutrient solution following quantitative management of solution KNO_3 or replacing it by a non-potassium fertilizer like HNO_3. Using this method, it is possible to reduce the potassium content of crops without inhibiting their normal growth. Recently, Aizu-fuji-kako Co., Ltd. (Tokyo, Japan) applied this cultivation method to produce low-potassium content leaf lettuce in larger scale. In the next section, we will describe the role of potassium in plants and humans, and recent advances in low-potassium vegetable production for dietary supplementation to dialysis patients.

Potassium as a Dietary Component

Potassium is necessary for the normal water balance between the cells and body fluids. Studies indicate that the average daily potassium intake is 2000-3900 mg,[4,20,21] which is too high for patients with kidney dysfunction to excrete. Thus, CKD patients with hyperkalemia are recommended to limit potassium intake to <1500 mg/day (Stage 5 patients) or 2000-2500 mg/day (Stage 3 and stage 4 patients).[21] Among all the vegetables, potato is the highest source of potassium and it is often suggested to adopt special preparation for making potato dishes. Moreover, potassium in foods is present with phosphate, sulfate, citrate, and many organic anions including proteins. Therefore, it is essential to bring some change in the general food habit of CKD patients. Potassium intake may be decreased with the agricultural revolution, when energy intake is shifted from a variety of plants including potassium-rich tubers to cereals and animal products with lower potassium content, and further decreased with a shift to highly refined processed foods.[22] At present, however, because dietary management is an important factor to improve outcomes in dialysis patients, clinical guidelines provide a recommended intake of micronutrients[23] to prevent hyperphosphatemia, hyperkalemia, hypertension, and water retention; and also reduced intake of protein, raw vegetables, and salt is recommended.[24-30]

Chronic Kidney Disease in Japan

CKD is defined by progressive decline in kidney function, documented by serum creatinine and the rate of creatinine clearance as measured by the rate of glomerular filtration.[31] It is a relatively common disorder with an increasing prevalence worldwide, and especially in Japan, where the prevalence of CKD has increased significantly over time.[32,33] Patients with chronic kidney disease suffer from a common life-threatening complication called hyperkalemia, abnormal increase in serum potassium.[34] Severe hyperkalemia may cause cardiac arrythmia and cardiac arrest.[29] Patients with diabetes, hypertension, and cardiovascular disease are of high risk for CKD.[35,36] Additional risk for development and progression of CKD may be conferred through lifestyle factors such as smoking and obesity.[37] Five stages of CKD are defined by reduced glomerular filtration rate and ranged from stage 1 to stage 5. Damage to the kidney may ultimately progress to stage 5 kidney failure, necessitating dialysis or kidney transplantation.[35] It also presents an economic burden, with the medical cost of end-stage renal disease representing approximately 4% of the total health care budget in Japan.[38]

In Japan, more than 300,000 CKD patients are treated with maintenance dialysis, and the number is gradually increasing. It is easily supposed that the number of CKD patients will grow even larger than ever and their health problems will become more complex as their age. Therefore, taking countermeasure against future pandemic of CKD is the most important task for the Japanese Nephrologists. It is estimated that the prevalence of CKD in Japan was 13.3 million and of CKD stages 1, 2, 3 and 4 + 5 were 0.6, 1.7, 10.7, and 0.2 million, respectively.[39] In this regards, the Japanese Society of Nephrology published guidelines for standardize treatment of CKD in 2007 and 2009.[40,41]

Potassium as Major Plant Nutrition

Potassium is one of the major nutrients, essential for normal growth and development of plants.[42] Plants absorb more

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potassium than any other mineral element with the exception of nitrogen.[43-46] It is the only monovalent cation that is essential for all higher plants, and is involved in three major functions such as enzyme activation, charge balance and osmoregulation.[46,47] Voogt and Sonneveld[48] found that with increases in tomato plant growth, potassium absorption increases to a relatively greater extent than that of other nutrients. In other studies, it was found that potassium requirement of greenhouse tomatoes is high for vegetative growth,[49,50] fruit production,[51] and fruit quality.[52,53]

Lower potassium levels in the culture solution may limit its assimilation into plant parts and retard plant growth, flower development, and fruit set.[51] It has direct effects on the partitioning of dry matter to the fruits and roots, and the growth of these organs is inhibited at lower potassium[54] and also fruit quality.[52,55] During the reproductive growth periods, fruits are the strongest sink for both carbon assimilates and potassium, therefore, quantitative management potassium in the culture solution is important option for producing low potassium fruits. In some determinate tomatoes, the demand for potassium during rapid fruit growth is above the uptake capacity such that leaf potassium is remobilized, resulting in foliar deficiency of the element.[56] Because of remobilization and recycling from old parts to new organs,[57,58] visible symptoms of injury do not appear on the growing sinks immediately in potassium deficient nutrient medium.[59] Thus, it is inevitable that reduced potassium supply will inhibit plant growth and yield. Therefore, investigation on minimal requirements of potassium in plants required for maintaining their normal growth and development is important. Additionally, production of melon fruits with low potassium content will also provide supplemental diet to the dialysis patients and may be of great interest for the patients, their family, hospitals, as well as the research community.

PRODUCTION OF LOW POTASSIUM CONTENT VEGETABLES IN JAPAN

Melon

Generally greenhouse cultured raw melon has higher potassium content of 340 mg/100 g fresh weight.[60] Significant decreases in potassium content in melon fruits would improve the diet of dialysis patients. Therefore, our research group used quantitative management of hydroponic culture solution for producing low potassium content melon and it was found that a reduction in KNO_3 in hydroponic solution could reduce the potassium concentration in melon fruit.[16] As hydroponic culture provides more precise control of growth conditions, it is easier to study various factors or parameters. Since a regular nutritional testing is conducted in the hydroponic growing system, so it can be easily defined whether the desired amount of nutritional content is present in the plants or not. It has also the precise control over concentration and composition of culture solution, which can be used for the production of both mineral enriched or deficient fruits and vegetables. Considering the above advantage of hydroponics, simple management of culture solution used for melon by reducing the potassium at lowest possible level would decrease fruit potassium content. Therefore, melon (*Cucumis melo* L. cv. Panna) were grown in nutrient solution with reduced KNO_3 concentrations from anthesis till harvest to investigate its impact on the fruit potassium content while maintaining normal growth, yield and other fruit qualities.

Three independent melon cultures were verified during the spring seasons from 2009 to 2011. In spring 2009, melon plants were grown in nutrient solution with $1/4^{th}$ KNO_3 decreased fruits potassium by 39% compared to fruits potassium in standard nutrient solution, whereas it was decreased by about 35% and 43% when melon plants were grown in nutrient solution with $1/16^{th}$ and O levels of KNO_3 in the spring 2010 and 2011, respectively. Compared to Standard Tables of Food Composition in Japan,[60] about 39% (207 mg/100 g FW) decreased potassium was found in melon fruits grown in nutrient solution without KNO_3 from fruit formation to final harvest during spring 2011. Fruit potassium content was not decreased expectedly even after limiting the potassium level to zero. The possible reason behind was the excessive absorption into the plant foliage during vegetative growth and storage before the start of potassium restriction, which in turn translocated into the melon fruits. Therefore, consideration of potassium translocation from leaves and stems to fruits during fruit developmental stage is an important issue for this study. Quantitative supply of KNO_3 to culture solutions of melon during vegetative growth would lower the fruit potassium level considerably. We have tried to serve low-potassium melon for lunch or dinner in CKD patients to verify the safety (Table 1) and evaluate the effectiveness (Figure 1). So far, we have a good response and need further investigation.

Strawberry

Hydroponic culture of strawberries has gained popularity for the commercial production of strawberries.[61] Generally, greenhouse-cultured fresh strawberries have a high K content of 170 mg/100 g FW of fruit.[60] Reducing this level in strawberry fruit would provide a good option in the diet for CKD patients. Therefore, our

Eating low-potassium melon	Serum K (mEq/L)	Serum Na (mEq/L)	Systolic BP (mmHg)	Diastolic BP (mmHg)	Pulse (/min)
Before	4.6±0.4	136.6±3.1	118.7±11.8	70.1±11.4	71.6±13.5
After	4.6±0.3	137.7±1.9	119.4±14.8	67.8±9.6	71.7±15.1
p-value	0.5	0.67	0.58	0.92	0.39

Table 1: Serum K and Na levels, blood pressure, and pulse before and after eating low-potassium melon. We served 100 g of low-potassium melon for dinner and measured serum K and Na levels, blood pressure, and pulse in 9 CKD patients (estimated GFR<45 ml/min/1.73 m^2) with mean age of 69 in a hospital. No significant change was determined before and 1 day after eating low-potassium melon in addition to their usual diet.

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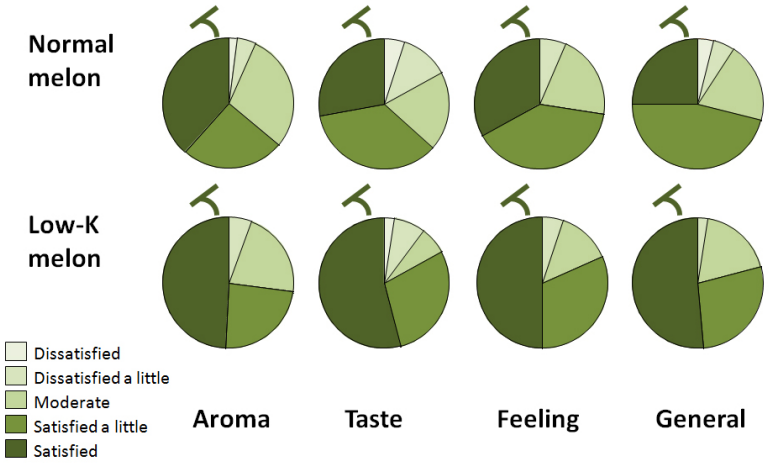

Figure 1: Results from a questionnaire regarding low-potassium melon in 76 dialysis patients. We served 50 g of low-potassium melon and 50 g of normal melon blindly in 76 maintenance dialysis patients in their lunch box. After eating melon, they answered some questions regarding the aroma, taste, and feeling without any information about melon. Interestingly, they satisfied with low-potassium melon at least as same as normal melon. Results were similar to those of healthy subjects (not shown).

research group attempted to produce low-potassium strawberry fruits through management of a KNO_3 fertilizer in nutrient solution from anthesis to the harvest period.[18] A general trend of decreasing potassium content in fruit was observed with the decrease of KNO_3 concentration in the nutrient solution. Among four strawberry cultivars, the fruit of the 'Toyonoka' exhibited a potassium reduction of about 64% when plants were grown in nutrient solution with KNO_3 at $1/16^{th}$ of the normal level. However, citric acid and ascorbic acid contents of 'Toyonoka' fruits were also reduced with decreasing KNO_3 concentrations in the nutrient solution. The use of $Ca(NO_3)_2$ in the nutrient solution to compensate the reduction in NO_3^- levels because of the use of low KNO_3 nutrient solution did not significantly affect the growth, yield and quality of these low-potassium strawberries. Compared with the standard potassium content in strawberry fruit, a 23.5% decrease (130 mg/100 g FW) in potassium was found in cultivars with $1/32^{nd}$ level of KNO_3. The potassium contents of plant parts suggested that the low KNO_3 level was responsible for the low potassium absorption, which may have affected the translocation and accumulation of potassium into fruit. Therefore, $1/32^{nd}$ of KNO_3 in nutrient solution lowers the fruit potassium content considerably.

Tomato

If low-potassium tomato fruit can be produced, it can improve the dietary options of dialysis patients. Effects of the amounts of potassium supply on the potassium content in fruits of cherry tomato and middy tomato were investigated in hydroponics for producing low-potassium content tomatoes.[62] A specific method of producing low-potassium content tomato (*Solanum lycopersicum* L.) fruit was investigated.[19] Several medium-sized tomato cultivars were tested, and the potassium supply was restricted using hydroponic production. Some plants were maintained through setting of a single fruit truss, and fresh fruit weight was not affected by potassium restriction. Total potassium uptake per plant decreased by 92% with lower potassium compared to conventional cultivation in which potassium content in fruit decreased by only 25%. Other plants were maintained until three trusses per plant were set, and fruit potassium content decreased 40% to 60% depending on cultivar because of potassium restriction. The variety "Aichan" was the most sensitive and variety "Frutica" was the least sensitive to potassium restriction. Total soluble solids content decreased slightly. Titratable acid content was affected by potassium restriction and decreased 20% to 40% depending on cultivar. In either case, potassium withdrawal in hydroponic culture following anthesis of the third truss was effective in producing low-potassium tomato fruit and could decrease fruit potassium content to at least 50% of expected tomato fruit potassium content.

Spinach

Hydroponic culture methods for spinach have been investigated with lower levels of potassium in the culture solution.[13] Spinach plants were grown hydroponically either with reduced potassium application throughout the growth period or without potassium applications during the last half of the growth period. There were no significant differences in fresh weight were observed in plants cultured with either of the solution. However, the potassium content in plants was reduced as much as 32% by reduced potassium application throughout growth period and 79% by without potassium application during the later half of growth period compared to control. These results suggest that it is possible to produce low-potassium spinach maintaining the normal plant growth. Other minerals like sodium and magnesium content increased with the decrease of potassium content, showing antagonistic role in osmotic pressure balance.

Lettuce

Lettuce is a popular potassium rich vegetable usually eaten raw in salad. CKD patients with hyperkalemia can't intake large

quantities of raw vegetables like lettuce, tomato, strawberry etc. To help these patients offset deficiencies in their vegetable intake, Fujitsu has started growing low-potassium lettuce. Capitalizing on know-how accumulated by manufacturing semiconductors to grow vegetables, Fujitsu has been carrying out agricultural management using ICT to optimize cultivation efficiency through developing infrastructure and performing data analysis. A patent has been given by Aizufujikako Co. Ltd. (Japan) for "Vegetable Having Low Potassium Content and Method for Culturing Said Vegetable". They reported that compared to normal leaf lettuce, low-potassium content lettuce has 100 mg or less of K/100 g fresh weights, while leaf lettuce contain 490 mg of potassium/100 g of fresh weight.[63] Fujitsu grows low potassium content lettuce at the Aizu-Wakamatsu Akisai Vegetable Plant located in Aizu-Wakamatsu City in Fukushima Prefecture. In this plant, the meticulous sanitary control and development management using ICT and Utilizing semiconductor fabrication technology allows production of lettuce that has approximately 80% less potassium. Vegetables are grown in an area of 2,000 square meters, which is the largest scale of plant factory growing low-potassium vegetables in Japan, where up to 3,500 heads of low-potassium lettuce per day. Therefore, it is possible to support dialysis patients under circumstances like that of the Great East Japan Earthquake in March 2011, where dialysis treatment is not available, by preparing a system to distribute low-potassium lettuce as an emergency relief supply across Japan. In this connection, agriculture, industry and medical fields work together at the cultivation and provision of low-potassium vegetables, with the Fujitsu Akisai agricultural cloud contributing to great food from Fukushima and Tohoku Region recovery.

In recent studies, it was found the low potassium lettuce contains a lower amount of potassium (-87%) than the normal leaf lettuce, while there was no significant difference in other nutritional contents, except for higher sodium and lower nitrate contents. Taste evaluation revealed that low potassium lettuce had lower bitterness and higher saltiness than the normal leaf lettuce. Soaking in water decreased the potassium content to 82% of that in the raw normal leaf lettuce. The overall preference score was significantly higher in low potassium lettuce, compared to that of the normal leaf lettuce.[14]

CONCLUSION

Currently there are 0.3 million dialysis patients, 2 million potential dialysis patients and 1.5 million of patients with kidney disease in Japan. This means 1 in 10 Japanese are suffering from renal disease, and most of them are following dietary restrictions of salt, protein, phosphate, and potassium. In addition, it is estimated that there are 2.0 million dialysis patients and 0.6 billion kidney disease patients around the world who need to follow dietary instructions. Therefore, our research and innovation on low potassium vegetable technology will change the boundaries of what is edible and what is not for such patients. Also, low potassium content vegetables are useful supplementation for increasing the variety of foods in diet of CKD patients who are apt to hyperkalemia. The studies highlighted in this article show that several Japanese researchers are successful in producing low-potassium melon, strawberry, tomatoes, spinach and lettuce. We hope that these products make dialysis patients happy, leading to an improvement of their quality of life. Indeed, many patients enjoyed these foods and gave us encouraging comments. Although our trial has just started, future work is necessary to provide these products to patients or hospitals with low cost and to produce other fruits and vegetables with low-potassium content.

CONFLICTS OF INTEREST: None.

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Review

*Corresponding author
Rakesh K. Mishra, PhD
Autoimmune Disease Center
The Feinstein Institute for Medical Research, 350 Community Drive Manhasset, New York, USA
E-mail: rakeshadams@gmail.com

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Involvement of NF-κB Signaling Pathway in the Pathogenesis of Systemic Lupus Erythematosus

Rakesh K. Mishra, PhD*

Autoimmune Disease Center, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, New York, USA

ABSTRACT

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by accumulation of anti-nuclear autoantibodies, hyperactivation. It can affect any organ, including brain, skin, joint, and kidney. The nuclear NF-κB pathway has long been considered a crucial pro- inflammatory signaling pathway. Its transcribed the genes involves in various autoimmune disease. Within the past year, many research studies have been conducted the role of NF-κB signaling in lupus. In this review, we will highlight some recent studies that support the potential link of NF-κB signaling pathway which play a crucial role in the pathogenesis of SLE.

KEYWORDS: Systemic lupus erythematosus (SLE); Nuclear factor kappa binding (NF-κB); Lupus; Toll-like receptors (TLRs).

INTRODUCTION

Systemic lupus erythematosus (SLE) is autoimmune disease characterised by a myriad of immune system aberrations that involve B-cells, T-cells, and cells of the monocytic lineage, resulting in polyclonal B-cell activation, increased numbers of antibody producing cells, hypergammaglobulinaemia, autoantibody production, and immune complex formation. It appears that excessive and uncontrolled T-cell help in the differentiation and activation of autoantibody forming B-cells is probably a final common pathway.[1] B-cell activation is abnormal in patients with SLE. The number of B-cells at all stages of activation is increased in the peripheral blood of patients with active SLE.[2]

Abnormalities in T-cell function are also evident in patients with SLE. The total number of peripheral blood T-cells is usually reduced, probably because of the effects of antilymphocyte antibodies[3] there is a skewing of T-cell function towards B-cell help, leading to enhanced antibody production.[3] Experiments have shown that the early events of T-cell activation are defective in patients with SLE compared with controls.

The NF-κB/Rel family includes NF-κB1 (p50/p105), NF-κB2 (p52/p100), p65 (RelA), RelB, and c-Rel). Most members of this family (RelB being one exception) can homodimerize, as well as form heterodimers with each other. The most prevalent activated form of NF-κB is a heterodimer consisting of a p50 or p52 subunit and p65, which contains transactivation domains necessary for gene induction.[1] The NF-κB target genes are involved in different aspects of immune functions, ranging from the development, activation, and differentiation of lymphocytes to the maturation and inflammatory functions of innate immune cells. The NF-κB factors are normally sequestered in the cytoplasm *via* association with a family of inhibitory proteins, including inhibitor of κB-alpha (IκBα) and related ankyrin repeat-containing proteins. In addition,the IκB family also includes the precursor proteins of NF-κB1 and NF-κB2, p105 and p100, which contain a C-terminal IκB-like structure and inhibitthe nuclear translocation of specific NF-κB members.[2] Proteasome-mediated processing of p105 and p100 involves selective degradation of their C-terminal IκB-like structure, leading to the generation of re-

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spective mature NF-κB subunits, p50 and p52, and the nuclear translocation of sequestered NF-κB proteins. The latent NF-κB complexes can be activated by various immune stimuli, which involves two major signaling pathways: the canonical and non-canonical pathways.[2] Both the canonical and noncanonical NF-κB pathways play a critical role in regulating immune activation and tolerance. Recent studies have emphasized diverse.

NF-κB has been implicated in the pathogenesis of autoimmune disease, such as rheumatoid arthritis (RA), type I diabetes, multiple sclerosis and SLE. During the SLE pathogenesis nuclear NF-κB promotes the aviation of T and B-cells in SLE.[3,4] Multiple number of evidences point out the crucial role of NF-κB signaling for the proper maturation and development of lymphocytes and dendritic cells. Abnormal NF-κB signaling lead to the secretions of auto reactive T-cells, which have a critical role in SLE and promotes plasma cell development, linking linear ubiquitination to multiple autoimmune diseases.[5]

Innate immunity may have a great influence in autoimmunity through Toll-like receptors. (Figure 1) *TLR7* and *TLR9* are expressed in endosomal compartments ligation induce signal transduction *via* the myeloid differentiation primary-response protein 88 (*MyD88*).[6,7] A common adaptor protein, which interacts with *IRAK1/4* (Interleukin-1 receptor-associated kinase 1/4) and *TRAF6* (TNF receptor-associated factor 6) to form the *MyD88*/*IRAK1*/*IRAK4*/*TRAF6* complex. Subsequently, *IRAK1* and *TRAF6* dissociate from the receptor complex and interact with kinases IKKβ (Iκβ kinases) resulting in the activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B-cells), permitting the expression of genes of proinflammatory cytokine and chemokines.[8] On the other hand, the transcription factor *IRF7* (Interferon regulatory factor 7) can bind to the *MyD88*/*IRAK1*/*IRAK4* complex, and its activation is dependent upon *TLR7* requiring the *TRAF3* (TNF receptor-associated factor 3) protein, which joins *IRAK1* and IKKα kinases to produce IFN-α. The activation of NF-κB is important for eliciting innate immune responses as well as for the subsequent development of adaptive immune responses.[9]

TLRs represent an important link between innate and adaptive immune responses.[10,11] Several mechanisms have been proposed to explain the production of autoantibodies in diseased B-cells, including impaired survival or apoptosis signalling that may prevent negative selection, dysfunctional complement or inhibitory Fc-receptors, and the activation of TLR in response to the accumulation of apoptotic bodies. Studies have shown that abnormal stimulation of innate immunity may have a great influence on immunopathogenesis of SLE through Toll-like receptors.[12,13] So far, 11 human TLRs have been identified, and *TLR7* and *TLR9* has been associated with SLE in both human and mouse models.[14,15] Both receptors are found on endosomes of several immune cells, mainly antigen-presenting cells, such as dendritic and B-cells. The recognition and internalization, through the B-cell receptor, of nuclear self-antigens released as a consequence of apoptosis in SLE patients, can activate *TLR7* in endosomes of B-lymphocytes supporting its role in the production of autoantibodies.[16,17] RNA-containing complexes must access the interior of the plasmacytoid dendritic cells (pDCs), through the Fc-receptors, thus providing a route of entry for RNA to reach *TLR7*, with the resulting INF-α production. INF-α influences the development, progression, and pathogenesis of SLE.[18-19]

Several studies have pointed to a relationship between NF-κB and lupus pathogenesis. Wen Zhang et al demonstrated that CD40-induced NF-κB signaling was constitutively activated in B-cells from active lupus patients. Including increased

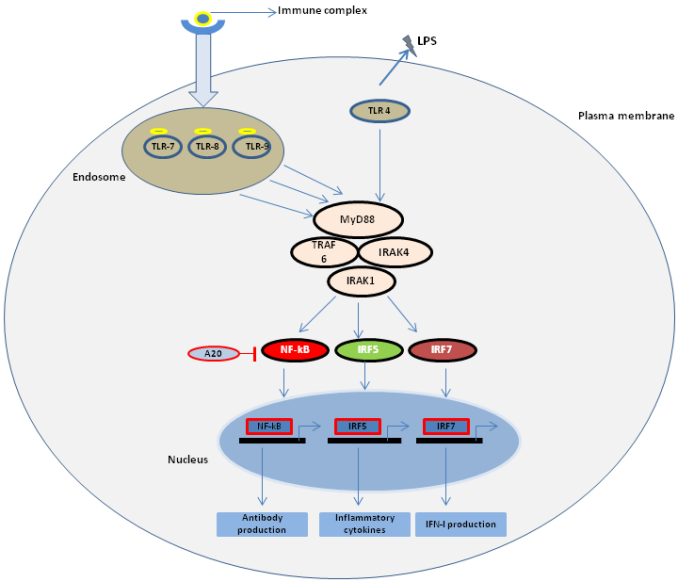

Figure 1: Overview of NF-κB signaling pathway.

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phosphorylation and degradation of IκB alpha, phosphorylation of P65 Aberrant CD40-Induced NF-κB Activation in Human Lupus B Lymphocytes.[20] Mayyan women are more suitable to get lupus disease. Pacheo et al[21] assess the role of *TLR7*, *MyD88*, and NF-κB p65 in B-lymphocytes of Mayan women with SLE and point out the increased expression of *TLR7*, *MyD88*, and NF-κB p65 in B-lymphocytes from Mayan women, which supports its role in the pathogenesis of SLE in this ethnic population of southeast of Mexico.[21]

Growing evidence suggests that recognition of nucleic acid motifs by Toll-like receptors may play a role in both the activation of antinuclear B-cells and in the subsequent disease progression after immune complex formation. TLRs expressed on various immune cells and upon detection of pathogens its trigger inflammation. For example, *TLR7* has been associated with SLE in both human and mouse models. This receptor is found on endosomes of several immune cells, mainly antigen-presenting cells, such as dendritic and B-cells. The recognition and internalization, through the B-cell receptor, of nuclear self-antigens released as a consequence of apoptosis in SLE patients, can activate *TLR7* in endosomes of B-lymphocytes supporting its role in the production of autoantibodies.[22]

Under basal conditions, NF-κB is maintained in the cytoplasm in an inactive state through inhibitors of κB (IκB). On activation, IκB rapidly undergoes phosphorylation and degradation, inducing nuclear translocation and gene expression. The A20-binding inhibitors of NF-κB (*ABINs1-3*) are suppressors of inflammation. Human polymorphisms in the gene encoding the *ABIN1* protein have been identified and are associated with a predisposition for autoimmune disease. *ABIN1*[D485N] knockin mice show significant expansion of myeloid cells in various organs and these mice show enhanced NF-κB and MAPK activation after TLR stimulation and display a SLE-like phenotype including expansion of myeloid cells, leukocyte infiltrations in different parenchymatous organs, activated T- and B-lymphocytes, elevated serum Ig levels, and the appearance of autoreactive antibodies. Kidneys develop glomerulonephritis and proteinuria, reflecting tissue injury.[23]

Inhibition of NF-κB reduced production of inflammatory cytokines IL-1 and TNFα in the RA model. NF-κB might also control B-cell function *via* BAFF and BAFF-R. This result would suggest that not only T-helper cells but also B-cells are connected by NF-κB pathways in SLE and RA. Excessive BAFF signaling through BAFF-R results in prolonged B-cell survival and costimulates B- and T-cells. Instead of blocking BAFF-R or decreasing BAFF, reduction of BAFF-R numbers would also, theoretically, reduce the effects of BAFF-BAFF-R signaling in inflammatory autoimmune diseases.

Thomas enzler et al,[24] examined which NF-κB pathway and which B-cell type are involved in development of SLE-like autoimmune disease in BAFF-Tg mice. In this study they have used genetic approach and found that both NF-κB signaling pathways contributed to disease development and possibility of controlling the amounts of BAFF-R and reducing the effects of BAFF-R signaling through NF-κB inhibition.

In other study conducted by Lee YH et al[25] determine whether polymorphisms of the Toll-like receptor (TLR) genes are associated with susceptibility to SLE and this study suggests that *TLR7*, *TLR8*, and *TLR9* polymorphisms are associated with the development of SLE in Caucasian, Asian, and African populations.[25]

Genetic approaches have gained much power and popularity in identifying the component mechanism(s) underlying the pathogenesis of common human diseases. (Table 1)

Gene	Function	Risk for disease
IRF5	Regulates type 1 IFN pathway	SLE, RA
IRF6	Regulates type 1 IFN pathway	SLE, RA
IRF7	Regulates type 1 IFN pathway	SLE, RA
STAT4	Regulates type 1 IFN pathway	SLE, RA
TRAF6	Regulates NF-κB pathway	SLE, RA
TNFAIP3	Regulates type 1 IFN pathway	SLE, RA
TNIP1	Regulates type 1 IFN pathway	SLE, RA
IRAK1	Innate immune signaling	SLE, RA
TLR7	Innate immune signaling	SLE, RA
TLR9	Innate immune signaling	SLE, RA
UBE2L3	Regulates NF-κB pathway	SLE, RA
SLC1514	Regulates NF-κB pathway	SLE, RA
PRKCB	Regulates NF-κB pathway	SLE, RA
TYK2	Regulates type 1 IFN pathway	SLE, RA

IFN: Interferon; NF-κB: Nuclear factor-κB; SLE: Systemic Lupus Erythematosus; TLR: Toll-like receptor. See text for complete gene names

Table 1: Pathway-associated SLE candidate genes.

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Genes that play a role in the NF-κB pathway downstream of TLR engagement have also been associated with increased SLE susceptibility. For example, both risk and protective haplotypes of *IRAK1* (interleukin-1 receptor-associated kinase 1) have been associated with SLE. The X-linked *IRAK1* gene encodes a kinase that acts as the *MyD88* complex on/off switch for activation of the NFκB inflammatory pathway. *TNFAIP3*, also associated with SLE and subphenotypes including renal disease, encodes A20, a deubiquitinating enzyme that inhibits NF-κB, leading to protein degradation and interactions that inhibit NF-κB activity and TNF-mediated programmed death. A dinucleotide polymorphism just downstream of the *TNFAIP3* promoter region was linked to the decreased expression of A20 in patients with SLE of Korean and European ancestry, and may be the risk haplotype functional variant. *TNIP1* (*TNFAIP3* interacting protein 1), encoding the A20-interacting protein, has also been associated with the risk of SLE. Additional genes within the NF-κB pathway associated with SLE susceptibility include: *SLC15A4* (solute carrier family 15, member 4) encoding a peptide transporter that participates in NOD1-dependent NF-κB signalling; *PRKCB* (protein kinase C, β), which is involved in B-cell receptor-mediated NF-κB activation and *UBE2L3* (ubiquitin-conjugating enzyme E2L 3), encoding the enzyme *UBCH7*, which participates in the ubiquitination of an NF-κB precursor, and may play a role in cell proliferation. A risk haplotype of *UBE2L3* confers increased *UBCH7* expression in patients with SLE; a variant contained in this haplotype has been associated with the presence of anti-dsDNA antibodies.[26] (Paragraph adapted from Ornella Josephine, Ann Rheum Dis 2012)

CONCLUSION

In this review, we have summarized that aberrant activation of NF-κB in lupus disease. It is worth to point out here that NF-κB may play even more roles than mentioned above in the development of SLE, as exemplified by multiples studies in both mice and human patients. The significance of NF-κB activation in SLE suggests that inhibition of this signaling pathway provides novel strategies for the prevention and treatment of disease. It is hopeful that as we increase our understanding of the regulation of the NF-κB pathways, insights into the better design of drugs that effectively target NF-κB will be gained that will ultimately lead to better prevention and treatment of the disease.

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Short Communication

Corresponding authors
Sheng Lang Zhu, MD
Director
Department of Nephrology
Nanshan Hospital
Affiliated Hospital of Shenzhen University
Taoyuan Road 89, Shenzhen City
P. R. 518060, China
Tel. + 86 755 26553111
Fax: +86 755 26565025
E-mail: nszhushenglang@163.com

Kunmei Ji, PhD
Professor
Department of Biochemistry and Molecular Biology
Health Science Center
Shenzhen University
Nanhai Ave 3688, Shenzhen City
Guangdong, P. R. 518060, China
Tel. +86 755 86671979
Fax: +86 755 86671906
E-mail: jkm@szu.edu.cn

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Suggestions to Improve Medical Education in China-Learning from Diagnostic Errors of Kidney Diseases

Jiuhong Li, MBBS[1,2#]; Pengfei Wei, MMed[1#]; Zhenfu Zhao, MD[1]; Meng Li, MSc[1]; Jin-Wen Hua, BSc[1]; Ni Xie, MD[3]; Xiaohua Xie, MMed[3]; Sheng Lang Zhu, MD[2*]; Kunmei Ji, PhD[1*]

[#]These authors contributed equally to this work

[1]Department of Biochemistry and Molecular Biology, Health Science Center of Shenzhen University, Shenzhen, China
[2]Department of Nephrology, Nanshan Hospital, Affiliated Hospital of Shenzhen University, Shenzhen, China
[3]Shenzhen Second People's Hospital, The First Affiliated Hospital of Shenzhen University, Shenzhen, China

ABSTRACT

A correct diagnosis is key to deliver the safe and effective medical interventions. However, diagnostic errors that can jeopardize patients' safety are common in clinical settings. Herein, we analyzed diagnostic errors in kidney disease cases based on our clinical experience, explored the factors underlying diagnostic errors, and discussed ways to improve medical education in China. In kidney disease, diagnosis is complicated by the kidney's physiological characteristics and the lack of sensitive early diagnostic methods. Additionally, there are shortcomings in Chinese healthcare service patterns and the Chinese medical education. There are not enough qualified General Practitioners (GPs) in China, resulting in a low-efficiency referral system. Clinical training for postgraduates is hindered by the pressure to publish papers. Continuing medical education in China seems to be oriented improperly, with insufficient focus on the progress of clinical practice. We believe that targeted measures should be enacted to address the aforementioned problems in China. Specifically, we recommend that medical education should incorporate evidence-based diagnostic mind maps and lessons from diagnostic errors.

KEYWORDS: Medical education; Diagnostic error; Kidney disease; Diagnostic mind map.

OBJECTIVE

Correct diagnosis is a prerequisite to providing proper medical treatment. However, *to err is human*.[1] Diagnostic errors occur often in clinical settings, which may bring about detrimental consequences such as treatment delays and even mortality. Employing our clinical experience, we analyzed diagnostic errors in kidney disease cases in China to explore how medical education system could be improved.

MISDIAGNOSIS OF KIDNEY DISEASES

Kidney diseases have high incidence rates in China. Zhang et al estimated that the prevalence of chronic kidney disease (CKD) in adults was about 10.8% in China, with only 12.5% of those affected being aware of the condition.[2] We have collected 1,662 cases involving chronic kidney failure misdiagnosis in 61 Chinese medical articles, published from 2004 to 2013. The time to rectify the misdiagnosis in these cases varied from less than a month to several years.[3] The prevalence of misdiagnosis is partly due to the kidney's physiological characteristics and a lack of early diagnostic methods. Owing to the kidney's strong compensatory ability, patients

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with early-stage renal dysfunction or injured kidneys may not have symptoms. Changes in serum creatinine (sCr) levels may not be clinically detectable until nearly 50% of kidney function has been lost.[4]

Suggestion 1: Maintain a Master-Apprentice System for Medical Practice and Enhance General Medical Education in China

China has a unique traditional medical practice system that is totally distinct from systems in western countries. The majority of physicians in China are practice semi-independently in public hospitals with their daily clinical activities being conducted under the supervision of senior physicians. It is customary that Chinese physicians undergo ongoing medical education from their supervising senior physicians throughout their careers. We suggest that this master-apprentice system be expanded and carried forward.

On the other hand, there are shortcomings in Chinese healthcare service patterns and medical education that may contribute to the commission of diagnostic errors. Before 1984, the three-tiered rural medicare system in China was one of the best healthcare systems in the world.[5] However, China has yet to establish an efficient dual referral system between community health centers and large hospitals. Patients in China usually seek the care of specialties at large hospitals directly as a first point of contact, without advice from a general practitioner (GP), even for common ailments. This circumstance produces an immense outpatient workload for large hospitals. A specialist may have only approx. 3-5 minutes to consult with each patient. Factors such as inappropriate specialist visits, specialists' limited knowledge of general medicine, time-limited communication, and limited knowledge of patients' history all contribute to diagnostic errors. Visiting GPs firstly is helpful for the choice of appropriate specialist if necessary. However, China now faces a severe shortage of registered GPs (only ~150,000) for its massive population.[6] Of the 168 medical schools in China, only ten offer bachelor's degrees in general medicine. To improve healthcare safety, China should enhance general medical education to cultivate more GPs for community health centers, so that these centers can act as more effective facilitators in the delivery of healthcare services and a more efficient referral system can be developed.

Suggestion 2: Emphasize Clinical Training in Postgraduate Medical Education

In China, prescription rights are usually granted after one finishes a 5-year bachelor's degree in medicine and one additional year of clinical practice, and then passes a medical license examination. Nevertheless, upon passing the examination, physicians' clinical abilities are still limited. Additionally, master and doctor of medicine programs are available. However, due to pressure to publish basic research papers, postgraduates focus on research activities, which compromise clinical training. An experience of the present paper's co-author Kunmei Ji exemplifies this problem. He sought care in a large Shenzhen hospital in 2005 due to hematuria, dizziness, and vomiting. Based on the findings of hematuria and proteinuria together with negative signs of renal calculi in ultrasound examination, he was diagnosed imprudently with renal lithiasis and medicated accordingly. His condition deteriorated within 2 days, and he was then diagnosed with kidney failure at another hospital with his sCr level reaching 1100 μmoI/L. The original physician had a medical doctorate with a good publication record, but did not know that it is essential to test for sCr levels in patients with hematuria, revealing a weakness in his medical education. Fortunately, the Chinese government became aware of the clinical shortcomings of Chinese medical undergraduates and graduates and has, consequently, instituted a policy requiring standardized medical residency training for 2-3 years, which represents important medical education advancement.

Suggestion 3: Refresh Clinical Knowledge through Continuing Medical Education

Continuing medical education (CME) seminars are an effective way to help physicians improve their clinical skills. Clinically related CME topics – such as new diagnostic methods, new treatment approaches, clinical experience, and clinical case analysis seminars – would be particularly beneficial. For example, there is an international consensus that estimated glomerular filtration rate (eGFR) is a much better indicator of renal function than sCr. However, many Chinese doctors still rely on sCr (normal: 44-133 μmol/L) and lack an appreciation for eGFR findings. To exemplify this point further, consider the case of a 65-year-old woman who was hospitalized for coronary artery disease and hypertension with comorbid diabetes. Her physicians concluded wrongly that her kidney function was normal based on her sCr level (127 μmol/L) and proceeded with coronary artery angiography (CAG). Tragically, three days after being subjected to CAG, the patient's sCr levels increased to 906 μmol/L and she exhibited anuria. The patient died within 15 days, although receiving hemodialysis. According to the Modification of Diet in Renal Disease equation, this patient's eGFR before CAG was only 37.44 ml/(min. 1.73 m^2), which indicates that she had phase-3 CKD, a contraindication for CAG. Such tragedies can be avoided if physicians are knowledgeable about eGFR. Hence, we believe that China's CME should focus on refreshing the clinical knowledge of Chinese physicians.

Suggestion 4: Establish Comprehensive Diagnostic Mind Maps During Medical Education

Standardization may reduce medical errors,[7] but given the complexities of diseases, it would be unrealistic for physicians to rely on rigid rubrics to diagnose patients. Here we suggest that medical education may help to build upon integrated evidence-based diagnostic mind maps and learn lessons from diagnostic errors. The maps should be reliable, memorable, and concrete, connecting the characteristics of related diseases. For example, hematuria is a sign of both kidney and urinary tract disorders,

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and is also associated with some systemic diseases and disorders of neighboring organs. In our clinical practice, a 35-year-old man presented with hematuria and proteinuria (8.0 g/ 24-hour urinary protein excretion) was initially suspected to have nephrotic syndrome. However, his serum albumin level was assayed within normal range. Considering other possibilities, we performed more laboratory tests. And then he was found to have low coagulation factor VIII levels. So he was supplied with coagulation factor VIII. Consequently, his hematuria and proteinuria disappeared. He was finally diagnosed as hemophilia A. The case revealed that a comprehensive diagnostic mind map could help physicians to seek more evidence towards the correct diagnosis.[8,9] Medical education and CME should include analysis of misdiagnoses so that medical students and physicians have the opportunity to learn from others' diagnostic errors and enhance their diagnostic proficiency.

CONCLUSIONS

In the past six decades, improvement of Chinese mass population's healthcare system is partially due to medical education. Here, we analyzed cases of misdiagnosis of kidney diseases in China and discussed the potential shortcomings in Chinese medical education system. Therefore, we suggest that China enhance general medical education, emphasize clinical training in postgraduate education, standardize medical residency training, and renew the clinical knowledge of all licensed physicians through CME. In addition, it would be worthwhile to help medical students establish evidence-based diagnostic mind maps and learn from prior diagnostic error experiences.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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NEPHROLOGY

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Research

*Corresponding author
Victoria A. Bennett, MBBS
St. George's Hospital
Blackshaw Road, Tooting
London SW17 0QT, UK
Tel. 07540 137 177
E-mail: v.bennett1@nhs.net

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Continuous Renal Replacement Therapy for Acute Kidney Injury Using Phosphate Containing Fluid is Associated With Greater Biochemical Derangement than Conventional Fluid

Victoria A. Bennett, MBBS[1*]; Richard Strickland, FCICM[2]; Mark Finnis, M. Biostat, FCICM[2,3]; Benjamin A. J. Reddi, PhD, FCICM[2,3]

[1]St. George's Hospital, Blackshaw Road, Tooting , London SW17 0QT, UK
[2]Intensive Care Unit, Royal Adelaide Hospital, Adelaide, SA 5000, Australia
[3]Discipline of Acute Care Medicine, University of Adelaide, Adelaide, SA 5000, Australia

ABSTRACT

Background: Continuous Renal Replacement Therapy (CRRT) is the preferred means of renal replacement therapy (RRT) in many intensive care units. Most units use only one type of RRT fluid or identify a standard 'default' fluid. Little data is available comparing the effects of using different RRT fluids. We aimed to identify the biochemical consequences of receiving CRRT with potassium and phosphate free fluid, Hemosol-B0, or potassium and phosphate containing fluid, Phoxilium, as a default RRT fluid.
Methods: Retrospective observational study in a mixed Level III Intensive Care Unit (ICU). A period of two-six months study were compared during which either Hemosol-B0 or Phoxilium were used as the unit's default fluid. Daily biochemistry results and K^+ and PO_4^{3-} supplementation were recorded in all patients over eighteen years requiring CRRT during the study period; data was collected on 35 patients in the Hemosol-B0 group and 31 patients in the Phoxilium group. Our primary outcome was the proportion of CRRT treatment during which $[PO_4^{3-}]$, $[Ca^{2+}]_{pl}$ and $[HCO_3^-]_{pl}$ were in the normal range, and secondary outcome the need for non-RRT fluid K^+ and PO_4^{3-} supplementation.
Results: Phoxilium was associated with a greater proportion of the CRRT delivery time spent with $[PO_4^{3-}]$ above the normal range. Furthermore, Phoxilium was associated with more time spent with ionised $[Ca^{2+}]_{pl}$ and $[HCO_3^-]_{pl}$ below the normal range. Phoxilium significantly decreased the requirement for phosphate but not potassium supplementation.
Conclusions: Patients receiving Phoxilium should be monitored to avoid hyperphosphataemia, hypocalcaemia and metabolic acidosis. Phoxilium use does not abrogate the need to handle concentrated potassium solutions.

KEYWORDS: Acute kidney injury; Dialysis**;** Hyperkalemia; Intensive care; Phosphataemia.

ABBREVIATIONS: CRRT: Continuous Renal Replacement Therapy; ICU: Intensive Care Unit; AKI: Acute Kidney Injury; CVVHDF: Continuous veno-venous haemodiafiltration; APACHE II: Acute Physiology and Chronic Health Evaluation II.

MAIN MESSAGE

There is limited data comparing fluids used for continuous renal replacement therapy (RRT) in the treatment of acute kidney injury. We undertook a retrospective observational study and identified that when the standard RRT fluid used in our institution was changed from a phosphate and potassium free fluid to a potassium and phosphate containing fluid, patients spent a significantly greater proportion of therapy time with hypocalcaemia, hyperphosphataemia and

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metabolic acidaemia. Our data suggest that phosphate containing fluids may not be ideal for unselected patients with acute kidney injury and their use may explain persistent acidaemia, and derangement of serum calcium and phosphate.

INTRODUCTION

Acute kidney injury (AKI) is increasingly common in critically ill patients and associated with significant morbidity and mortality.[1] One large multicenter study showed that over 16% of critically ill patients are diagnosed with AKI within forty-eight hours of ICU admission with a 22% overall incidence of AKI during ICU admission, as defined by the (risk, injury, failure, loss of kidney function, and end-stage kidney disease) RIFLE classification.[2] AKI is an independent risk factor for death.[3] In-hospital mortality for patients with AKI exceeds 25%[4] and mortality amongst ICU patients requiring renal replacement therapy approaches 70%.[5]

Continuous renal replacement therapy (CRRT) is the favored treatment modality for AKI in critically ill patients in Australasia. Many units, including ours, use Hemosol-B0 as their standard CRRT fluid.[5] Hemosol-B0 does not contain PO_4^{3-} or K^+, leaving patients susceptible to hypophosphataemia and hypokalaemia unless plasma levels ($[PO_4^{3-}]_{pl}$ and $[K^+]_{pl}$) are assiduously monitored and maintained.[6] Depending on CRRT intensity and duration the incidence of hypophosphataemia with CRRT can exceed 65%.

Phosphate is required to form the high-energy bond that provides the major energy currency required for metabolism. Complications of hypophosphatemia include respiratory muscle dysfunction and prolonged respiratory failure,[7] cardiac dysrhythmia (particularly ventricular tachycardia), reduced myocardial contractility and neuromuscular depression. Longer term, hypophosphataemia is associated with delayed recovery from AKI and higher incidence of chronic renal failure one year after dialysis commencement.[8] Hypophosphataemia is independently associated with worse ICU survival; the duration of hypophosphataemia predicts mortality.[9]

Phoxilium is a phosphate and potassium containing CRRT fluid, introduced to mitigate the risk of hypophosphataemia and need for potassium supplementation.[10] The composition of Hemosol-B0 and Phoxilium are compared in Table 1. Concerns regarding the use of Phoxilium as a standard RRT fluid include (i) metabolic acidosis promoted by the lower $[HCO_3^-]$ and [lactate], and increased $[HPO_4^{2-}]$ in Phoxilium, and (ii) suppression of ionized plasma $[Ca^{2+}]$ ($_i[Ca^{2+}]_{pl}$) as a consequence of increased plasma $[PO_4^-]$ and low $[Ca^{2+}]$ in Phoxilium.

Previous work identified that after administering Phoxilium for 42 hours to selected patients median $[HCO_3^-]_{pl}$ and $_i[Ca^{2+}]_{pl}$ were reduced but $[PO_4^{3-}]_{pl}$ maintained relative to a matched cohort who continued to receive Hemosol-B0.[10] It is common practice in most intensive care units to identify a default fluid to be used for CRRT. The biochemical consequences of replacing Hemosol-B0 with Phoxilium as the default CRRT fluid to an unselected patient cohort, and the potential benefit of minimizing handling of concentrated K^+ solutions (identified as a high risk intervention by organizations in Australia, Canada, the US and UK[11, 12]), remain unknown.

We undertook a retrospective observational cohort study to investigate the hypothesis that using Phoxilium, rather than Hemosol-B0, as a standard CRRT fluid would be associated with more normal and consistent phosphate levels and lower requirements for non-RRT electrolyte supplementation. The primary study end-point was the proportion of RRT duration spent with plasma $[PO_4^{3-}]$ within the normal range. Secondary analysis were performed to identify (i) differences in the proportion of the RRT period spent with $[HCO_3^-]_{pl}$ and $_i[Ca^{2+}]_{pl}$ in the normal range and (ii) differences in the requirement for non-RRT fluid K^+ and PO_4^{3-} supplementation.

METHODS

Study Population

Critically ill patients over 18 years old admitted to the Royal Adelaide Hospital Intensive Care Unit between October 2011 and October 2012 who required renal replacement therapy were recruited into the study.

Intervention

Patients underwent CRRT with continuous veno-venous haemodiafiltration (CVVHDF) with 40 ml/kg/hr effluent rate. This relatively high RRT dose was used to ensure minimum achieved doses of 25 mls/kg/hr (previous local audit data identified that

	Hemosol-B0	Phoxilium
Phosphate	0	1.2
Potassium	0	4
Bicarbonate	32	30
Lactate	3	0
Calcium	1.75	1.25
Magnesium	0.5	0.6
Sodium	140	140
Chloride	109.5	115.9

Table 1: Composition of RRT fluids (mmol/l).

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patients prescribed CRRT receive CRRT for an average 16 hours/day). Two six-month treatment periods were compared: during the first period, from October 2011 to March 2012, the standard renal replacement fluid was Hemosol-B0, (Hemosol-B0 standard period) with K^+ added to RRT fluid bags at the clinicians discretion. During the second period, from May 2012 to October 2012, Phoxilium was the default CVVHDF fluid (Phoxilium standard period). Both fluids were manufactured by Gambro, Lundia AB, Lund, Sweden. Patients receiving RRT fluid other than the designated default for that period were excluded from data analysis. The study was performed with local ethics committee approval.

Data Collection

Demographic, clinical and outcome data were recorded and mean delivered dialysis dose calculated. Daily plasma $[PO_4^{3-}]_{pl}$, $i[Ca^{2+}]_{pl}$, $[HCO_3^-]_{pl}$, $[K^+]_{pl}$ and $[Mg^{2+}]_{pl}$ results were documented throughout the duration of RRT. Supplementation with K^+ and PO_4^{3-} was recorded. Normal ranges refer to those used by the SA Pathology laboratory (SA Pathology, Rundle Mall, Adelaide, SA 5000, Australia) who were responsible for blood chemistry analysis during the study period.

Data Analysis

Categorical variables are expressed as number (percentage) and compared using Chi-squared or Fisher's exact test as indicated. Continuous variables are expressed as mean and standard deviation (SD) or when non-normally distributed as median (interquartile range), with between group comparisons performed by *t*-test or Wilcoxon rank-sum test. A two-tailed *p* value <0.05 was considered statistically significant. All analysis were undertaken using Stata/MP 14.0, Stata Corp LP software.

RESULTS

There were 86 patients admitted to the Royal Adelaide Hospital Intensive Care Unit between October 2011 and 2012 requiring RRT. Of these 7 patients were excluded from the Hemosol-B0 group and 4 from the Phoxilium due to unavailability of dialysis records or absent biochemical data. A further 9 patients were excluded as they received Hemosol-B0 during the Phoxilium standard period; no patients received Phoxilium during the Hemosol-B0 period as Phoxilium was not available. Data was collected on 35 patients in the Hemosol-B0 group and 31 patients in the Phoxilium group.

There was no significant difference in patient demographics, presence of chronic kidney disease, illness severity, length of stay, time ventilated or hospital mortality between the study groups (Table 2). Initial electrolytes prior to CRRT were not statistically different. There were no significant differences in the aetiology of AKI between the groups, in both groups sepsis was the most common precipitant (51% in the Hemosol-B0 group *vs.* 57% in the Phoxilium group) followed by cardiogenic shock, hypovolaemia and drug toxicity. There was no between-group difference in the cumulative insulin dose administered whilst receiving CRRT (p=0.591).

Plasma biochemistry values during CRRT administration are illustrated in Figure 1. For clarity only the first 5 days are included in the figure since only eight patients in each group underwent more than 5 days of CRRT. Data for the entire duration of CRRT is included in the data analysis.

Phosphate

Compared to patients receiving CRRT with Hemosol-B0, Phoxilium recipients spent a greater proportion of CRRT duration with $[PO_4^{3-}]_{pl}$ outside the normal range (Hemosol-B0 45.3% *vs.* Phoxilium 66.9% of patient days; p<0.001). In the Phoxilium group the median $[PO_4^{3-}]_{pl}$ exceeded the upper limit of the normal range on days 2-5 (Normal range: 0.65-1.45 mmol/l).

Ionised Calcium

Patients in the Phoxilium group spent more time with $_i[Ca^{2+}]_{pl}$ below the normal range than patients receiving Hemosol-B0 (Hemosol–B0 45% *vs.* Phoxilium 77.7% of patient days; p<0.001).

	Hemosol - BO	Phoxilium	*p* value
Age (years)	59.2 (54-67)	59.5 (46-70)	0.81
Gender - number % male	21 (54)	18 (58)	0.72
APACHE II	27.5 (23-33.75)	26.5 (20.25-31)	0.50
Pre-existing renal disease number (%)	13 (33.3)	12 (38.7)	0.64
Hours receiving RRT	50 (23-89)	45 (34-105)	0.69
CRRT dose (ml/kg/hr)	37.8 (37.8-39.63)	38.7 (38.7-39.95)	0.87
ICU length of stay median (hours)	205 (74-684)	155 (70-235)	0.47
Ventilated days post dialysis initiation – median	4 (1-12)	3 (0-5)	0.19
Hospital mortality - number (%)	24 (61.5)	21 (67.7)	0.59

Table 2: Patient clinical and outcome data: median (IQR) unless otherwise stated.

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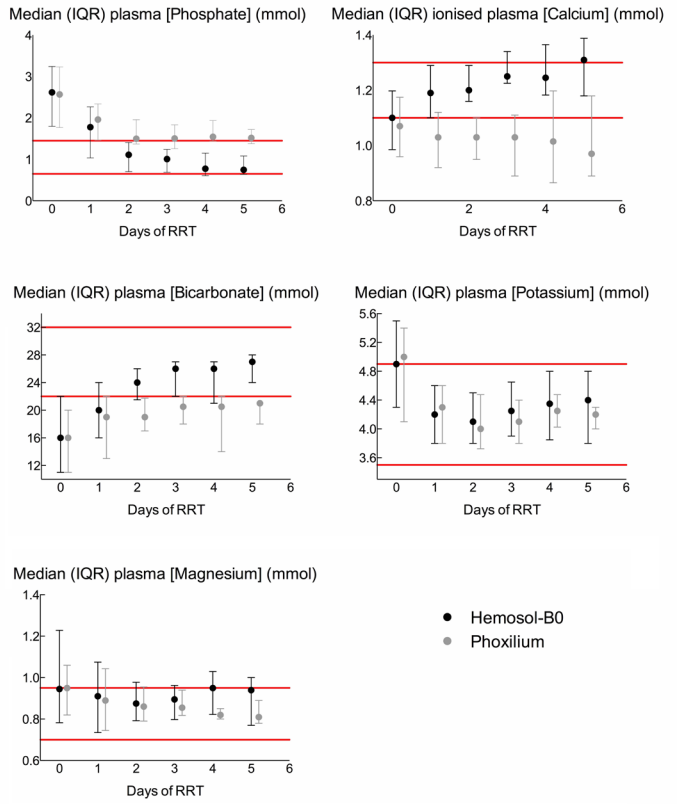

Figure 1: Median and interquartile ranges of plasma phosphate, calcium, bicarbonate, potassium and magnesium concentrations over the first five days of renal replacement therapy with either Hemosol-B0 (black circles) or Phoxilium (grey circles). Since only eight patients in each group underwent >5 days of RRT their data has been omitted from the figure for clarity. Their data is, however, included in all statistical analysis.

(Normal range: 1.1-1.3 mmol/l).

Standard Bicarbonate

In the Phoxilium group $[HCO_3^-]_{pl}$ was abnormally low for a greater proportion of time than in the Hemosol-B0 group (Hemosol-B0 26% *vs.* Phoxilium 68.5% of patient days; $p<0.001$). Starting from similar initial values, median $[HCO_3^-]_{pl}$ remained low on day 1 in both groups; however, median $[HCO_3^-]_{pl}$ normalized on days 2-5 in the Hemosol-B0 group whilst remaining low in the Phoxilium group. (Normal range: 22-32 mmol/l).

Potassium and Magnesium

There was no difference between the groups in the proportion of RRT duration spent with $[K^+]_{pl}$ or $[Mg^{2+}]_{pl}$ in the normal range (Hemosol-B0 85.8% and 61.8% *vs.* Phoxilium 89.4% and 73.1% of patient days; $p=0.30$ and 0.27 respectively); in both groups median $[K^+]$ and $[Mg^{2+}]$ were normal on each day of CRRT. (Normal ranges: K^+ 3.5-4.9 mmol, Mg^{2+} 0.7-0.95 mmol/l).

Phosphate and Potassium Supplementation

Phoxilium use abrogated the need for phosphate supplementation – no patients in the Phoxilium group required phosphate supplementation whilst 26% of patients in the Haemosol-B0 group required PO_4^{3-} supplementation. KCl was added to Hemosol-B0 in all but four of the Hemosol-B0 group. In the Phoxilium group, 45% of patients required non-RRT fluid potassium supplementation *vs.* 41% of patients receiving Hemosol-B0 ($p>0.05$).

DISCUSSION

When used as a standard CRRT fluid in a tertiary metropolitan intensive care unit, treating AKI with CRRT using Phoxilium, compared to Hemosol-B0, was associated with a greater proportion of treatment time spent with abnormally high $[PO_4^{3-}]_{pl}$, low ionized $[Ca^{2+}]_{pl}$ and low $[HCO_3^-]_{pl}$. Phoxilium use reduced the need for phosphate but not non-RRT fluid potassium supplementation.

Phoxilium use was associated with a significant period of time spent with $[PO_4^{3-}]_{pl}$ above the normal range. The median $[PO_4^{3-}]_{pl}$ was minimally elevated and mild hyperphosphataemia is generally well-tolerated in the short-term, although likely to contribute to $_i[Ca^{2+}]_{pl}$ suppression and promotion of metabolic acidaemia, both deleterious to the critically ill. Chronic hyperphosphataemia is associated with cardiovascular risk and mortality in end-stage renal failure[13,14]; further study is needed to identify whether analogous processes occur during short-term

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hyperphosphataemia.

Low $[Ca^{2+}]$ and increased $[PO_4^{3-}]$ in Phoxilium contribute to lowering $_i[Ca^{2+}]_{pl}$. Potential consequences include vasopressor resistance, decreased myocardial performance, arrhythmia, neuromuscular dysfunction and impaired coagulation.[15,16] Patients in our study were critically ill, as indicated by their Acute Physiology and Chronic Health Evaluation II (APACHE II) scores, and even mild manifestations of these complications would be deleterious.

Potential mechanisms by which Phoxilium might suppress $[HCO_3^-]_{pl}$ include: (i) lower $[HCO_3^-]$ and absent lactate (the metabolism of which consumes hydrogen ions) relative to Hemosol-B0 (ii) relatively high $[HPO_4^{2-}]_{pl}$ in patients receiving Phoxilium.[17] Consequences of acidaemia *in vivo* are complex[18]; acidaemia is associated with impaired myocardial performance, arrhythmia and vasopressor insensitivity as well as altered drug pharmacology and immune and metabolic dysregulation.[19] Again, given the severity of illness of our patient population, these complications are poorly tolerated.

Although Phoxilium contains K^+, its use in preference to Hemosol-B0 did not reduce the need for non-RRT fluid K^+ supplementation. This suggests that a policy of adding K^+ to RRT fluid at clinicians discretion is at least as effective at maintaining $[K^+]_{pl}$ as using RRT fluid containing KCl as standard. Indeed two potential advantages of an RRT fluid not containing KCl as standard are (i) use in hyperkalaemic patients (the probable reason for 9 patients in the Phoxilium period receiving Hemosol-B0) and (ii) the option to use a potassium salt with a weak anion such as acetate instead of chloride. Nevertheless, Hemosol-B0 requires more handling of KCl concentrate when supplementation to the RRT fluid is considered. Hospital surveillance systems identify KCl administration as the most frequent source of fatal drug errors[11]; guidelines recommend limiting KCl availability in clinical areas, and storage in a locked cupboard with meticulous (and time consuming) safety check procedures in place prior to its use.[20]

Using Phoxillium removed the need for phosphate supplementation. Our data are consistent with the important data published by Chua et al[10] that identified metabolic acidosis and hypocalcaemia amongst selected patients receiving CRRT in whom Phoxilium had been introduced to at clinicians discretion. However, in contrast to their finding that plasma $[PO_4^{3-}]$ was better maintained in patients receiving Phoxilium, we found that that the use of Phoxilium was associated with a greater proportion of time spent outside the normal plasma range of $[PO_4^{3-}]$. The difference can be explained by the different way in which the RRT fluids were used. In our study, the RRT fluids were used as unit defaults rather than at the discretion of the treating consultant (although clinicians could actively choose to use either fluid). This reflects common practice in many intensive care units, whereby a default CRRT fluid is identified, with the option to select an alternative at clinician's discretion. Notably, the default fluid was used in 89% of cases underlining the importance of 'default' clinical pathways and suggesting limited selection bias.

The use of potassium and phosphate containing renal replacement fluids in patients with AKI with hyperkalaemia and hyperphosphataemia is potentially harmful. In our study 9 of the Phoxilium group with the highest $[K^+]$ received Hemosol-B0 and the plasma $[K^+]$ following initiation of RRT was the same in both groups, suggesting that clinicians, appropriately, avoided Phoxilium in patients with high baseline $[K^+]$. However, several patients with high baseline $[HPO_4^{2-}]_{pl}$ were initiated on RRT with Phoxilium, reflecting the common observation that establishing a 'default' clinical pathway can inadvertently impede the application of clinical judgement. These data reinforce the view that a patient centred RRT strategy is preferable to a 'one size fits all' approach.

Our study further builds on previous work by standardizing the dialysis modality and dose, extending the study period to encompass the duration of RRT and measuring the important safety outcome of K^+ supplementation.

LIMITATIONS

As a retrospective study the possibility of selection bias is acknowledged, in particular 9 patients received Hemosol-B0 during the 'Phoxilium standard' period. Clinicians may have favoured Hemosol-B0 in patients with high $[K^+]$ or $[PO_4^{3-}]$. In a randomized study K^+ containing CRRT fluid might be expected to promote hyperkalaemia precluding the safe undertaking of a randomized, blinded comparison.

Although obtained retrospectively, biochemical datasets resist ascertainment bias. This was a single-centre study albeit in a large metropolitan hospital with broad casemix.

FUTURE DIRECTIONS

Our outcomes are primarily biochemical, clinical outcomes associated with Hemosol-B0 *vs.* Phoxilium, or indeed RRT fluids in general, remain to be evaluated. We reviewed all RRT for AKI, the majority being short term. Studying a subgroup of patients requiring longer-term intensive care RRT might identify particular benefits of Phoxilium as hypophosphataemia becomes more problematic and other drivers of acidosis defervesce. Although units frequently designate a 'standard' RRT fluid, and indeed in the well-regarded RENAL study all participating centres agreed to use Hemosol-B0 for CVVHDF,[5] with the availability of alternative RRT fluids such as Phoxilium trials evaluating patient centred RRT prescription are warranted.

CONCLUSION

In critically ill patients treated for AKI with continuous venovenous haemodiafiltration, Phoxilium was associated with hy-

perphosphataemia, hypocalcaemia and metabolic acidosis compared to Hemosol-B0. Phoxilium use reduced supplementary phosphate requirements; the presence of KCl in Phoxilium offers both advantages and disadvantages. These results inform clinicians of anticipated biochemical consequences of using these RRT fluids, and highlight the importance of patient-centred RRT fluid selection.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Mini Review

Corresponding author
Xin-Ming Chen, PhD
Senior Lecturer
Renal Research Laboratory
Kolling Institute of Medical Research
Level 9, Kolling Building;
Royal North Shore Hospital
University of Sydney
St. Leonards, NSW 2006, Australia
Tel. 61-2-9926 4780
Fax: 61- 2-9926 5715
E-mail: xin-ming.chen@sydney.edu.au

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Biomarkers and Next Generation Sequencing in Chronic Kidney Disease

Ying Shi, MD; Hao Yi, MD; Chunling Huang, PhD; Carol A. Pollock, MD, PhD; Xin-Ming Chen, PhD*

Kolling Institute of Medical Research, Royal North Shore Hospital, University of Sydney, St. Leonards, NSW 2006, Australia

INTRODUCTION

Biomarkers are valuable for early diagnosis, predicting prognosis and monitoring therapeutic efficacy in medicine, and particularly important in 'personalized medicine'.[1] Next generation sequencing technologies and their application in human diseases to foster human healthcare and personalized medicine have been recognized.[2] To date, reliable biomarkers for chronic kidney disease (CKD) are not available in clinic and the application of next generation sequencing technologies in CKD has not been well studied.

BIOMARKERS

Biomarkers are becoming increasingly important for predicting disease prognosis and enabling personalized therapy (precision medicine). As we previously reviewed,[3] biomarkers are increasingly being investigated for their utility in predicting patients most at risk of decline in renal function in order to rationalize and target care because of the escalating cost of monitoring and follow-up required in the care of patients with CKD. However, to date no reliable biomarker is available in clinical practice.

Current renal biomarkers include glomerular filtration rate (GFR) measurement, cardiovascular disease prediction, CKD progression, inflammatory and fibrotic markers.[4] The currently utilized biomarkers of CKD include estimated glomerular filtration rate (eGFR), albuminuria, cystatin C, nitric oxide, asymmetric dimethylarginine and neutrophil gelatinase-associated lipocalin (NGAL), especially eGFR. However, as the golden standard parameter, eGFR is based on creatinine levels, which could be influenced by the metabolic variants.[5] And those biomarkers are not sensitive and specific enough for early stage CKD patients, which results in the delayed intervention on CKD patients.[3] Besides, most of them can't distinguish the risk of developing CKD and estimate the kidney function accurately. Although, eGFR and albuminuria are used to define CKD, however they do not accurately indicate the renal function and injury in all forms of CKD.[6,7] For example, a proportion of patients with diabetic nephropathy develop renal failure without proteinuria. Moreover in kidney transplantation, the poor performance of biomarkers may lead to missing opportunity to receive potential kidneys because of the inaccurate low eGFR of living donors. In a word, current renal diagnosis and treatment situation requires more specific, stable and precise biomarkers for CKD.

By analyzing the endogenous metabolites from biofluids, tissue extracts and intact tissues, some metabonomic biomarkers were identified, such as glycocholic acid from plasma, valine from serum.[8] And the sequencing technique also is used in biomarker related research. Urinary microRNA (miRNAs) were demonstrated representing a crucial role on early detection and predicting progression.[9] Besides, there are studies identifying many key molecules in renal progression. For instance, tumor necrosis factor-alpha (TNF-α) is demonstrated as a biomarker to estimate the level of inflammation.[10] Transforming growth factor-β1 (TGF-β1) and bone morphogenetic protein-7 (BMP-7) were identified as the biomarkers of interstitial fibrosis by our group. The combination use of TGF-β1 (total and active) and BMP-7 showed a better prediction on renal end point than the conventional biomarkers.[11]

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Although several new biomarkers (NGAL, KIM-1, FGF23, miRNAs) have emerged, however, none of them have been validated to make clinical decisions upon their positivity.[7] They still remain to be tested before being used in clinical practice. Therefore, more promising biomarkers and validation cohort are both needed in the future renal research.

NEXT GENERATION SEQUENCING

After the automated sanger sequencing, a new sequencing method called the next generation sequencing (NGS) is wildly used in research. Compared to the conventional method, NGS is a low-cost and high-efficient way for various applications. Basing on different purpose of research, NGS enables to sequence the interested regions or whole genomes, viral or eukaryotic genomes or genetic mutation, and is applied in studies of transcriptomes (RNA sequencing (RNA-seq)), epigenetic modifications by using seq-based methods (ChIP-sequencing (ChIP-seq), methylation sequencing (methyl-seq) and DNase I hypersensitive sites sequencing (Dnase-seq)), and metagenomics.[12,13] With this revolutionary technique, the research method is fundamentally changed and researchers are capable of studying further mechanism and doing more personalized researches. Since precision medicine being proposed, NGS becomes the best way to implement.

It is reported that NGS has been used in renal research. The studies of renal diseases using NGS could be divided into four main directions: renal disease diagnosis, treatment, predicting prognosis and pathogenesis study. In regard to diagnosis, genetic variants are considered to be responsible for many genetic renal diseases, including autosomal dominant polycystic kidney disease (PKD), congenital nephrotic syndrome, congenital anormalies of the kidney and urinary tract and so on. However, the conventional diagnosis approach is costly, time-consuming limited by the technique.[14-17] Identifying those variants and develop specific molecular diagnosis to improve the accuracy and cost-efficiency of diagnosis is urgently needed. Using NGS to facilitate the routine diagnostics and find the potential biomarkers will be beneficial.[14,18] In term of treatment, many factors influence the therapy effect, like drug-resistance, individual sensitivity. For instance, drug-resistance includes virus drug-resistance and pharmacogenomics of immunosuppressive drugs for kidney transplant patients. For the former, genetic mutation of those viruses is verified for the drug-resistance. NGS has been used to optimize the tacrolimus dosage in renal transplanted patients by examining ABCB1/MDR1 gene variants and to elucidate variants over the entire BKV genome and at CD8 T-cell epitopes in pediatric hematopoietic cell transplant and kidney transplant recipients with BKV infection.[19,20] In addition, detecting genetic mutation in renal cell carcinoma could be used to predict the prognosis and monitor therapeutic response.[15,21] Moreover, studying pathogenesis by NGS revealed and identified several key molecules and pathways in initiation of renal fibrosis or kidney disease progression and renal cell function.[22-24] Therefore, NGS will be useful to identify novel therapeutic targets for kidney disease and consequently catalyze the precision medicine on renal diseases as an innovational tool.

Collectively, comprehensive studies to identify reliable biomarkers and apply NGS for personalized medicine in chronic kidney disease are definitely necessary.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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