IJKD IRANIAN JOURNAL OF KIDNEY DISEASES

Official Journal of the Iranian Society of Nephrology

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*Summarized Version**
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Review

Pharmacotherapy Considerations in CKD Patients With COVID-19, A Narrative Review

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Keywords. CKD, COVID-19, pharmacotherapy, SARS-CoV-2

Treatment of coronavirus disease 2019 (COVID-19) among patients with CKD requires special pharmacotherapy considerations that are reviewed here.

Literature review was done for several pharmacotherapy aspects in CKD patients including selection and modification of COVID-19 treatment, drug interactions, nephrotoxicity of drugs that are used for treatment of COVID-19 and potential risks/benefits of routine medications of CKD patients during COVID-19 pandemic.

CKD patients should be treated according to local or national COVID-19 protocols as other patients. But, there is no data on using remdesivir in patients with severe CKD. Oseltamivir and ribavirin require dose modification in patients with moderate to severe CKD. Nephrolithiasis, CKD, and acute interstitial nephritis have been reported with protease inhibitors. Acute kidney injury has been reported with remdesivir in patients with severe COVID-19. Pharmacokinetic-enhanced protease inhibitors increase the concentration of some drugs such as statins, cinacalcet, steroids, calcineurin inhibitors (CNIs). Some hypothetical benefits and harms have been suggested for statins and renin-angiotensin-aldosterone system inhibitors in COVID-19 patients. Continuing guideline-directed administration of these drugs is recommended. Among different immunomodulating/immunosuppressive drugs, hydroxychloroquine and CNIs are the safest ones during COVID-19. Antimetabolites are suggested to be withheld during moderate to severe COVID-19. Fluid therapy and anticoagulant prophylaxis/treatment need special attention in CKD patients with COVID-19. CKD patients with COVID-19 are treated as other patients, with some dose modifications if needed. Be mindful for management of drug interactions as well as modification of immunosuppressive drugs in patients with moderate to severe COVID-19.

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INTRODUCTION

New coronavirus pandemic named coronavirus disease 2019 (COVID-19) causes by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] Patients with diabetes mellitus, hypertension, and cardiovascular diseases have been introduced as high risk patients for COVID-19.[2] Diabetes and hypertension are common causes of chronic kidney disease (CKD).[3] Hence, it is expected that patients with CKD be at increased risk for COVID-19. Treatment of COVID-19 among CKD patients requires special pharmacotherapy considerations that have been reviewed here in several aspects including modification of COVID-19 treatment

regimen for patients with impaired kidney function, management of drug interactions in these patients due to common polypharmacy among patients with CKD, attention to nephrotoxicity of COVID-19 treatment regimen and possible need for modification of commonly used drugs among CKD patients. Pharmacotherapy considerations in kidney transplant patients are not discussed here.

RISK FOR COVID-19 AMONG CKD PATIENTS

There is no exact data on the incidence of CKD as comorbidity among patients with COVID-19. A survey on 1099 patients with laboratory-confirmed COVID-19 from 552 hospitals in 30 provinces, China revealed that only 0.7% of the patients had pre-existing CKD disease.[2] Despite this low reported rate of CKD among patients with COVID-19, a meta-analysis on 4 studies consisting 1389 patients showed significant association between CKD and severe COVID-19 (OR = 3.03, 95% CI: 1.09 to 8.47).[4] Angiotensin converting enzyme (ACE) 2 that is the functional receptor for SARS-CoV-2 and its cellular invasion is expressed in podocytes and proximal convoluted tubules, therefore, kidney is expected to be an important organ for SARS-CoV-2 induced cytopathic and inflammatory damage.[5,6] But, the rate of acute kidney injury (AKI) is not so high in hospitalized patients with COVID-19. In a report on 1099 patients from China, AKI happened in 0.5% of hospitalized patients.[2] A systematic review and meta-analysis on 19 studies consisting 660 patients with COVID-9 showed that 7.9% of patients experienced AKI.[7] Another retrospective, observational, multicenter study on 193 patients with laboratory-proven COVID-19 in China showed that at hospital admission 59% of the patients had proteinuria, 44% had hematuria, 14% had increased blood urea nitrogen (BUN), and 10% showed elevated serum creatinine concentration. Since previous medical histories of the patients were not exactly available, all of these findings cannot be readily diagnosed as AKI due to COVID-19; however, a multivariate analysis revealed that proteinuria, hematuria, and elevated BUN and creatinine concentrations were significantly correlated with death in patients with COVID-19.[8] AKI has been reported as a lethal complication among patients with COVID-19 in another survey as well.[9]

TREATMENT OF COVID-19 IN CKD PATIENTS

There is no specific pharmacologic treatment for SARS-CoV-2. Several antimicrobial and anti-inflammatory/adjuvant drugs are being used under clinical trial or compassionate use protocols. These drugs have been chosen based on in vitro activity against SARS-CoV-2 or other members of coronavirus family and/or some limited clinical experiences. These drugs include chloroquine/hydroxychloroquine, remdesivir, protease inhibitors (lopinavir/ritonavir, atazanavir, darunavir/cobicistat), favipiravir, arbidol (umifenovir), oseltamivir, azithromycin, sofosbuvir, tocilizumab, interferon (alpha and beta), and intravenous immunoglobulin.[10] Patients with creatinine clearance of less than 50 (NCT04292899) or 30 mL/min (NCT04257656, NCT04280705, NCT04323761) have been excluded from remdesivir clinical trials in patients with COVID-19.[11] Tocilizumab has not been studies in patients with creatinine clearance of less than 30 mL/min.[12] Other drugs are not contraindicated in patients with underlying kidney disease but some of them needs dose modification based on the level of kidney function.[12] Therefore, patients with underlying kidney diseases can be treated according to local or national COVID-19 protocols as other patients; but, more data is needed before using remdesivir and tocilizumab in patients with severe CKD.

DOSE ADJUSTMENT OF COVID-19 TREATMENT REGIMEN IN CKD PATIENTS

Lopinavir/ritonavir, atazanavir, darunavir/cobicistat, arbidol, sofosbuvir, and azithromycin do not require dose adjustment in patients with CKD.[12,13] Fifty percent dose reduction has been suggested for hydroxycholoquine in lupus nephritis patients with creatinine clearance of less than 30 mL/min.[12,14] Half routine dose of chloroquine has been suggested for patients with creatinine clearance of less than 10 mL/min and those on maintenance hemodialysis or peritoneal dialysis.[12] Dose modification of hydroxychloroquine has been proposed for patients who are taking these drugs chronically[12,14] and may not be extrapolated to short-course treatment of COVID-19.

Oseltamivir dose has to be reduced from 75 mg twice daily to 30 mg twice daily in patients

with creatinine clearance of less than 60 mL/min and to 30 mg/d in CKD patients with creatinine clearance of less than 30 mL/min. For patients on intermittent maintenance hemodialysis doses of 75 mg and 30 mg after each dialysis session have been proposed for dialysis with high-flux and low-flux membranes, respectively. For patients on continuous ambulatory peritoneal dialysis it has been postulated that a single 30 mg dose is sufficient for a 5-day treatment course.[12]

Remdesivir had not been previously approved by US food and drug administration (FDA) or European Medical Agency (EMA) for any indication. It is administered intravenously with a dose of 200 mg in the first day and then 100 mg daily for 9 days under clinical trial or compassionate protocols. Patients with creatinine clearance of less than 50 (NCT04292730, NCT04292899) or 30 mL/min (NCT04323761, NCT04252664) have been excluded from remdesivir clinical trials in patients with COVID-19. Therefore, no data would be available for remdesivir in patients with severe CKD.[11,15]

Favipiravir has been approved for treatment of influenza in Japan. It has not been approved by US FDA or EMA for any indication. So, enough data on its dose adjustment in CKD patients is not available. At least three clinical trials have been submitted for using favipiravir in COVID-19. Different doses have been applied *e.g.* 1600 mg twice daily for the first day of treatment, followed by 600 mg twice daily thereafter usually for 1 week (NCT04310228, NCT04333589) or 2400-2400-1200 mg for the first day, 8 hours apart followed by 1200 mg twice daily from the second day of treatment (NCT04303299). These studies have not excluded CKD patients from the study with these favipiravir doses. Only one of them excluded patients with unstable kidney function (NCT04333589) (probably AKI not CKD).[11]

Ribavirin was used in the regimen of COVID-19 at the beginning of SARS-CoV-2 outbreak; however, it is not used in newer COVID-19 regimens. It has been used with doses of 1000 to 1200 mg twice daily or 600 to 800 mg three times a day. Dose reductions of 50% and 75% have been proposed for patients with creatinine clearance between 30 to 50 mL/min and less than 30 mL/min, respectively in patients with SARS.[16] Tocilizumab has been approved for rheumatoid arteritis and cytokine release syndrome. It has not been studied in patients with creatinine clearance of less than 30 mL/min.[12]

NEPHROTOXICITY OF DRUGS THAT ARE USED FOR TREATMENT OF COVID-19

Some drugs that are used in COVID-19 treatment regimen (such as remdesivir, favipiravir, arbidol) have not been approved previously by US FDA or EMA, so; there is no data on their potential nephorotoxicity. Chloroquine/hydroxychloroquine, azithromycin, oseltamivir, and interferons have no considerable nephrotoxicity.[12]

Ritonavir boosted protease inhibitors such as lopinavir/ritonavir and atazanavir/ritonavir have been associated with increased risk of CKD in less than 1% of patients taking these drugs. This side effect has been seen in the median time of 4.5 years (inter-quartile range (IQR) 2.7 to 6.1 years) of follow-up.[17] Atazanavir can cause crystalluria/urolithiasis in a median time of 24.5 months (IQR 14.7 to 34.6 months) after commencement.[18] In addition, acute interstitial nephritis has been reported with atazanavir.[19] However, short duration of administration of these drugs for treatment of COVID-19 decreases the risk of renal side effects of these protease inhibitors.

Sofosbuvir may induce AKI in the form of acute interstitial nephritis with varying incidence of 1 to 15%. The median time of 9 weeks from the beginning of sofosbuvir to AKI occurence has been reported.[20] Therefore, short treatment period of COVID-19 eliminates the concern of sofosbuvir induced acute interstitial nephritis.

AKI has been reported as a major side effect of remdesivir among patients with severe COVID-19. This adverse effect has been mostly seen in patients under invasive mechanical ventilation.[21]

Nephrolithiasis has been reported in less than 2% of the patients taking tocilizumb, however; most of the patients with this side effect were those with rheumatoid arteritis who were taking methotrexate concomitantly. Methotrexate has been well known for inducing nephrolithiasis.[12]

Acute kidney injury has been reported in less than 1% of patients who receive intravenous immunoglobulin. In patients with underlying kidney dysfunction the infusion rate and concentration of the intravenous immunoglobulin solution have to be reduced.[12]

MANAGEMENT OF INTERACTION BETWEEN ROUTINE DRUGS OF THE CKD PATIENTS WITH COVID-19 TREATMENT REGIMEN

Some protease inhibitors (*e.g.* lopinavvir, darunavir) and their pharmacokinetic enhancers (ritonavir and cobicistat) are potent inhibitors of both cytochrome (CYP) 450 3A isoenzymes and P-glycoprotein efflux pump. These two systems play major roles in the metabolism and cellular distribution of several drugs; some of them are widely used by CKD patients.[12,22]

Cinacalcet

Cinacalect is metabolized by CYP450 3A. Cinacalcet concentration and exposure increase in patients taking lopinavir/ritonavir, atazanavir/ritonavir or darunavir/cobicistat. Although, serum level of parathyroid hormone may not change rapidly during only several days coadministration of cinacalcet with these ritonavir/ cobicistat -boosted protease inhibitors, rapid presenting side effects of cinacalcet such as hypocalcemia and hypomagnesemia may develop. These electrolyte abnormalities intensify the QT prolongation adverse effect of lopinavir/ritonavir or atazanivir/ritonavir especially if these antiviral drugs are used in a regimen containing chloroquine/hydroxychloroquine as well.[12,22]

Statin

Many patients with nephrotic syndrome take statins. CKD patients with different types of cardiovascular diseases also receive statins.[3] Most statins are metabolized by CYP450 3A and their exposure increases if coadministered with pharmacokinetic-enhanced protease inhibitors. Coadministration of simvastatin or lovastatin with ritonavir/cobicistat boosted protease inhibitors should be avoided. Due to increased systemic exposure to atorvastatin and rosuvastatin by about 490% and 108%, respectively when coadministered with pharmacokinetic-boosted protease inhibitors; maximum daily doses of 20 mg for atorvastatin and 10 to 20 mg for rosuvastatin have been proposed in patients taking these two drug classes concomitantly. Systemic exposure to pitavastatin and pravastatin increases by about 30% if taken with protease inhibitors. No dose adjustment was recommended for these two statins in combination with pharmacokinetic-enhanced protease inhibitors. It is prudent to monitor patients taking statins in combination with ritonavit/cobicistat-boosted protease inhibitors regarding myopathies, creatine phosphokinase elevation and possibly rhabdomyolysis and AKI. Keep in mind that these signs and symptoms are common between COVID-19 and statin toxicity.[12,22,23]

Corticosteroids

Patients with glomurelunephritis or vasculituies are usually treated with intravenous methylprednisolone pulse and oral prednisone/prednisolone. Pharmacokinetic-boosted protease inhibitors increase steroid exposure and adverse effects.[12,22]

Calcineurin Inhibitors

Pharmacokinetic-boosted protease inhibitors impede metabolism of calcineurin inhibitors (CNIs). A significant decline of 80% to 95% in cyclosporine dose or dose reduction of tacrolimus to about 1mg weekly and close blood concentrations monitoring of CNIs are recommended. Chloroquine/hydroxychloroquine also can increase CNIs blood concentration by inhibiting CNIs metabolism. In contrast, tocilizumab decreases CNIs concentrations by inducing CYP450 3A.[12,22]

Azathioprine

Hematologic toxicities of azathioprine may be enhanced in combination with chloroquine/hydroxychloroquine. Ribavirin can interfere with azathioprine metabolism and increase myelotoxic metabolites of azathioprine. Ribavirin has its own hematologic toxicity as well that exacerbate cytopenia in patients treating with these two drugs concomitantly. Enhanced myelotoxicity may also happen in coadministration of azathioprine with tocilizumab or interferons.[12,22] Patients should be monitored for blood cells counts.

Mycophenolate Mofetil/Sodium

Mycophenolate[12] and COVID-19[2] have hematologic side effects in common. Although chloroquine/hydroxychloroquine has hematologic side effects[12] and despite long term use of mycophenolate and hydroxychloroquine in patients with lupus nephritis,[13] no interaction has been reported between these two drugs in the literature.[12,22]

HYPOTHETICAL BENEFITS/HARMS OF SOME ROUTINE DRUGS OF CKD PATIENTS ON SEVERITY OF COVID-19

Renin-angiotensin-aldosterone system inhibitors

SARS-CoV-2 uses ACE2 as a functional receptor for cell entry. ACE2-bound SARS-CoV-2 internalization causes ACE2 down-regulation and subsequent overexpression of angiotensin II (AngII) and AT1 receptor in the lung and heart and damages to these tissues. Based on above findings, some researchers hypothesized that ACE inhibitors or angiotensin receptor blockers (ARBs) could be possible options to reduce SARS-CoV-2 induced lung injury.[24] This hypothesis has not been assessed in experimental or clinical studies. While ACE2 down-regulation by the virus may promote AngII and AT1 receptor expression, using ACE inhibitors and ARBs also increase AngII expression by several times. In addition, ACE2 is not inhibited by ACE inhibitors.[25] Therefore, using renin-angiotensin system inhibitors to prevent or treat COVID-19 may provide more ACE2 available for SARS-CoV-2 spike protein to bind and invade the lung and cardiac cells. Some investigators concern regarding harmful effects of these drugs in COVID-19 patients because of increased ACE2 levels in the lungs and heart by these drugs.[26] There is no scientific evidence to support this suggestion as well. Taken together, at this time it is recommended not initiating ACE inhibitors or ARBs to prevent or treat COVID-19. Several specialty societies recommend continuing these drugs during COVID-19 outbreak in patients who were taking them due to definite cardio- and nephroprotective indications such as history of myocardial infarction, heart failure, hypertension or proteinuria.[27] Another aspect that should be taken into account is AKI as a severe complication among patients with COVID-19. It may be at least partly due to high expression of ACE2 in podocytes and proximal convoluted tubules that make kidney as an important organ for SARS-CoV-2 induced cytopathic and inflammatory damage.[5] ACE inhibitors and ARBs may also cause AKI especially during initiation and dose escalation. Dose adjustment or even temporary discontinuation of ACE inhibitors/ARBs may be required in patients with severe AKI and subsequent hyperkalemia.[28]

Statins

Some human and animal studies revealed lung injury improvement by statins due to anti-inflammatory effects of these drugs.[29,30] In contrast, a retrospective study on the efficacy of rosuvastatin against infection-induced ARDS showed higher mortality in statin treated patients possibly because of increased IL-18.[31] During current COVID-19 pandemic some US hospitals included statin in their treatment regimen[32] and some suggested their use.[33,34] On the other hand, some others worry about statin-induced increase IL-18 level and worsening of SARS-CoV-2 induced ARDS and mortality.[35]

Large number of CKD patients suffers diabetes or cardiovascular diseases and should receive statin. Therefore, guideline-directed continuations of statin therapy among COVID-19 patients with history of atherosclerotic cardiovascular disease or diabetes and guidance-directed initiation of statin in patients with COVID-19 who show acute cardiac injury have been recommended. But, staring statin for management of COVID-19 infection outside clinical trial protocols is not suggested.[23]

FLUID THERAPY IN CKD PATIENTS WITH COVID-19

In the absence of shock, conservative fluid management is recommended in COVID-19 patients with severe acute respiratory infection or acute respiratory distress syndrome (ARDS). Excessive fluid administration may worsen oxygenation in these patients. For patients in septic shock, bolus dose of 250 to 500 mL crystalloid solutions over 15 to 30 minutes is recommended. Additional fluid should be administered after assessment for signs of overload.[36]

MODIFICATION OF IMMUNOSUPPRESSIVE/IMMUNOMODULATORY DRUGS IN CKD PATIENTS WITH COVID-19

Hydroxychloroquine

Almost all patients with lupus nephritis are treated with hydroxychloroquine.[14] Hydroxychloroquine has been used in COVID-19 treatment regimens as well.[10] So, CKD patients who are treated with hydroxychloroquine should continue this drug during COVID-19 pandemic and infection episode.

Corticosteroids

Large number of CKD patients with glomerulonephritis or vasculitis takes maintenance oral prednisolone/prednisone and during disease flare receives intravenous steroid pulse.[3,14] Steroids may prolong viral shedding, therefore, are not recommended for the treatment of patients with mild to moderate COVID-19 unless other indication such as exacerbation of asthma or obstructive pulmonary disease is present. Steroids are used for treating severe COVID-19 patients with septic shock or ARDS.[36] Some experts propose administering least effective maintenance dose of prednisolone/prednisone during COVID-19 pandemic in CKD patients who were already treating with these drugs. Increased steroid dose or changing oral steroid to intravenous one during severe COVID-19 infection and ARDS is recommended in kidney disease patients on chronic steroid therapy.[37]

CNIs

Some *in vitro* antiviral activities have been reported for cyclosporine against some members of coronavirus family.[10,38] Considering risks of the flare of underlying kidney disease, it seems prudent to continue CNIs especially cyclosporine with the lowest effective dose.[37] Balancing the risk of flare of the underlying kidney disease and severity of COVID-19 infection, one may consider switching from other immunosuppressive drugs to cyclosporine if there is efficacy for cyclosporine in that situation. However, AKI is a complication that may be seen with both COVID-19[2] and CNIs.[39]

Mycophenolate / Azathiopurine

The results regarding antiviral effects of mycophenolate are conflicting.[38,40] Fatal outcome has been reported with this drug during previous viral outbreaks.[38] On the other hand, mycophenolate has adverse hematologic effects including leukopenia and thrombocytopenia that may exacerbate hematologic complications of COVID-19.[2,38] Since available data shows no higher incidence of COVID-19 among CKD patients compared with other populations, it seems logical to continue mycophenolate in CKD patients taking this drug for glomerulonephritis or vasculitis diseases. In patients with severe COVID-19, it is suggested to stop antimetabolites including mycophenolate and azathioprine.[37]

Cyclophosphamide / Rituximab

ERA/EDTA recommends postponing the administration of maintenance cyctotoxic drugs/rituximab in patients with glomerulonephritis or vasculitis, however, disease flare may be detrimental.[37]

ANTICOAGULATION IN CKD PATIENTS WITH COVID-19

Patients with severe COVID-19 are at increased risk for thrombosis because of inflammation, immobility, hypoxia-induced thrombosis, and possibly invasion of the virus into the endothelial cells.[41] In a retrospective study on 449 patients with severe COVID-19, 28-day mortality was compared between patients who received and not received prophylactic doses of unfractionated (UFH) or low molecular weight heparin (LMWH). In general, mortality did not differ between these two groups of the patients. But, in patients with sepsis induced coagulopathy score[42] of more than 4 or D-dimer of more than 6 times of upper normal limit, 28-day mortality was significantly lower in heparin product users.[43] In addition to anticoagulation effects, heparin derivatives possess anti-inflammatory effect[44] that may be of benefit in COVID-19 patients who fulfill criteria for receiving prophylactic or treatment doses of heparins. World health organization recommends thromboembolism prophylaxis using LMWH (preferred) or UFH for critically ill patients with COVID-19 with no contraindication for heparin administration.[36] Based on available data, International Society of Thrombosis and Haemostasis (ISTH) has recommended the prophylactic dose of LMWH for every patient with COVID-19 who requires hospitalization. ISHT considered only active bleeding and platelet count of less than 25×10^9 /L as contraindications for LMWH administration.[45] CKD is a double-edged sword situation for thrombosis and hemorrhagic events.[46] Due to renal elimination of LMWH, unfractionated heparin is usually preferred in CKD patients with creatinine clearance of less than 30 mL/min or those who experience AKI during infection episode. However, dose reduction to 1 mg/kg/d for treatment and 20 to 30 mg/d for thrombosis prophylaxis has also been proposed for enoxaparin in CKD patients with creatinine clearance of less than 30 mL/min.[12,46] ISTH did not exclude patients with severe

CKD from its recommendation for thrombosis prophylaxis using LMWH and only recommended patient and laboratory monitoring.[45]

CONCLUSION

CKD patients should be treated according to local or national COVID-19 protocols as other patients. Due to lack of data in patients with severe CKD, remdesivir and tocilizumab are not suggested in patients with severe CKD. Oseltamivir and ribavirin require dose modification in patients with moderate to severe CKD.

Nephrolithiasis, CKD and acute interstitial nephritis have been infrequently reported with some protease inhibitors; however, due to the short period of COVID-19 treatment, there is no concern for these renal side effects. Pharmacokinetic-boosted protease inhibitors increase the concentration of some drugs such as statins, cinacalcet, steroids, and CNIs that are frequently used in patients with kidney diseases. The dose of these drugs should be reduced and their side effects be monitored. Some hypothetical benefits and harms have been proposed for statins and inhibitors of renin-angiotensin-aldosterone system in patients with COVID-19. Specialty societies recommend continuing these drugs in CKD patients who were already taking them during COVID-19 infection. They also recommend guideline-directed starting of these drugs in patients with COVID-19; however their side effects that are in common with clinical/laboratory characteristics of COVID-19 (such as AKI or myopathy) should be kept in mind. CKD patients who are taking hydroxychloroquine should continue this drug during COVID-19 pandemic and infection. Among different immunosuppressive drugs, CNIs are the safest ones during COVID-19 pandemic and infection. Antimetabolites are recommended to be withheld during moderate to severe COVID-19 infection. Some specialty societies recommend postponing administration of cytotoxic drugs and rituximab. But, clinicians should be mindful for the risk of underlying disease flare. Fluid therapy in hospitalized CKD patients with COVID-19 should be done conservatively. Fluid resuscitation in patients with shock should be done with small bolus of crystalloid solutions. Excessive fluid administration deteriorates oxygenation and increases the risk of ARDS. Prophylactic anticoagulation with heparin derivatives is recommended in CKD patients with COVID-19 who are admitted in the hospital. Report and studies on the efficacy and safety of drugs that are used in COVID-19 treatment regimen in CKD patients are emergently needed. Appropriate modification of immunosuppressive drugs requires sharing experiences of different hospitals worldwide.

CONFLICT OF INTEREST

Authors declare no competing interest.

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AUTHORS CONTRIBUTIONS

SDK contributed in conceptualization, literature review, data gathering and interpretation, manuscript writing, drafting, and finalization.

HK contributed in literature review, data gathering and interpretation, supervision, manuscript writing, drafting and finalization.

AN contributed in literature review and manuscript drafting.

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Review

Effects of Carvedilol on Cardiovascular Events and Mortality in Hemodialysis Patients, A Systematic Review and Meta-Analysis

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Carvedilol, the third generation of vasodilators; serves as the blocker of non-selective beta-adrenergic receptor and alpha1 adrenergic receptor. It could protect the cardiovascular system of patients receiving dialysis treatment. However, current clinical trials discussing the therapeutic benefit of carvedilol on patients receiving dialysis treatment remain inconsistent. Consequently, we decided to perform a meta-analysis to evaluate the clinical efficacy of carvedilol on patients receiving dialysis treatment.

A search was conducted using EMBASE, Pubmed, Cochrane Central Register of Controlled Trials, Wanfang database, Chinese National Knowledge Infrastructure (CNKI), and VIP information database up to February 2020. We research publications (include English and Chinese language) that discuss the effects of carvedilol on cardiovascular events, all-cause mortality, hospitalizations or left ventricular ejection fraction (LVEF) in dialysis population.

Our analysis included 4 randomized control trials and 2 observational studies. We discussed the therapeutical effects of carvedilol on all-cause mortality, cardiovascular events, hospitalizations, and LVEF of patients receiving dialysis treatment. Totally, this analysis reported 2998 hemodialysis (HD) patients. We found a significant association between carvedilol and reduced incidence of all-cause mortality, cardiovascular events and hospitalizations in HD patients. In addition, carvedilol significantly improves LVEF (n = 241; WMD = 6.95; 95% CI, 0.54 to 13.36; I^2 = 90%) in HD population.

Our systematic review and meta-analysis demonstrates that carvedilol is associated with a reduced incidence of cardiovascular events, all-cause mortality and hospitalizations in patients on HD. Besides; carvedilol significantly improves LVEF in HD population. Nevertheless, high-quality and well-powered evidence is still needed, so as to further confirm the impacts of carvedilol on HD patients.

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Keywords. carvedilol, cardiovascular events, mortality, hemodialysis

INTRODUCTION

Cardiovascular events is the major killer of Hemodialysis (HD) patients because of the following reasons.[1-3] First, approximately 80% of HD patients have one or more types of cardiac diseases,[4] which increases the possibility of cardiovascular events.

The mortality rate of chronic kidney disease (CKD) patients receiving dialysis is 6.1 to 7.8 times higher than the general population. Second, intermittent HD sessions expose patients to a high variability in hemodynamics, heart rate and electrolytes, which also increase the risk of cardiovascular events and mortality. Third, over activated sympathetic nervous system in HD patients can trigger cardiovascular events.[5-7] Finally, due to the lack of evidence-based drug therapy strategies and the complex pathophysiology in dialysis patients, cardiovascular events remains a big challenge to improve the survival rate of patients receiving dialysis.[8] Carvedilol, the blocker of non-selective beta-adrenergic receptor and alpha1 adrenergic receptor; offers multiple favorable effects such as antioxidant, antiapoptotic, and antiarrhythmic actions.[9-11] Thus, it may theoretically play a unique cardiovascular protective role in the patients receiving dialysis. However, only a clinical trial demonstrated that carvedilol improved survival rate of chronic dialysis patients with severe heart failure,[12] while other studies failed to demonstrate that carvedilol could help improve the survival rate[13,14] in the dialysis population. Considering the fact that the effect of carvedilol on dialysis patients still remains controversial, we thus aimed to perform a meta-analysis to evaluate the effects of carvedilol on patients requiring dialysis.

MATERIALS AND METHODS

We perform the systematic review and meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Ethical approval is not required because this meta-analysis does not directly involve any patient.

Data Sources and Search Strategy

A search was conducted using EMBASE, Pubmed, Cochrane Central Register of Controlled Trials, Wanfang database, Chinese National Knowledge Infrastructure (CNKI), and VIP information database up to February 2020. We research publications (include English and Chinese language) that discuss the effects of carvedilol on cardiovascular events, all-cause mortality, hospitalizations or left ventricular ejection fraction (LVEF) in dialysis population. The search strategy comprised a combination of free text terms and MeSH terms, primarily including: "Carvedilol", "Hemodialysis", "Hemodialyses", "Dialysis", and "Renal Replacement Therapy". We also reviewed the reference lists in order to search additional relevant studies.

Inclusion and Exclusion Criteria

Inclusion criteria was considered: a) participants: adult HD patients, b) study design: randomized controlled trial and observational studies, c) outcomes: cardiovascular events, all-cause mortality, and hospitalizations, d) intervention: the intervention group received standard care + carvedilol treatment, while the comparison group received standard care + placebo therapy or only standard care.

Exclusion criteria was considered: abstracts, reviews, duplicate publications, editorials, comments, case reports, publications without available data, and cell or animal experiments.

Data Extraction and Study Quality Assessment

Data from the included studies were extracted and recorded independently by two authors (D.Y. and J.H.) and disagreements were resolved by consensus. The following information recorded in each included study were extracted for both RCTs and observational studies: first author, year of publication, study design, dosage of carvedilol, sample size, follow-up, cardiovascular events, all-cause mortality, hospitalizations, mean and SD of LVEF (if the LVEF data was presented as mean and SE, it was converted to mean and SD). For RCTs, two reviewers (L.L. and M.P.) evaluated risk of bias of studies with the Cochrane collaboration risk of bias (ROB) tool.[15] For observational studies, the Newcastle-Ottawa Scale[16] was used to assess the quality of our included studies by the reviewers (L.L. and M.P.). Conflicts were resolved by the third reviewer (G.K.).

Statistical Analysis

We used the risk ratio (RR) and weighted mean difference (WMD) to compare dichotomous and continuous variables respectively. All results were reported with a 95% confidence intervals (CIs). Heterogeneity among studies was assessed using the I^2 statistics ($I^2 > 50\%$ suggested substantial heterogeneity). We used fixed effects or random effects model because it takes into account the heterogeneity across studies. Pre-stratified subgroup

analysis was performed to investigate possible sources of heterogeneity, including study design. The presence of publication bias was also evaluated with Egger's tests and funnel plots. If the all-causes mortality were present merely in figures, two authors (L.L. and G.K.) would use Engauge Digitizer 10.8 to collect data from the statistical graphs independently. Then, the mean values of all-cause mortality would be used to perform meta-analysis.[17] All analyses were performed using RevMan 5.3 and Stata 12.0. We considered $P < .05$ as statistically significant.

RESULTS

Search Results

In total, our comprehensive search yielded 248 articles. First, 36 duplicate articles were excluded and 212 articles were remained for screening. Then, we excluded 167 of the 212 articles after examining the title and abstract in more detail. We scrutinized the full texts of the remaining 45 studies, of which 39 were excluded, due to a lack of necessary data related to our study. Eventually, after a careful selection based on our above-mentioned inclusion criteria, 6 studies (Figure 1) with a total of 2998 participants were included in this meta-analysis (4 RCTs[7,12,14,18] and 2 observational studies[13,19]).

Study Characteristics

The main characteristics of the 6 studies included are shown in Table 1 and 2. Patients in these 6 studies had a long-term HD history. The intervention groups received standard care + carvedilol treatment, while the control groups received standard care + placebo therapy or only standard care. All LVEF measurements were estimated by echocardiogram. The author's judgments over the risk of bias for each included study were shown in Supplementary Table 1 and 2. Four RCTs and 2 observational

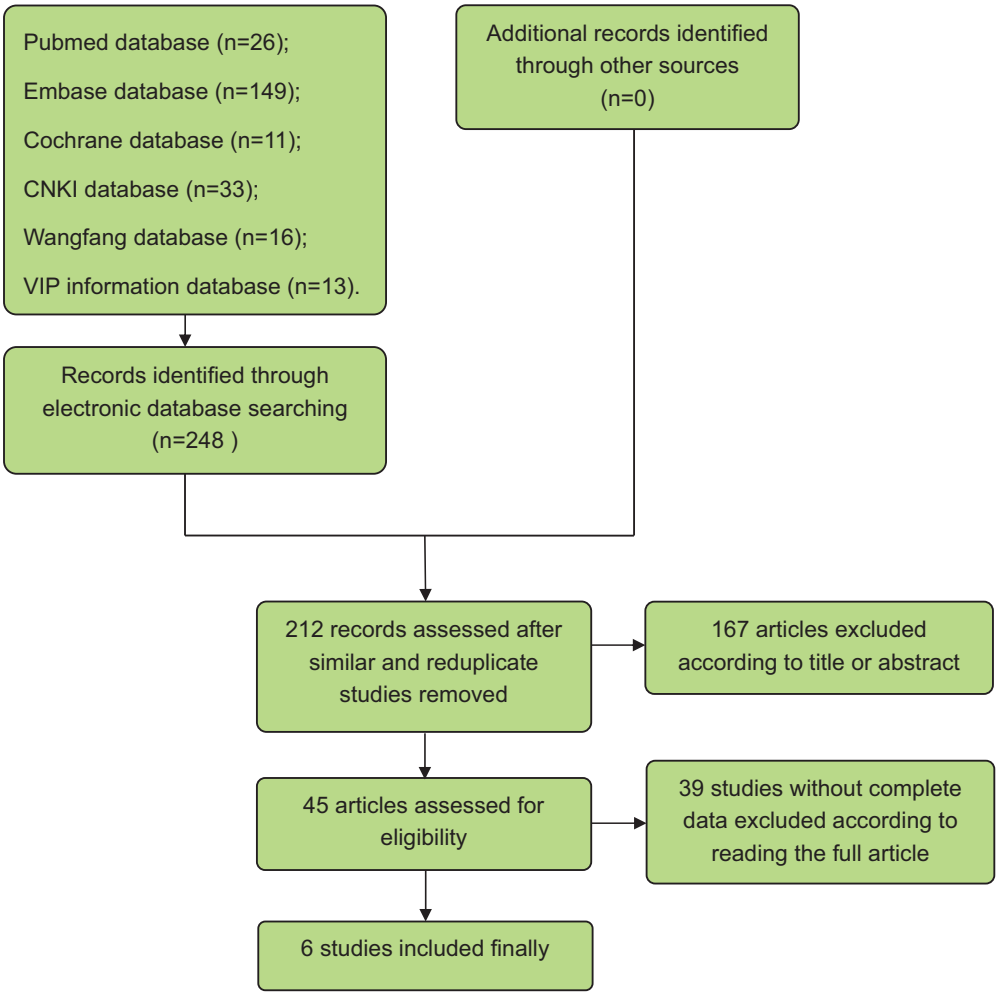


Figure 1. It shows flow diagram illustrating the selection of studies for this meta-analysis.

Table 1. Characteristics of Included Studies

Study	Center	Study Design	Age Carvedilol/Placebo	N Carvedilol/ Placebo	Interventions Carvedilol / Placebo		F/U (m)	Outcomes reported relevant to this meta-analysis
Cice et al, 2003	Italy	RCT	55.1 ± 7.6	58/56	1) Received 25mg bid Carvedilol; 2) Dialyzed four times a week; 3) Digitalis, ACEI, angiotensin II receptor antagonists, and nitrates.	1) Dialyzed four times a week; 2) Digitalis, ACEI, angiotensin II receptor antagonists, and nitrates.	24	All-cause mortality, Cardiovascular events, Hospitalizations, LVEF
Kojima et al, 2007	Japan	RCT	62.5 ± 7.16	10/10	1) Received 2.5 mg carvedilol a day. Dose was doubled every week until reaching 10 mg/d; 2) Conventional therapy; 3) Dialyzed 3 times weekly	1) Conventional therapy; 2) Dialyzed 3 times weekly .	3	All-cause mortality, Cardiovascular events, Hospitalizations, LVEF
Tang et al, 2008	China	RCT	38 ± 9	18/17	1) Received 10mg carvedilol bid; 2) Dialyzed 2-3 times weekly; 3) ACEI, angiotensin II receptor antagonists, nifedipine, iron agent, calcium agent, vitamin D3, erythropoietin.	1) Received 10 mg oryzanol bid; 2) Dialyzed 2-3 times weekly; 3) ACEI, angiotensin II receptor antagonists, nifedipine, iron agent, calcium agent, vitamin D3, erythropoietin.	2	All-cause mortality, Cardiovascular events, LVEF
Roberts et al, 2016	Australia	RCT	56.1 ± 10.3/61.4 ± 13.0	26/23	1) Receive carvedilol from 6.25mg twice daily to 25mg twice daily or to the maximum tolerated dose; 2) Standard treatment without carvedilol (detail not mentioned); 3) Dialyzed regularly.	1) Standard treatment without carvedilol (detail not mentioned); 2) Dialyzed regularly.	12	All-cause mortality, Cardiovascular events, Hospitalizations
Ma et al, 2018	China	Observational study	65.42 ± 9.83/63.66 ± 8.42	14/58	1) Received 5mg carvedilol bid. Dose reached 10 mg bid in 1-2 weeks; 2) CCB, ACEI, angiotensin II receptor antagonists, and nitrates; 3) Dialyzed regularly (more than 10h a week).	1) CCB, ACEI, angiotensin II receptor antagonists, and nitrates; 2) Dialyzed regularly (more than 10h a week).	43	All-cause mortality Cardiovascular events LVEF
Tang et al, 2016	China	Observational study	65.6 ± 11.5	1008/1700	1) Received 16.4mg carvedilol a day; 2) Standard treatment (detail not mentioned); 3) Dialyzed regularly.	1) Standard treatment (detail not mentioned); 2) Dialyzed regularly.	60	All-cause mortality

Data shown as mean [± SD]. N, number; F/U, follow-up; m, months; RCT, randomized controlled trial; LVEF, left ventricular ejection fraction; CCB, calcium channel blocker; ACEI, angiotensin-converting enzyme inhibitor.

Table 2. All-cause Mortality, Cardiovascular Events, Hospitalizations, and LVEF in Studies Using Carvedilol

Study	All-cause Mortality		Cardiovascular Events		Hospitalizations		LVEF	
	Carvedilol	Placebo	Carvedilol	Placebo	Carvedilol	Placebo	Carvedilol	Placebo
Cice et al, 2003	30 (52%)	41 (73%)	17 (29%)	39 (70%)	20 (34%)	33 (59%)	37 [10]	24 [10]
Kojima et al, 2007	0	0	0	0	0	0	63.5 [5.4]	66.4 [5.1]
Tang et al, 2008	0	0	2 (11%)	1 (6%)	Unclear	Unclear	46.8 [5.4]	38.8 [5.3]
Roberts et al, 2016	1 (4%)	0	1 (4%)	3 (13%)	14 (54%)	14 (61%)	Unclear	Unclear
Ma et al, 2018	5 (36%)	19 (36%)	3 (21%)	10 (17%)	Unclear	Unclear	68.6 [8.0]	59.2 [9.7]
Tang et al, 2016	555 (55%)	1190 (70%)	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear

Data shown as mean [± SD] or absolute (percentage). LVEF, left ventricular ejection fraction.

Supplementary Table 1. Risk of bias assessment quality of included RCTs

Study	Adequate random sequence generation	Allocation concealment	Blinding of participants and personnel	Adequate assessment of each outcome	Selective outcome reporting avoided	Free of Other Bias
Cice 2003	Yes	Unclear	Unclear	Yes	Yes	Yes
Kojima 2007	Yes	Unclear	Unclear	Yes	Yes	Yes
Roberts 2016	Yes	Unclear	Unclear	Yes	Yes	Yes
Tang 2008	Yes	Unclear	Unclear	Yes	Yes	Yes

Note: Risk of bias was assessed with use of the Cochrane risk-of-bias tool. The overall risk of bias of a study was considered "high" if more than 1 item was rated as "high risk" or if fewer than 2 items were rated as "low risk"; The overall risk of bias of a study was considered "moderate" if 2 or 3 items were rated as "low risk"; The overall risk of bias of a study was considered "low" if more than 4 items were rated as "low risk".

Supplementary Table 2. Risk of Bias in Observational Studies Using Newcastle-Ottawa Scale

Study	Selection				Comparability	Outcome			Total score
	Exposed cohort	Nonexposed cohort	Ascertainment of exposure	Outcome of interest		Assessment of outcome	Length of follow-up	Adequate follow-up	
MA 2018	*	*	*	-	**	*	*	*	8
Tang 2016	*	*	*	*	**	*	*	*	9

Note: A higher overall score corresponds to a lower risk of bias, a study awarded with ≥ 5 stars was defined as a high-quality study.

studies were at low risk.

Association of Carvedilol Therapy with All-cause Mortality

In the pooled analysis of 6 studies (n = 2998), compared with the patients with no carvedilol treatment, carvedilol reduced all-cause mortality in HD patients (RR = 0.79, 95% CI: 0.74 to 0.84; $P < .01$ in the fixed effects model, Figure 2a). There was no heterogeneity among studies ($P > .05$, $I^2 = 0\%$). Besides, subgroup analysis also showed that the results of 4 RCTs and 2 observational studies were consistent.

Association of Carvedilol Therapy with Cardiovascular Events

Meta-analysis of 5 studies (n = 290, 4 RCTs and 1 observational study) showed a significant decline in cardiovascular events of patients who received carvedilol treatment (RR = 0.51, 95% CI: 0.35 to 0.75; $P < .01$ in the fixed effects model, Figure 2b). Heterogeneity was detected among studies ($P > .05$, $I^2 = 34\%$). Since we only use one observational study, we did not perform subgroup-analysis.

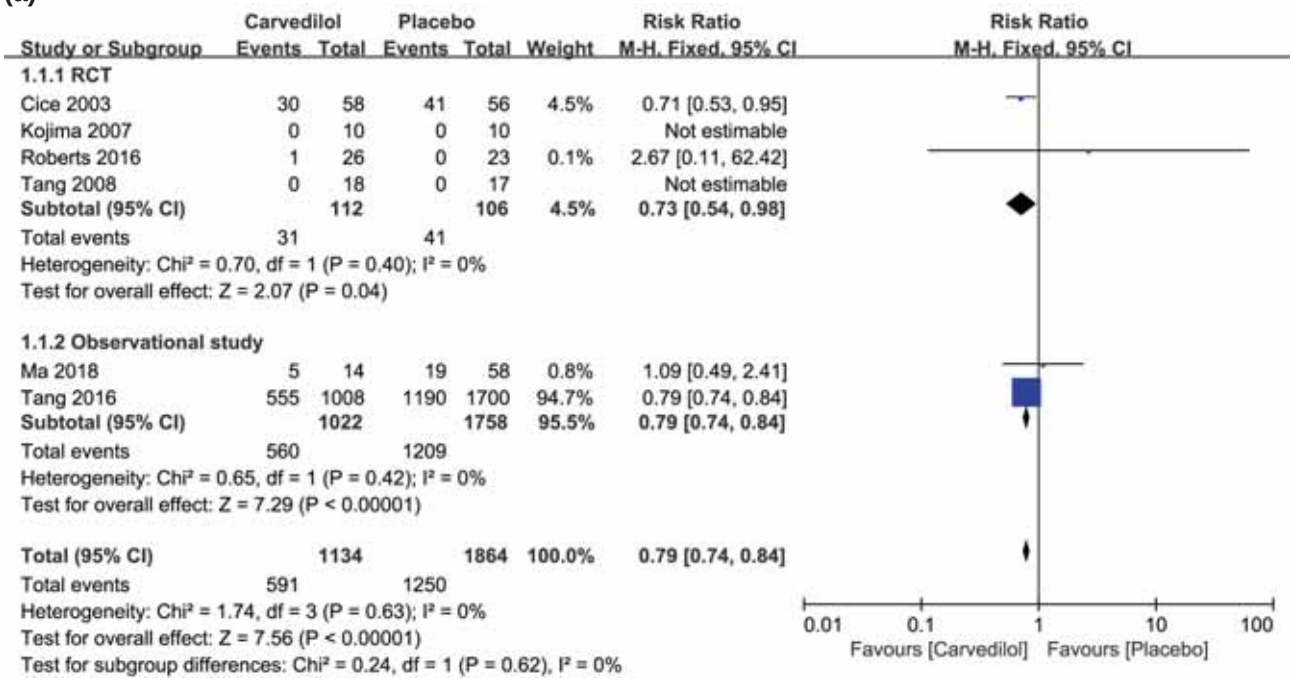
Association of Carvedilol Therapy with Hospitalizations

Meta-analysis of 3 RCTs (n = 183) showed a noticeable reduction in hospitalizations with carvedilol treatment (RR = 0.68, 95% CI: 0.49 to 0.93; $P < .05$ in the fixed effects model, Figure 2c). Heterogeneity was found among studies ($p = 0.2$, $I^2 = 39\%$).

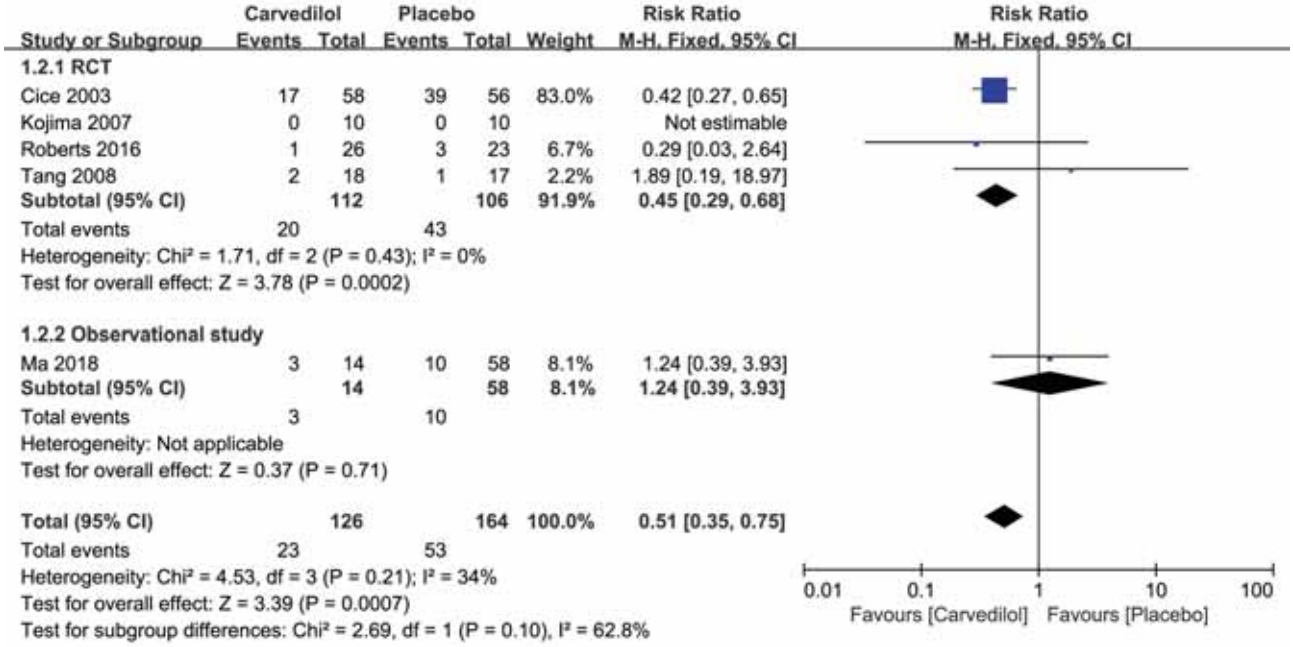
Association of Carvedilol Therapy with LVEF Change

Meta-analysis of 4 studies (n = 241, 3 RCTs, 1 observational study) showed carvedilol significantly improves LVEF (WMD = 6.95, 95% CI: 0.54 to 13.36; $P < .05$ in the random effects model, Figure 2d) in HD patients. However, heterogeneity was detected among studies ($P < .01$, $I^2 = 90\%$). Similarly, since

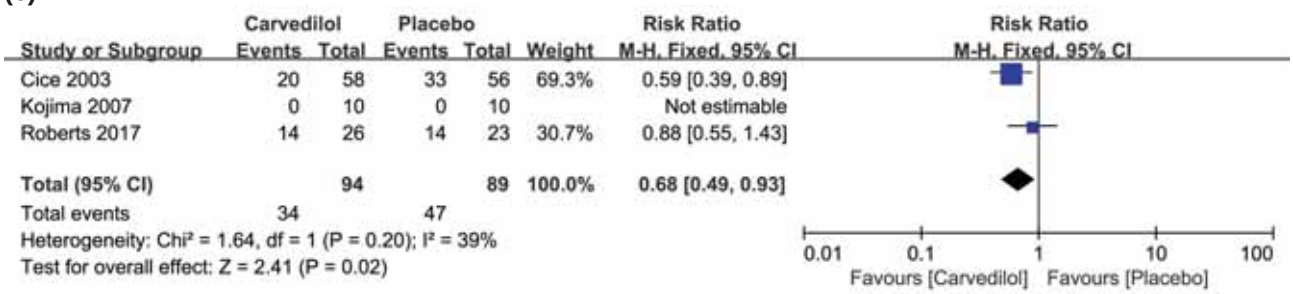
(a)


Carvedilol
Placebo
Risk Ratio
Risk Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
1.1.1 RCT
Cice 2003 30 58 41 56 4.5% 0.71 [0.53, 0.95]
Kojima 2007 0 10 0 10 Not estimable
Roberts 2016 1 26 0 23 0.1% 2.67 [0.11, 62.42]
Tang 2008 0 18 0 17 Not estimable
Subtotal (95% CI) 112 106 4.5% 0.73 [0.54, 0.98]
Total events 31 41
Heterogeneity: Chi² = 0.70, df = 1 (P = 0.40); I² = 0%
Test for overall effect: Z = 2.07 (P = 0.04)
1.1.2 Observational study
Ma 2018 5 14 19 58 0.8% 1.09 [0.49, 2.41]
Tang 2016 555 1008 1190 1700 94.7% 0.79 [0.74, 0.84]
Subtotal (95% CI) 1022 1758 95.5% 0.79 [0.74, 0.84]
Total events 560 1209
Heterogeneity: Chi² = 0.65, df = 1 (P = 0.42); I² = 0%
Test for overall effect: Z = 7.29 (P < 0.00001)
Total (95% CI) 1134 1864 100.0% 0.79 [0.74, 0.84]
Total events 591 1250
Heterogeneity: Chi² = 1.74, df = 3 (P = 0.63); I² = 0%
Test for overall effect: Z = 7.56 (P < 0.00001)
Test for subgroup differences: Chi² = 0.24, df = 1 (P = 0.62), I² = 0%
0.01 0.1 1 10 100
Favours [Carvedilol] Favours [Placebo]

(b)


Carvedilol
Placebo
Risk Ratio
Risk Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
1.2.1 RCT
Cice 2003 17 58 39 56 83.0% 0.42 [0.27, 0.65]
Kojima 2007 0 10 0 10 Not estimable
Roberts 2016 1 26 3 23 6.7% 0.29 [0.03, 2.64]
Tang 2008 2 18 1 17 2.2% 1.89 [0.19, 18.97]
Subtotal (95% CI) 112 106 91.9% 0.45 [0.29, 0.68]
Total events 20 43
Heterogeneity: Chi² = 1.71, df = 2 (P = 0.43); I² = 0%
Test for overall effect: Z = 3.78 (P = 0.0002)
1.2.2 Observational study
Ma 2018 3 14 10 58 8.1% 1.24 [0.39, 3.93]
Subtotal (95% CI) 14 58 8.1% 1.24 [0.39, 3.93]
Total events 3 10
Heterogeneity: Not applicable
Test for overall effect: Z = 0.37 (P = 0.71)
Total (95% CI) 126 164 100.0% 0.51 [0.35, 0.75]
Total events 23 53
Heterogeneity: Chi² = 4.53, df = 3 (P = 0.21); I² = 34%
Test for overall effect: Z = 3.39 (P = 0.0007)
Test for subgroup differences: Chi² = 2.69, df = 1 (P = 0.10), I² = 62.8%
0.01 0.1 1 10 100
Favours [Carvedilol] Favours [Placebo]

(c)


Carvedilol
Placebo
Risk Ratio
Risk Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
Cice 2003 20 58 33 56 69.3% 0.59 [0.39, 0.89]
Kojima 2007 0 10 0 10 Not estimable
Roberts 2017 14 26 14 23 30.7% 0.88 [0.55, 1.43]
Total (95% CI) 94 89 100.0% 0.68 [0.49, 0.93]
Total events 34 47
Heterogeneity: Chi² = 1.64, df = 1 (P = 0.20); I² = 39%
Test for overall effect: Z = 2.41 (P = 0.02)
0.01 0.1 1 10 100
Favours [Carvedilol] Favours [Placebo]

(d)

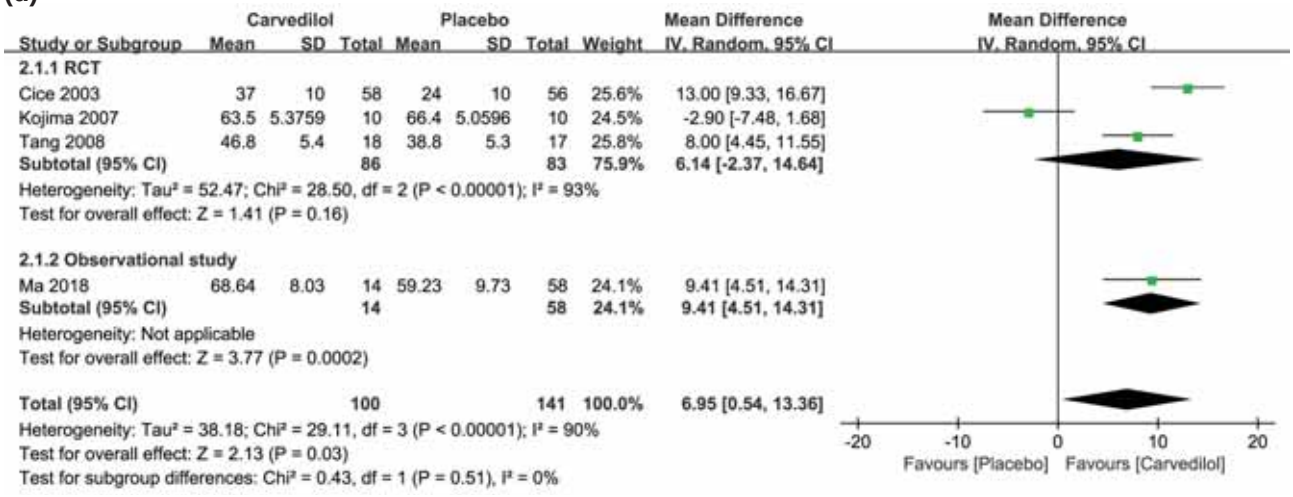


Figure 2. It demonstrates forest plots for all-cause mortality, cardiovascular events, hospitalizations, and LVEF outcomes; respectively: carvedilol associated with reduced all-cause mortality (a), cardiovascular events (b), and hospitalizations (c) in HD patients. Furthermore, carvedilol significantly improves LVEF (d) in dialysis population (LVEF, left ventricular ejection fraction; HD, hemodialysis).

we only use one observational study, we did not perform subgroup-analysis.

Publication Bias

The potential publication bias was detected by Egger's test and funnel plots (Figure 3a and 3b). We found no publication bias for carvedilol on all-cause mortality (Egger's test, $P > .05$) and cardiovascular events (Egger's test, $P > .05$). Besides, apart from all-cause mortality and cardiovascular events, we do not draw the funnel plots for other parameters in this meta-analysis, due to the small size of these parameters in our included studies.

DISCUSSION

To the best of our knowledge, the present research is the first meta-analysis that evaluated the clinical efficacy of carvedilol on HD patients. Our analysis included 4 RCTs and 2 observational studies, reporting 2998 HD patients. First, Carvedilol was associated with a 49% reduction in cardiovascular events, a 21% reduction in all-cause mortality and a 32% reduction in hospitalizations in HD patients. Besides, carvedilol significantly improves LVEF in HD population. Our research outcome could help update the information over the unique role of carvedilol in protecting patients receiving HD.

Approximately 80% of HD patients have one or more types of cardiac disease.[4] Also, intermittent HD sessions (usually three times a week) expose patients to a high variability in hemodynamics, heart rate and electrolytes. What's more, overactivated sympathetic nervous system in HD patients further triggers off cardiovascular events.[5-7] Given the high incidence of cardiovascular events, HD patients may benefit from β-blockers therapy,[20,21] especially the carvedilol, which is widely used in patients with heart failure (HF),[22,23] chemotherapy-induced cardiotoxicity,[24] arterial stiffness,[25] left ventricular function dysfunction,[26] acute coronary syndrome,[27] and hypertension[28]. However, few high-quality and well-powered studies have evaluated cardiovascular therapy's effects on HD patients. Most studies have excluded patients with advanced CKD due to the risk of side effects, such as hyperkalemia, hypotension, fluid overload, anemia and so forth.[29-32] Wali *et al*. reported a meta-analysis on RCTs addressing the efficacy and safety of carvedilol in HF treatment on CKD patients.[33] They suggested that treatment with carvedilol in CKD patients reduced the relative risks for all-cause, cardiovascular, and HF mortality in HF patients with CKD.[33] However, their finding did not determine whether carvedilol therapy could benefit advanced CKD or HD patients. Our meta-analysis filled such a gap by including data of 2998 HD patients and extracted from six studies. The pooled result suggested that carvedilol might play a unique cardiovascular protective role in the patients receiving dialysis.

First, our analysis focused on the association of carvedilol therapy with mortality rate, in that

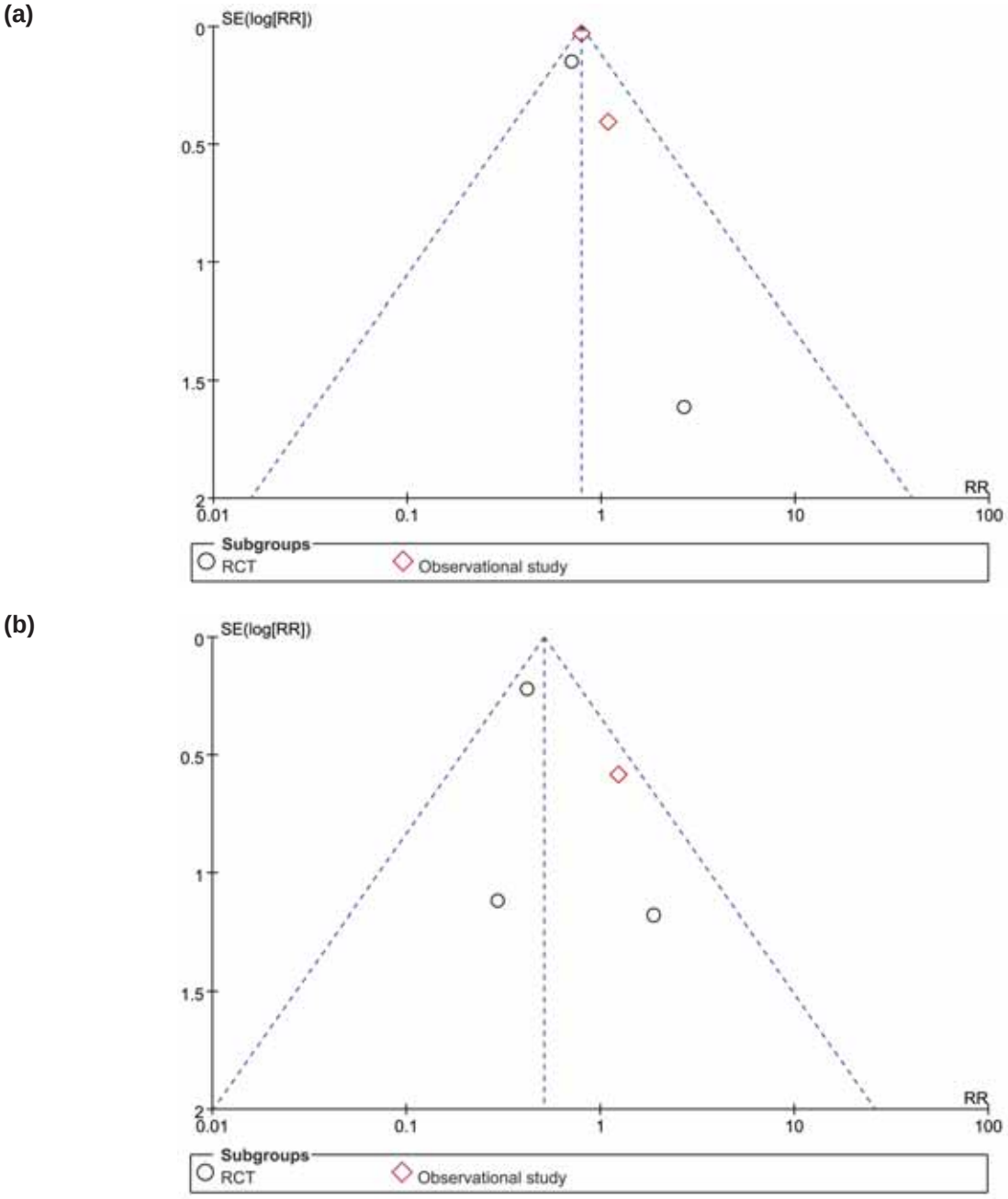


Figure 3. It shows publication bias assessment by funnel plot for all-cause mortality (a) and cardiovascular events(b).

mortality rate is one important clinical index, and mortality rates are high for HD patients. For HD patients initiating their renal replacement therapy within 3 years, the mortality rate almost reached 50%.[34] In addition, we also discussed the association of carvedilol therapy with Cardiovascular events, because Cardiovascular events is the leading killer of HD patients.[2,3,35] Moreover, we continued to analyze the association between carvedilol therapy and LVEF, as LVEF is the most frequently used parameter to define left ventricular systolic (dys-) function[36] and is strongly associated with the increased mortality rate in CKD patients.[37,38] Volume overload, chronic pressure and non-hemodynamic, such as oxidative stress and abnormal renin-angiotensin-aldosterone system (RAAS) activation, lead to the development of left ventricular systolic and diastolic dysfunction[39] of CKD patients. Our meta-analysis showed that carvedilol significantly improves LVEF in HD population, and thus was

consistent with the findings which showed that carvedilol was associated with a 49% reduction in cardiovascular events, a 21% reduction in all-cause mortality, and a 32% reduction in hospitalization of HD patients.

In sum, carvedilol is associated with a reduced possibility of cardiovascular events, all-cause mortality and hospitalizations in patients receiving HD. Besides; carvedilol significantly improves LVEF in dialysis population. Carvedilol can block sympathetic neural and RAAS activation, antioxidant, antiapoptotic, antiarrhythmic actions and so forth. Hence, it can provide a potential protective mechanism for HD patients.

Limitations of this systematic review and meta-analysis are as follows. Firstly, we were unable to minimize the heterogeneity's impacts through stratified analyses or subgroup, especially in LVEF comparisons, because of the limited number of included studies. The random effects model might reduce the effect of heterogeneity, but does not minimize it. Secondly, the included RCTs have a relatively small sample size and a short-term follow-up, which may lack strong persuasiveness. Thirdly, because of the limited number of studies (such as metoprolol, nebivolol, and bisoprolol), we could just quantitatively assess the effects of carvedilol. Hence, further clinical trials are needed to test the effects of other beta-blockers. Fourthly, different doses, different lengths of intervention time in each study might cause a potential bias. Also, different experiments had different designs, and the condition of patients also differed. Moreover, the small number of included studies could afford modest ability to detect the presence of publication bias.[40] Thus, high-quality and well-powered evidence is needed for future study.

CONCLUSION

The results of this meta-analysis support the argument that treatment with carvedilol can reduce rates of cardiovascular events, all-cause mortality and hospitalization in HD patients. Besides, carvedilol significantly improves LVEF in HD population. Nevertheless, high-quality and well-powered evidence is still needed to confirm the therapeutic impacts of carvedilol on HD patients.

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DISCLOSURE STATEMENT

The authors declare no conflicts of interest.

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Clinical and Radiologic Characteristics of COVID-19 in Patients With CKD

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Keywords. chronic kidney disease; COVID-19; computed tomography; mortality

Introduction. In this study, we aimed to evaluate the presentation and outcome of COVID-19 in patients with chronic kidney disease (CKD).
Methods. We included 43 patients with a past history of CKD and confirmed diagnosis of COVID-19. Patients were evaluated for demographic characteristics, clinical and laboratory data and findings of initial chest computed tomography (CT) and were followed until either death or discharge occurred. Then, study variables were compared based on final outcome and stage of CKD.
Results. Mean age ± SD of patients was 60.65 ± 14.36 years; 65.1% were male. Five of 43 patients (11.6%) died on follow-up and the rest were discharged. Disease outcome did not differ across CKD stages ($P > .05$). More than half of the patients (58.1%) presented with severe disease on admission. Clinical symptoms were similar to those of non-CKD individuals. Mean duration of hospitalization was higher in those who died, although not significant (16.6 ± 8.38 vs. 11 ± 6.26, $P > .05$). The only hematologic parameter that significantly differed between survivors and non-survivors was lactase dehydrogenase level ($P < .05$). Ground-glass opacification and reticular pattern were the most frequent patterns on CT and pleural effusion existed in about one-fifth of all patients. A greater lower zone score was noted in deceased patients ($P < .05$).
Conclusion. Patients with CKD are vulnerable to a more severe form of COVID-19 and experience a higher mortality rate than the general population; however, higher CKD stage is not related to worse prognosis or different imaging manifestation compared with lower stage.

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Original Paper

INTRODUCTION

In December 2019, an outbreak of pneumonia caused by a novel beta-coronavirus, currently named as the "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2), occurred in Wuhan, China. The disease caused by this virus was subsequently named Coronavirus Disease 2019 (COVID-19).[1] The most common clinical presentations of COVID-19 include fever, cough, dyspnea, and fatigue along with ground glass opacification (GGO) on chest computed tomography (CT) imaging.[2,3] Although the majority of patients with COVID-19 develop mild form of the disease, specific patient populations are at a risk of severe disease and require more attention. According to published studies, patients with underlying

conditions such as diabetes, cardiovascular disease, liver cirrhosis, and chronic kidney disease (CKD) are not only at a higher risk of infection, but also prone to a more serious outcome once infected. These patients are more likely to progress to forms of disease requiring admission to intensive care unit, mechanical ventilation or death.[4,5] Of note, patients with CKD are most likely to suffer from other concurrent comorbidities, such as hypertension, diabetes, and cardiovascular diseases.

Hence, it is likely that patients with CKD, in particular those on dialysis, will be at an excessive risk by the current COVID-19 pandemic[5] and as the crisis tends to remain, increased emphasis should be given to understanding disease presentation in high-risk subgroups for better patient management. While there is relatively extensive data regarding acute kidney injury triggered by COVID-19,[6,7] only few studies have investigated the characteristics of COVID-19 in patients with pre-existing kidney failure. Thus, in this study, we aimed to evaluate the clinical, laboratory and imaging findings, as well as disease outcome of a series of CKD patients with confirmed diagnosis of SARS-CoV-2 infection.

MATERIALS AND METHODS

Study Population And Design

This was a single-center study conducted on 43 consecutive patients with confirmed COVID-19 who were admitted from 20 February, 2020 to 15 April, 2020 to our academic tertiary hospital. Figure 1 shows the flowchart for patient enrollment. Inclusion criteria were as follows: 1) confirmed diagnosis of COVID-19 through real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay with samples obtained from nasopharyngeal swab; 2) chest CT images suggestive for COVID-19 pneumonia; 3) confirmed diagnosis of CKD based on Kidney disease: Improving Global Outcomes (KDIGO) CKD Work Group (2012) classification;[8] and 4) age older than 18 years old**.** All patients were receiving standard therapy for CKD based on national and international guidelines. On admission, patients' information regarding demographic data, past medical history, and presenting signs and symptoms was obtained through a pre-designed questionnaire filled by an independant investigator. Also, vital signs including patients' pulse rate and oxygen saturation (SpO2) were measured by a fingertip pulse oximeter and respiratory rate was measured by counting chest movements in one minute. Furthermore, the imaging findings of patients' initial chest CT were recorded. According to diagnosis guidelines of COVID-19 in Iran, all patients had undergone at least one low-dose CT scan at admission as part of their initial work-up.[9]

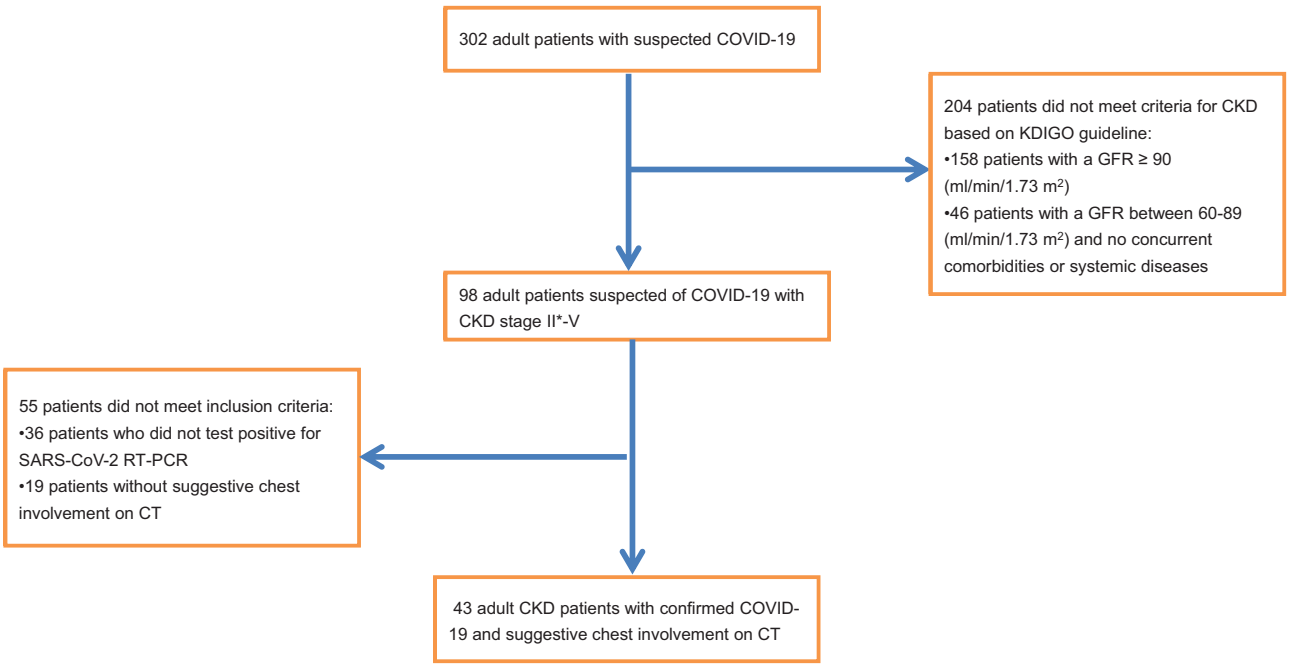

Figure 1. It shows flowchart of patient enrollment.
*G2 category with concurrent comorbidity/systemic disease

All patients were followed until one of the study endpoints (determined as either death or complete recovery and discharge) were reached. Estimated glomerular filtration rate (eGFR) was calculated for each case according to the Modification of Diet in Renal Disease (MDRD) equation.[10] According to the interim guideline of the WHO, published on 13 March, 2020, severe disease was defined as fever or suspected respiratory infection, plus either respiratory rate > 30 breaths/min; severe respiratory distress; or SpO2 ≤ 93% on room air.[11] Lymphocytopenia was considered as absolute lymphocyte count < 1×10^9, thrombocytopenia as platelet count < 150×10^9, neutrophilia as absolute neutrophil count > 7.5×10^9, and eosinopenia as < 0.01×10^9 in per liter of blood.

Ethical Considerations

The study protocol was approved by the ethics committee of our institutional review board. Informed consent was obtained from all patients prior to enrollment and all personal data was anonymized. All procedures performed in this study was in accordance with 1964 Helsinki declaration and its later amendments.

Chest CT Imaging

As part of national COVID-19 guidelines, all patients underwent non-contrast chest CT scan with a low-dose protocol.[18] All CT scans were performed using a 64-slice scanner (Siemens sensation; Siemens Healthineers, Erlangen, Germany) in a supine position during end-inspiration. For every patient, a low-dose CT protocol with the following scanning parameters was performed: gantry rotation time of 0.5 seconds, 0.625 mm × 64-detector array, pitch of 1.4, table speed of 45.2 mm/rotation, 20 mAs, 120 kVp, and a 300 × 300 matrix. CARE Dose4D; CARE kV scanning parameters were off. For the purpose of sagittal and coronal image reconstruction, 1 mm slice thickness and 1 mm reconstruction intervals were used. All of the machine surfaces were disinfected with ethanol and didecyldimethylammonium chloride (DDAC). After every CT, passive air ventilation was performed for at least 30 minutes.

DICOM data were transferred onto a picture archiving and communicating system (PACS) and two expert radiologists with 9 and 18 years of experience interpreted the images. Both radiologists were blinded to the lab data, clinical features, and patients' diagnosis. A final CT score was reached by consensus of the two radiologists. The opinion of a third radiologist was used to resolve dual-reader disagreements. All the CT scans were reviewed in axial, sagittal and coronal planes. In patients with more than one CT scan at admission, only the initial CT was evaluated. For classifying lung zone involvement, three zones were defined as follows: upper zone: above the carina region; middle zone: the area between the carina and inferior pulmonary vein; and lower zone: below the inferior pulmonary vein.[2] Predominant pattern of involvement was assessed and classified as GGO, consolidation, reticular or mixed. In addition, lesion distribution (peripheral, central or diffuse) and predominant zonal involvement (upper, middle, lower or diffuse) were recorded. In addition, the presence of other imaging features including crazy-paving, reverse halo sign, airway thickening, dilated vessels, airway dilatation, air bronchogram and lymphadenopathy (defined as a lymph node with a short-axis diameter > 10 mm) was assessed. The percentage of lung involvement was scored using the following system: 0: no involvement, 1: < 25%, 2: 26% to 50%, 3: 51% to 75%, and 4: > 75%.[26] The scores of each specific zone (upper, middle, and lower) of both lungs were summed up to calculate the zonal score (maximum score = 8) and the total score was calculated by summing scores of the upper, middle, and lower zones (maximum score = 24).

Laboratory Procedures

At admission, nasopharyngeal swab samples were taken from all patients with suspected SARS-CoV-2 infection and RT-PCR (DAAN gene Co Ltd device) was performed for every patient. Laboratory tests including biochemistry, complete blood count (CBC) and indices such as neutrophil/ lymphocyte ratio (NLR) and platelet/ lymphocyte ratio (PLR) ratios, and inflammatory markers such as C-reactive protein (CRP) were recorded. CRP levels were measured using the Rondox essay kit with immunoturbidimetric techniques. To evaluate CBC, NLR and PLR, venous blood samples were collected in potassiumethylene diamine tetraacetic acid tubes (dipotassium EDTA tubes) and the Sysmex-XE 2000i automated blood cell analyzer (Sysmex, Kobe, Japan) was used to for measurement within an hour. This is the standard duration time

for our laboratory, since it prevents EDTA-induced swelling.

Statistical Analysis

Continuous variables are reported as mean (± SD) and categorical variables are expressed as frequency (percentage). Variables were compared across outcome groups (death vs discharge) and also CKD stages. Normality assumptions were tested using the Shapiro-Wilk test. Student t-test was used for comparison of continuous data and Chi-square or Fischer's exact test was applied to compare categorical variables. All statistical analyses were performed by SPSS version 23 (IBM corp., Chicago, IL, USA). $P < .05$ was considered statistically significant.

RESULTS

Demographic Data

Table 1 shows demographic characteristics of patients at baseline. The mean age ± SD of patients was 60.65 ± 14.36 years (range: 27 to 87); 65.1% were male. Age and sex were equally distributed across the two groups of outcome ($P > .05$, $P > .05$; respectively). The most frequent CKD stage was stage IIIa and the least common was stage IV. Of the five patients with ESRD, three were already on dialysis and in the other two cases; dialysis was initiated for the first time after SARS-CoV-2 infection. Majority of patients (n = 32, 74.4%) had a positive history of cardiovascular diseases.

Clinical and Laboratory Findings

Table 2 shows clinical and laboratory data of patients at the time of admission. Of the total 43 patients, 5 (11.6%) died on follow-up; including four patients with an estimated GFR < 60 cc/min/ 1.73m^2 and one patient with stage II CKD. The rest of the cases (88.4%) were discharged. Disease outcome was not significantly different across different stages of CKD ($P > .05$). On admission, severe disease had developed in 58.1% of the patients. Mean duration of hospitalization was 11.65 ± 6.67 (range: 2 to 33), which was not significantly different across outcome groups ($P > .05$, $P > .05$; respectively, Table 2). Overall, the most common clinical presentation was dyspnea (65.1%), followed by cough (60.5%). Mean respiratory rate, temperature, and oxygen saturation did not vary significantly between those who died and those who were discharged (Table 2). Leukopenia, leukocytosis, and thrombocytopenia were observed in 7 (16.3%), 4 (9.3%), and 12 (27.9%) patients; respectively. The mean lymphocyte, neutrophil and eosinophil count was 1.29 ± 0.57 × 10^9 /L, 4.56 ± 2.81 × 10^9 /L, 0.083 ± 0.065 × 10^9 /L which did not display a significant difference between the two groups of outcome. Evaluation of serum biochemistry revealed that mean serum C-reactive protein and lactase dehydrogenase (LDH) level were higher

Table 1. Comparison of Patients' Baseline Characteristics Based on Final Disease Outcome.

Variables	Total Patients (n = 43)	Discharged (n = 38)	Death (n = 5)	*P*
Mean Age, years	60.65 ± 14.36 27 to 87	60.63 ± 14.50 27 to 87	60.8 ± 14.61 38 to 76	> .05
Sex				
Male	28 (65.1)	23 (60.5)	5 (100)	> .05
Female	15 (34.9)	15 (39.5)	-	
Comorbidities				
IHD	14 (32.6)	11 (28.9)	3 (60)	> .05
HTN	18 (41.9)	16 (42.1)	2 (40)	
Diabetes Mellitus	16 (37.2)	15 (39.5)	1 (20)	
Asthma	1 (2.3)	1 (2.6)	-	
CKD stage				
II	10 (23.3)	10 (26.6)	-	> .05
IIIa	19 (44.2)	17 (44.7)	2 (40)	
IIIb	7 (16.30)	5 (13.2)	2 (40)	
IV	2 (4.7)	2 (5.3)	-	
V	5 (11.6)	4 (10.5)	1 (20)	

Continuous variables are reported as mean ± SD and range. Categorical variables are reported as n (%). P values are calculated by χ^2 test, Fisher's exact test, or Student t-test.
Abbreviations: IHD, ischemic heart disease; HTN, hypertension; CKD, chronic kidney disease.

Table 2. Comparison of Patients' Baseline Clinical Presentation and Laboratory Findings Based on Final Disease Outcome

Variables	Total Patients (n = 43)	Discharged (n = 38)	Death (n = 5)	*P*
Clinical Presentation				
Dyspnea	28 (65.1)	25 (65.8)	3 (60)	
Fever	21 (48.8)	19 (50)	2 (40)	
Cough	26 (60.5)	23 (60.5)	3 (60)	
Sore Throat	5 (11.6)	4 (10.5)	1 (20)	
Chilling Sensation	9 (20.9)	9 (23.7)	-	
Headache	4 (9.3)	3 (7.8)	1 (20)	> .05
Myalgia	14 (32.6)	13 (34.2)	1 (20)	
Nausea	6 (14)	5 (13.1)	1 (20)	
Abdominal Pain	6 (14)	5 (13.1)	1 (20)	
Diarrhea	4 (9.3)	3 (7.8)	1 (20)	
Duration of Hospitalization, days	11.65 ± 6.67 2 to 33	11 ± 6.26 2 to 33	16.6 ± 8.38 7 to 29	> .05
Oxygen saturation, %	88.73 ± 6.52 68 to 98	89.31 ± 5.21 74 to 98	85.5 ± 12.15 68 to 96	> .05
Respiratory rate,/min	17.77 ± 3.80 12 to 30	17.73 ± 3.71 12 to 30	18 ± 4.9 12 to 24	> .05
Temperature, ºC	37.17 ± 0.90 35 to 39	37.13 ± 0.91 35.5 to 39	37.4 ± 0.88 35 to 38.3	> .05
Leukocyte Count				
< 4 × 10^9 /L	7 (16.3)	6 (15.8)	1 (20)	
× 10^9 /L	32 (74.4)	28 (73.6)	4 (80)	> .05
> 4 × 10^9 /L	4 (9.3)	4 (10.6)	-	
Platelet count				
< 150 × 10^9 /L	12 (27.9)	10 (26.3)	2 (40)	
150-450 × 10^9 /L	29 (67.4)	26 (68.2)	3 (60)	> .05
> 450 × 10^9 /L	2 (4.7)	2 (5.2)	-	
Hemoglobin Level, g/dL	13.56 ± 2.87 6.7 to 18.9	13.59 ± 2.54 6.7 to 18.9	13.26 ± 2.53 9 to 15.3	> .05
Differential Count				
Neutrophilia	4 (9.3)	4 (10.5)	-	
Lymphocytopenia	23 (53.5)	20 (52.6)	3 (60)	> .05
Eosinopenia	3 (7)	3 (7.9)	-	
NLR	4.03 ± 2.99	4.11 ± 3.15	3.36 ± 0.93	> .05
PLR	179.57 ± 73.52	184.5 ± 74.4	142.7 ± 60.44	> .05
NLR*CRP	174.8 ± 199.1	176.5 ± 210.6	160.1 ± 28.0	> .05
Serum Creatinine, mg/L	1.89 ± 1.59 0.8 to 7.3	1.80 ± 1.53 0.80 to 7.30	2.58 ± 2.06 1.26 to 6.24	> .05
Serum BUN, mg/L	51.2 ± 47.3 10 to 231	47.56 ± 45.05 10 to 231	90 ± 64.08 43 to 163	> .05
CRP, mg/dL	39.42 ± 20.08 1 to 73	38.56 ± 2.24 1 to 73	46.7 ± 17.58 33 to 69	> .05
Creatine Phosphokinase, IU/l	183.2 ± 140.59 15 to 1008	174.11 ± 201.3 15 to 1008	240.2 ± 210.1 82 to 531	> .05
Lactase Dehydrogenase, IU/l	408.4 ± 232.7 10.93 to 1413	355 ± 127.5 10.93 to 571	740.2 ± 452.9 430 to 1413	< .05
Serum Calcium, mmol/L	8.59 ± 0.68 6 to 9.9	8.6 ± 0.7 6 to 9.9	8.65 ± 0.65 7.8 to 9.3	> .05
Serum Phosphorus, mg/dL	3.55 ± 0.86 2.2 to 5.1	2.85 ± 1.61 2.2 to 4.7	3.7 ± 1.97 2.3 to 5.1	> .05
Serum Magnesium, mmol/L	2.06 ± 0.73 1.2 to 4.8	2.08 ± 0.77 1.2 to 4.8	1.86 ± 0.2 1.7 to 2.1	> .05
25 (OH) Vitamin D, ng/mL	37.93 ± 26.2 5 to 126	37.76 ± 26.75 5 to 126	39.33 ± 27.46 17 to 70	> .05

Continuous variables are reported as mean ± standard deviation and range. Categorical variables are reported as n (%). P values are calculated by χ^2 test, Fisher's exact test, or Mann-Whitney U test.
Abbreviations: NLR, Neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein; BUN, blood urea nitrogen.

than normal in all patients. Also, baseline LDH level was significantly higher in patients who had experienced death ($P < .05$). Blood urea nitrogen and serum creatinine showed borderline significance in regards to disease outcome.

Imaging Findings

Table 3 shows chest CT findings of patients

Table 3. Comparison of Patients' Initial Chest CT Findings Based on Final Disease Outcome

Variables	Total Patients (n = 43)	Discharged (n = 38)	Death (n = 5)	*P*
Upper Zone Score	2.63 ± 1.77 0 to 6	2.65 ± 1.79 0 to 6	2.44 ± 1.81 0 to 5	> .05
Middle Zone Score	3.98 ± 2.12 0 to 8	3.78 ± 2.15 0 to 8	4.4 ± 1.14 4 to 7	> .05
Lower Zone Score	4.3 ± 2.31 0 to 8	4.05 ± 2.28 0 to 8	6.2 ± 1.64 4 to 8	< .05
Total Score	10.91 ± 5.67 1 to 22	10.5 ± 5.75 1 to 22	14 ± 4.18 8 to 19	> .05
Pattern of Involvement				
Ground Glass Opacification	15 (34.9)	12 (31.5)	3 (60)	> .05
Consolidation	4 (9.3)	3 (7.9)	1 (20)	
Reticular	7 (16.3)	7 (18)	-	
Mixed	4 (9.3)	3 (7.9)	1 (20)	
Lesion Distribution				
Axial				
Central	2 (4.7)	2 (5.2)	-	> .05
Peripheral	35 (87.4)	32 (84.2)	3 (60)	
Diffuse	6 (14)	4 (10.5)	2 (40)	
Craniocaudal				
Upper	3 (7)	3 (7.8)	-	> .05
Middle	8 (18.6)	8 (21)	-	
Lower	20 (46.5)	18 (47.4)	2 (40)	
Diffuse	12 (27.9)	9 (23.7)	3 (60)	
Anteroposterior				
Anterior	2 (4.7)	2 (5.2)	2 (40)	> .05
Posterior	29 (67.4)	29 (76.3)	-	
Diffuse	12 (27.9)	9 (23.7)	3 (60)	
Lung Involvement				
Bilateral	40 (93)	35 (92.1)	5 (100)	> .05
Unilateral	3 (7)	3 (7.9)	-	
Other Imaging Features				
Pleural Effusion	9 (20.9)	8 (21)	1 (20)	> .05
Pericardial Effusion	6 (14)	6 (15.8)	-	
Emphysema	1 (2.3)	1 (2.6)	-	
Fibrosis	1 (2.3)	1 (2.6)	-	
Bronchiectasis	1 (2.3)	1 (2.6)	-	
Bronchial Wall Thickening	37 (86)	32 (84.2)	5 (100)	
Crazy-paving Pattern	7 (16.3)	6 (15.8)	1 (20)	
Reversed-halo Sign	-	-	-	
Dilated Vessel	32 (74.4)	27 (71)	5 (100)	
Airway Dilatation	18 (41.9)	17 (44.7)	1 (20)	
Air Bronchogram	13 (30.2)	12 (31.5)	1 (20)	
Cavitation	-	-	-	
Interseptal Thickening	4 (9.3)	4 (10.5)	-	
Cyst	3 (7)	3 (7.8)	-	
Lymphadenopathy	3 (7)	3 (7.8)	-	

Continuous variables are reported as mean ± standard deviation and range. Categorical variables are reported as n (%). P-values are calculated by χ^2 test, Fisher's exact test, or Student t-test.

in detail. As shown, the mean ± SD score of the upper zone, middle zone, and lower zone were 2.63 ± 1.77, 3.98 ± 2.12, and 4.3 ± 2.31; respectively. The total lung score was 10.91 ± 5.67, which was not different across outcome groups ($P > .05$); however, in patients who died, lower zone score was higher ($P < .05$). We observed bilateral involvement in 93% of patients. Interestingly, bilateral lung involvement was seen in all patients who eventually experienced COVID-19-related mortality. The most common pattern of involvement was GGO followed by reticular pattern. Lesions were mainly distributed in the posterior and lower parts of the lungs. Moreover, these lesions were commonly found in the periphery of lungs. Among other imaging features, vessel dilatation was a frequent finding, observed in approximately 75% of patients. Also, we observed that airway thickening and vessel dilatation existed in all patients who had expired. Pleural effusion was seen in approximately one-fifth of patients and pericardial effusion was seen in 14% of our patients (Figure 2).

Comparison of Clinical, Laboratory, and CT Findings Based on CKD Stage

As shown in Table 4, patients with ESRD experienced the longest duration of hospitalization, however; when comparing across groups, no statistically significant different was seen. Mean oxygen saturation also did not vary in patients with different stages of CKD. Evaluation of biochemical parameters revealed that leukocyte count is associated with borderline difference across CKD stages ($P > .05$); however, this difference was not observed in platelet count and level of serum hemoglobin**.**

As shown by the total lung score, the extent of lung involvement did not differ across patients with different stage of CKD ($P > .05$, Table 4). However, the presence of consolidation and GGO was significantly different across CKD groups ($P < .05$). Although lymphadenopathy was more commonly observed in patients with a higher GFR ($P < .05$), the presence of other imaging features did not vary significantly between patients with different CKD stages.

DISCUSSION

Many recent studies have emphasized the role of SARS-CoV-2 infection in inducing acute kidney damage but evidence regarding the characteristics of COVID-19 infection in patients with a history of chronic renal disease is sparse.[6,7] Besides, no study has specifically investigated disease outcome in these patients based on stage of CKD. The results of our study showed that more than half of patients with CKD developed severe form of COVID-19. However, lower GFR and subsequently, higher stage of CKD, was not associated with a poorer prognosis and outcome.

To date, several risk factors have been proposed to increase the chance of developing progressive COVID-19 disease, among them the presence of co-existing morbidities. Recent reports have stated that CKD is significantly associated with increased COVID-19 severity and mortality.[5,12] Our results

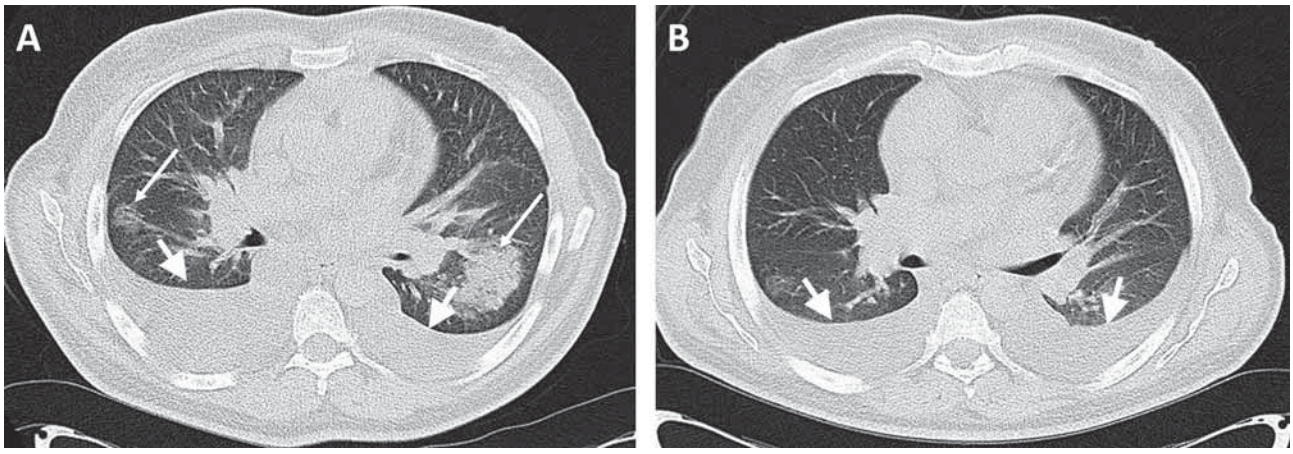

Figure 2. A) A 55-year-old male patient with a history of underlying chronic kidney disease (GFR = 9.1, stage V) presented with dry cough and dyspnea which had started since 5 days ago. Initial computed tomography (CT) imaging showed bilateral moderate pleural effusion predominantly in right side, ground glass opacity and area of consolidation in middle zones. B) CT imaging obtained 22 days later show bilateral mild pleural effusion and complete lesion absorption. The patient was discharged after 7 days.

Table 4. Comparison of Clinical and Laboratory Data and CT Scores Based on CKD Stage

Variables	Stage II (n = 10)	Stage IIIa (n = 19)	Stage IIIb (n = 7)	Stage IV (n = 2)	Stage V (n = 5)	*P*
Days of Hospitalization	13 ± 8.5	10.2 ± 6.2	10.6 ± 2.8	10.5 ± 2.1	15.4 ± 6.4	> .05
Oxygen Saturation, %	90.5 ± 5.1	88.6 ± 5.7	90.0 ± 4.7	92.0 ± 2.3	82.0 ± 12.7	> .05
Leukocyte Count, × 10^9 /L	5.32 ± 1.36	6.09 ± 3.64	5.25 ± 1.98	4.25 ± 0.07	9.06 ± 3.03	> .05
Platelet Count, × 10^9 /L	214.2 ± 78.6	206.3 ± 89.2	170.7 ± 96.7	134.5 ± 96.8	337.1 ± 181.2	> .05
Hemoglobin Level, g/dL	14.4 ± 2.6	13.7 ± 2.4	13.9 ± 3.93	12.0 ± 1.37	11.2 ± 3.3	> .05
Lymphopenia	7 (70)	8 (42.1)	2 (28.6)	2 (100)	4 (80)	> .05
Neutrophilia	-	2 (10.5)	-	-	2 (40)	> .05
Eosinopenia	-	1 (5.3)	1 (14.3)	-	1 (20)	> .05
NLR	3.07 ± 1.35	4.3 ± 3.9	4.3 ± 1.7	2.3 ± 0.1	5.2 ± 3.2	> .05
PLR	168.4 ± 64.5	175.9 ± 46.5	185.3 ± 94.3	147.4 ± 44.7	236.9 ± 128.1	> .05
NLR*CRP	138.5 ± 100.5	207.3 ± 274.1	181 ± 67.8	108.9 ± 73.1	114.1 ± 101.5	> .05
C-reactive Protein, mg/dL	38.4 ± 19.3	41.3 ± 22.8	39.3 ± 6.44	66.0 ± 15.3	29.0 ± 27.6	> .05
Lactase Dehydrogenase, IU/l	392.5 ± 75.5	330.3 ± 142.1	619.4 ± 484.5	450.0 ± 43.8	378.6 ± 78.3	> .05
Upper Zone Score	3.5 ± 1.9	2.8 ± 1.8	1.85 ± 0.89	2.0 ± 0.0	1.41 ± 1.67	> .05
Middle Zone Score	4.1 ± 2.6	4.6 ± 1.9	3.1 ± 2.1	2.5 ± 0.7	3.0 ± 2.0	> .05
Lower Zone Score	4.5 ± 2.75	5.1 ± 1.9	2.8 ± 2.4	4.5 ± 0.7	3 ± 2	> .05
Total Zone Score	12.1 ± 7.0	12.5 ± 5.0	7.8 ± 5.1	9 ± 1.4	7.9 ± 4.9	> .05

Continuous variables are reported as mean ± standard deviation and range. Categorical variables are reported as n (%). P values are calculated by χ^2 test, Fisher's exact test, or Student t-test.
Abbreviations: NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein.

approved this finding, showing that the risk of developing severe disease is twice as higher as compared with the general patient population.[13,14] Also, mortality rate was considerably higher (11.6%) in our patients than the rate reported for the general population.[13-5] The comorbidities associated with increased odds of COVID-19-related death are prevalent in patients with CKD. A large study in China estimated mortality rate of COVID-19 to be 10.5% in patients with cardiovascular diseases.[16] In our cohort of patients, more than 75% of cases had a positive history of cardiovascular diseases, possibly justifying the high mortality rate that was observed.

Our patients mostly manifested with dyspnea and cough, which is similar to that of non-CKD patients.[3,17] In contrast to other studies, we did not find a significantly worse outcome in the elderly.[17-9] A recent study on kidney transplant patients reported fever in 80% of cases, however; we observed fever in less than half of our patients. Furthermore, in the mentioned study, patients who required hospitalization were more likely to have reported dyspnea.[20] However, the result of our study failed to show any relationship between disease presentation and final outcome.

We observed a borderline significant difference in patients' outcome based on serum creatinine and BUN level. In a recent study, it was reported that elevated levels of BUN and serum creatinine are significantly associated with the death of patients with COVID-19.[6] In anothor study, these factors were found to be predictive of in-hospital death.[7] Despite this, our study did not display a difference in disease severity, outcome, or duration of hospitalization as the level of GFR decreased. In this study, the only biochemical factor which demonstrated a significant relationship with outcome was level of LDH. This finding had previously been reported by several studies.[3,21-3] CRP was elevated in 69.7% of our patients, which was very close to the rate (60.7%) reported by Guan and his colleagues,[3] however it was not predictive of disease outcome.

Normal white blood cell count was a more frequent finding in our series of patients compared with leukopenia, which is consistent with the results of another study conducted on kidney transpalnt patients.[24] Although we did not perform a subset analysis on lymphocyte count, a study on hemodyalis patients with COVID-19 pneumonia showed that T-cell count was significantly less as compared to non-hemodyalisis patients.[5]

Lymphocytopenia has been addressed as a marker of diseaese severity in COVID-19.[25] Despite the higher mortality rate obsereved in our study, lymphocytopenia existed in just a little more than half of our patients on admission, which is

lower than the rate reported for general patient population.[18,21,25] Moreover, unlike other studies reporting NLR and PLR as important indicators of predicting disease progression,[26-9] we did not observe such a finding in CKD patients.

The CT imaging features of COVID-19 pneumonia resemble various other conditions such as organizing pneumonia or inflammatory lung processes.[30,31] Trujillo *et al*. recently evaluated kidney transplant patients infected with SARS-CoV-2, reporting no significant difference between imaging features of survivors and non-survivors.[32] Our study also displayed the same results. However, we found that predominant radiologic patterns among CKD patients are slightly different to those of other patients. In this study, the most frequently observed patterns were GGO followed by reticular pattern, while in other studies, GGO and consolidation have been reported as the typical chest CT features of COVID-19 pneumonia.[19,33] It has been reported that reticular pattern is a late finding; however, even in late stages, reticular pattern has been observed in 3% to 6% of the general population,[34] which is considerably lower than the rate observed in our study. Another interesting finding was the high prevalence of vessel dilatation in the CT imaging of our patients, in particular those who died. Vascular enlargement has been reported to convey prognostic information, thus, this observation might be justified by the fact that our patients had presented with a more severe form of disease.[35,36] Also, of note, pleural effusion was seen in more than one-fifth of our patients. Generally, the observation of pleural effusion in a patient with acute respiratory distress, especially in early stages, is not in favor of COVID-19 pneumonia and leads away from its diagnosis.[37-9] However, based on the results of our study and also considering the fact that pleural effusion is a common complication in patients with impaired renal function, in particular in those with ESRD,[40,41] the presence of pleural effusion should not exclude the possibility of COVID-19 diagnosis in this specific patient population. Other imaging findings were similar to studies investigating the general population; for example, our study demonstrated bilateral lung involvement, mainly in the peripheral posterior lobes and with lower lung zone predilection, which is consistent with the majority of published studies.[42,43] Regarding CT score, lower zone score was found to be higher in patients who died, however, total CT score did not differ based on patients' CKD stage or disease outcome. Therefore, we suggest that the extent of lower zone involvement could be considered as a marker of disease burden.

Our study had some limitations. First, the time from symptom onset to presentation was not evaluated in this study. Second, the sample size in our study was relatively small, which could possibly affect the reults.

CONCLUSION

In conclusion, the results of this study showed that compared to the general population, patients with CKD are vulnerable to a more severe form of COVID-19 and experience a higher rate of death. Thus, the presence of CKD should be considered as an important factor in risk stratification of COVID-19 patients and imply the need for close monitoring and timely management of these patients. Nevertheless, higher stage of CKD is not related to worse prognosis or more extensive lung involvement .

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DECLARATION OF INTEREST

The authors report no conflict of interest.

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Urinary System and Renal Involvement in Children With Cystic Fibrosis

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Keywords. cystic fibrosis, renal involvement, children

Original Paper

Introduction. A few data on the prevalence of renal involvement in cystic fibrosis and its spectrum in childhood is available. In the present study, we conducted a prospective study on children who had cystic fibrosis and evaluated their renal involvement. In fact, the aim of the study was to provide data on the clinical consequences of proper identification of kidney disease in a group of children with cystic fibrosis.
Methods. This prospective study was conducted on 55 consecutive patients with previous diagnosis of cystic fibrosis during a three-year period and at least 3 months to over 5 years or more follow-up. The inclusion criteria was the diagnosis of cystic fibrosis which was made by clinical presentation of cystic fibrosis and laboratory results. Initially, patients' medical records were reviewed and relevant data were collected. A 24-hour urine collection (or a random urine sampling in very young infants) was used to assess crystalluria and renal function was evaluated by blood sampling.
Results. Totally, 55 patients with cystic fibrosis were admitted in two hospitals with the mean age of 8.22 ± 5.66 years. GFR totally reduced in 34.5%. The overall prevalence of hypercalciuria was estimated to be 60%, while hyperoxaluria, hypocitraturia, and hyperuricosuria in 41.8%, 24.5%, and 47.3%; respectively.
Conclusion. Crystalluria is a common consequence of cystic fibrosis in childhood. The prevailing crystalluric finding includes hypercalciuria followed by hyperuricosuria, and hyperoxaluria. During disease GFR may be decreased due to several reasons such as nephrotoxic drugs usage.

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INTRODUCTION

Recent evidences have emphasized the association between cystic fibrosis and appearance of kidney injuries especially among children.[1,2] Following significant development in treatment of respiratory disorders and pancreatitis in cystic fibrosis, the life expectancy among patients has been considerably increased from 2 years to more than 30 years.[3,4] One of the main underlying factors affecting the progression as well as prognosis of the affected patients includes the presence of the CFTR gene polymorphism that encodes a polyprotein cystic fibrosis trans-membrane conductance regulator (CFTR), which functions as an ATP-responsive chloride channel in apical membrane of epithelial cells.[5] Because this gene is abundantly findable in various segments of the nephron especially in proximal tubule, the inactivation of CFTR can lead to renal insufficiency presents with proteinuria as well as nephrocalcinosis and hypercalciuria.[6] In this

regard, the overall prevalence of nephrocalcinosis and hypercalciuria is estimated to be 90% and 30% of affected patients, respectively emphasizing high risk for renal impairment in cystic fibrosis patients.[7] More interestingly, urolithiasis in these patients may result from hyperoxaluria originated from other clinical abnormal conditions such as fat malabsorption. Along with urolithiasis, patients with cystic fibrosis may be also found, but less commonly, with other renal disorders such as glomerulonephritis, and AA amyloidosis.[8-10] Totally, renal involvement in cystic fibrosis should be considered as an emergence; however a few data on the prevalence of renal involvement and its spectrum in childhood is available. In the present study, we report a series of children who had cystic fibrosis and some degree of renal involvement. In fact, the aim of this study was to provide data on proper identification of kidney disease in a group of children with cystic fibrosis.

MATERIALS AND METHODS

This prospective study was conducted on 55 consecutive patients with final diagnosis of cystic fibrosis who referred to Masih Daneshvari and Mofid Children's hospital in Tehran during 2012 to 2015. In this study, the diagnosis of cystic fibrosis was made if there were clinical presentations in addition to chloride concentration more than 60 mmol/L in sweet test according to the guidelines by the Gibson & Cooke methods in two separate tests. In addition, we confirmed pancreatic malabsorption by quantification of elastase-1 activity and fat droplet in stool sample. False positive cases such as anorexia nervosa, congenital adrenal hyperplasia, adrenal insufficiency, glucose-6-phosphatase deficiency, familial hypoparathyroidism, hypothyroidism, nephrogenic diabetes insipidus, pseudohypoaldosteronism, and Klinefelter syndrome were ruled out by history, physical examination, and appropriate laboratory tests. Initially, patients' medical records were reviewed, and relevant data were collected regarding baseline characteristics, anthropometric parameters, blood pressure on admission, and laboratory indices. Also, kidney ultrasonography was done and findings were highlighted. A 24-hour urine collection (or a random urine sampling in very young infants) was done to assess crystalluria. The methods of measurement of crystals in urine was as following: peroxidase-TOOS [N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine] method for uric acid, photometric method for calcium and citrate, colorimetric oxidase method for oxalate, and Jaffe's reaction for creatinine. GFR was calculated using the Schwartz formula and serum creatinine was measured by Jaffe method: "GFR (mL/min/1.73m^2) = (K) (height in cm) / serum creatinine (mg/dL).[11]

In this formula, "K" coefficient in infants under one year of age with LBW and infants with birth weight above 2.5 Kg under one year is 0.35 and 0.45, respectively. This number for young children and female patients in pubertal age is 0.55 and for male patients is 0.7.[12,13]

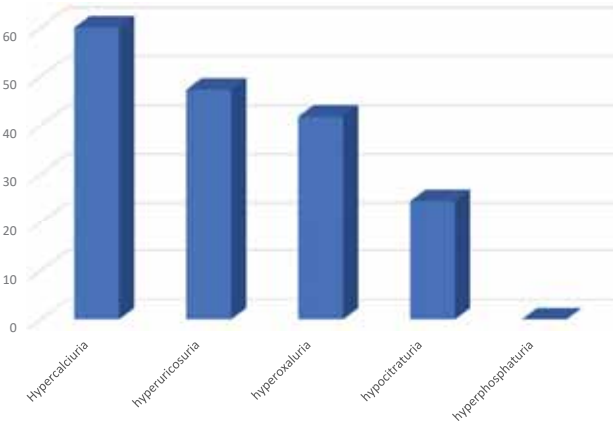
To assess the electrolyte abnormalities, their concentration was determined and venous blood gas analysis was done. The findings related to the *CFTR* mutation were also recorded if available. In ten cases genetic study confirmed the diagnosis.

Results were presented as mean ± SD for quantitative variables and were summarized by absolute frequencies and percentages for categorical variables. For the statistical analysis, the statistical software SPSS version 16 for windows was used.

RESULTS

Within three years of study, 55 patients with cystic fibrosis were admitted to these two hospitals with the mean age of 8.22 ± 5.66 years (ranged 4 months to 22 years). Follow up duration was five years. Even in some cases the follow up period was longer. Patients were visited on a monthly basis or every two months averagely. Cases with exacerbation or worsening of their condition were hospitalized. Treatments included antibiotics based on patients age and cultured microbial species. Nebulized treatments which were used were as following: inhaled antibiotics, hypertonic saline, and in some cases recombinant DNAase. Appropriate supplements, vitamins and diet were prescribed for CF patients. Physiotherapy was done and based on the patients' condition, traditional methods or appropriate devices were used. During admission, psychologic or psychiatric consultation might have been required. Appropriate vaccination was considered. We took patients' or parents' consent in advance. The study was approved by ethics committee of SBMU (The registration number is: IR.SBMU.MSP.REC.1397.559). Among patients,

52.7% were male (with a mean age of 7.20 ± 5.34 years) and 47.3% were female (with the mean age of 9.55 ± 5.81 years). The blood pressure was in the normal range in all subjects with no evidences of hypertension. Hematuria was a prominent finding in 5 patients (9.1%). Urine culture was negative in all patients. One patient (1.8%) showed bilateral fullness in ultrasonography. None of the patients had electrolyte disturbances. The details on demographic data and laboratory indices are summarized in Table. The most common electrolyte abnormalities were hyponatremia and hypokalemia.Venous blood gas analysis showed normal condition in 28 patients, while 5 patients were diagnosed as respiratory acidosis, 10 patients as respiratory alkalosis, 5 patients as metabolic alkalosis, and 7 patients as a mixed blood gas abnormality. GFR totally reduced (according to patients' age) in 14 patients (25.4%), while ranged 60 to 89 in 23.6%, and 15 to 30 in only 1.8%. The status of crystalluria is shown in Figure. The overall prevalence of hypercalciuria was estimated to be 60.0%, while hyperoxaluria, hypocitraturia, and hyperuricosuria diagnosed in 41.8%, 24.5%, and 47.3%; respectively. Decreased urine output was found in only one patient (1.8%).



It shows frequency of crystalluria in children with cystic fibrosis.

DISCUSSION

Despite low prevalence of symptomatic urolithiasis among patients with cystic fibrosis, the prevalence of hypercalciuria was shown to be notably high causing unexplained morbidities in these patients. It is thus mandatory to identify different aspects of renal involvement in the patients because of its adverse effect on life expectancy particularly among children due to their lower tolerability. In most studies the incidence and clinical status of renal involvement in cystic fibrosis

Details on Demographic Data and Laboratory Serum Indices in Children with Cystic Fibrosis

Parameter	Mean	SD	Minimum	Maximum
Weight, kg	20.53	11.39	2.8	52.7
Height, cm	116.7	29.98	55	161
Percentage of FTT	63.63 (35 cases)	-	-	-
Sweat Chloride, mmol/L	81.67	11.38	65	110
Urine Output, mL	1290.38	682.35	200	2700
Sodium, mEq/L	137.96	2.72	131	144
Potassium, mEq/L	4.24	0.45	3.1	5.5
Urea, mg/dL	18.34	6.33	4	34
Cr, mg/dL	0.65	0.15	0.3	0.9
Uric Acid, mg/dL	4.29	0.96	2.9	6.8
Calcium, mg/dL	8.87	0.59	7.6	10
Phosphorus, mg/dL	4.21	1.02	2.1	6.6
Alkaline Phosphatase	491.45	207.84	156	1124
Bicarbonate	24.98	24.56	16.6	44.4
PH	7.41	7.41	7.3	7.53
PCO_2	37.05	11.04	22	76.2
Urine Calcium	11	197	92.84	55.03
Urine Uric Acid	384.84	252.77	59	1072
Urine Citrate	324.7	221.71	74	902
Urine Phosphorus, mg	512.8	323.96	132	1620
Urine Protein, mg	92.69	72.44	11	282
Urine Cr, mg	385.61	200.02	66	743
GFR, mL/min/ 1.73 m^2	100.87	27.62	20.16	162

was based on histological assessments using renal biopsy. In one of the main studies by Abramowsky and Swinehart[11] on autopsies from both pediatric and adult patients, the main histological findings were related to glomerulomegaly, a mesangiopathic lesion, and tubulointerstitial disease frequently associated with acute and chronic tubular injury that were significantly associated with the severity of renal dysfunction. In the present study and aided by laboratory findings and sonography assessment, we showed high prevalence of crystalluria especially hypercalciuria and hyperuricosuria in children with cystic fibrosis. In our study, the dominant crystalluric finding was hypercalciuria found in about two-third of patients, while hyperoxaluria or hyperuricosuria was found in less than half of them. In fact, it seems that the existence of exocrine pancreatic dysfunction as a major risk factor for enteric hyperoxaluria may be revealed in about half of our patients. In contrast with other reports that showed increased prevalence of calcium oxalate and medullary nephrocalcinosis,[12] our results were negative.

Regarding renal functional status, reduced GFR was found in about a quarter of the children that was significant. We estimated the GFR based on creatinine clearance; however we showed reduced urinary output only in 1.8% of the patients. It is now agreed that the estimation of GFR using creatinine is not a reliable estimation of renal function. In fact, the assessment of renal sclerotic lesions as a serious renal change following cystic fibrosis may not be followed by only GFR estimation based on creatinine. Because accurate assessment of renal sclerotic lesions is of great help for clinicians who care for these patients, employing suitable and more valid tools to assess these changes is essential particularly in those who require receiving nephrotoxic immunosuppressive agents; As a result, limitation of our study was that we could not use an accurate method for GFR measurement such as DTPA (diethylenetriamine pentaacetate).

In conclusion, crystalluria is a common consequence of cystic fibrosis in childhood. The prevailing crystalluric finding includes hypercalciuria followed by hyperuricosuria, and hyperoxaluria. Thus, assessing the risk of crystalluria and also determination of its main predictors is essential to prevent deleterious effects on renal function especially in affected children.

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High Neutrophil/Lymphocyte Ratio as an Independent Risk Factor for the First Occurrence of Stroke in Peritoneal Dialysis Patients

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Introduction. Though neutrophil/lymphocyte ratio (NLR) level appears to be related with stroke events in general population, its relationship with stroke in peritoneal dialysis (PD) patients is still uncertain. This study aims to investigate the association between NLR and the first occurrence of stroke in PD patients.
Methods. In this retrospective cohort study, 1507 PD patients were enrolled from four centers in China and stratified into tertiles of NLR levels. The incidence of the first occurrence of stroke was analyzed by Kaplan-Meier cumulative incidence curve among different NLR tertiles, competing risk analysis was used to calculate the incidence of the first occurrence of stroke in the presence of competing risk of other events, multivariable COX regression analysis was performed to estimate the hazard ratios (HRs) for the first occurrence of stroke, as well as forest plot was utilized to describe the relationship between NLR and the first occurrence of stroke in different subgroups.
Results. During follow-up, 84 new-onset stroke events were recorded. Kaplan-Meier cumulative incidence curves showed significant differences in the incidence of the first occurrence of stroke among three groups (log-rank test: $P < .001$). In competing risk analysis, the cumulative incidence curves for tertiles of NLR levels were highly significant for the first occurrence of stroke ($P < .001$), but they were not statistically different for the occurrence of other events. Compared to the lowest tertile of NLR level, the highest tertile was associated with increased risk of the first occurrence of stroke in the adjusted Cox model (HR = 2.39, 95% CI: 1.37 to 4.15; $P < .05$). As for forest plot, there was no interaction in all subgroups.
Conclusion. High NLR was an independent risk factor for the first occurrence of stroke in PD patients.

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Original Paper

INTRODUCTION

Stroke is the second leading cause of death worldwide and currently the leading cause of death in China, which contributes to a heavy disease burden.[1-4] It seems that people with chronic kidney diseases have the highest risk in suffering from subsequent cardiovascular disease (CVD).[5,6] Moreover, CVD become the main causes of mortality in end-stage renal disease (ESRD) patients maintaining dialysis.[7] Stroke is one of the major causes of cardiovascular mortality in the group.[8] What's more, patients relying on maintenance dialysis with ESRD have

remarkably greater stroke incidence and higher mortality of stroke than non-dialysis patients do.[9,10] Therefore, reliable prognostic factors, which could help to estimate patients at high risk of stroke for ESRD population, are needed.

Neutrophil to lymphocyte ratio (NLR) as a novel index could be used to predict stroke and stroke prognosis.[11] Fang YN *et al*. suggested NLR was one of the credible biomarkers, which had the advantage of predicting prognostic outcome among patients who had suffered acute ischemic stroke.[12] Tao C *et al*. also found that increased level of NLR was associated with poor 90-day outcome independently after intracerebral hemorrhage, while NLR may serve as a novel inflammatory biomarker after intracerebral hemorrhage.[13]

Since NLR has been associated with increased risk of stroke prognosis in non-dialysis patients, it may probably also predict the risk of stroke in PD patients. However, there is no published paper having indicated NLR level associates with the risk of stroke in PD patients. In this study, we aim to investigate the association of NLR and the first occurrence of stroke in PD patients.

MATERIALS AND METHODS

Participants

From January 1, 2010 to May 31, 2016, a total of 1652 patients were recruited from four PD centers. Of them, 145 were excluded for the following reasons: age younger than 18 years or older than 80 years (n = 34), PD was maintained for less than 3 months (n = 32), clinical evidence of active infection that happened in a month before returning to hospital (n = 37), history of hematological or autoimmune disease and taking glucocorticoid or immunosuppressive (n = 42). Above patients were excluded because those factors may influence NLR level. Finally, this study included 1507 patients. The Institutional Review Board of the four PD centers approved this retrospective study. Written informed consent was not required because we retrospectively collected available medical records in the hospital.

Baseline Investigations

Baseline demographic and clinical data were collected at the initiation of PD therapy. Biochemical parameters were collected 3 months after PD therapy was initiated. Patients who reported current use of insulin or oral hypoglycemic agents and/or who had a clinical diagnosis of type 1 or type 2 diabetes mellitus were considered to have diabetes mellitus.[14] Hypertension was recorded if the patient took antihypertensive drugs or had 2 separate blood pressure measurements ≥ 140/90 mmHg. CVD was defined as including coronary heart disease, myocardial infarction, angioplasty, coronary artery bypass or heart failure. Stroke was defined as including cerebral infarction, intracerebral hemorrhage and subarachnoid hemorrhage.

Laboratory measurements were obtained using standard methods in the clinical laboratory. Total Kt/V were calculated using PD Adequest software 2.0 (Baxter, Deerfield, IL). Medicine use was recorded based on prescriptions. The patients returned to these centers for quarterly evaluation, and trained nurses interviewed the patients by telephone monthly to assess general conditions.

Study Outcome

The outcome was the first occurrence of stroke since PD therapy. Stroke was defined as including cerebral infarction, intracerebral hemorrhage, and subarachnoid hemorrhage. Trained nurses asking about previous medical events during monthly phone interviews identified strokes, and then experienced doctors confirmed the diagnosis of stroke again or via review of medical records. All patients were followed until death, transfer to hemodialysis therapy, kidney transplantation, transfer of care from four centers or censoring on May 31, 2017.

Statistical Analysis

Participants were divided into tertiles of NLR levels (tertile 1 [lowest], < 2.74; tertile 2 [middle], 2.74 ≤ NLR < 4.00; and tertile 3 [highest], ≥ 4.00). Summary statistics by tertile of NLR level were presented. Based on the results of the normality test, all continuous variables are skewed distribution. The values for skewed variables were described as median (25^{th} to 75^{th} percentile) and categorical data were given as frequency and percentages. Differences among the tertiles of NLR level were tested using $\chi 2$ test for categorical variables, Mann-Whitney U test for skewed continuous variables. A univariable logistic regression model was used to examine the association between patients' characteristics and new-onset stroke events since PD therapy with lower category as reference,

and then a multivariable logistic regression was used to examine patients' characteristics with predictive odds of the first occurrence of stroke, which adjusted for covariates with ($P < .05$ in univariable logistic analysis). Kaplan-Meier cumulative incidence curves were used to analyze the incidence of the first occurrence of stroke, and differences among distributions of incidence of new-onset stroke events were assessed by log-rank test. Competing risk analysis was used on the first occurrence of stroke and other events, and differences were assessed by Gray's test. Cox regression models were used to evaluate the relationship among the tertiles of NLR level with the first occurrence of stroke in PD patients, initially without adjustment and subsequently adjusting for several groups of covariates. The multivariable Cox regression model was constructed using eligible covariates that demonstrated significant or near-significant association with the first occurrence of stroke ($P < .05$) on multivariable analysis or characteristics ($P < 0.01$) list in Table 1 or for

Table 1. Demographic and Baseline Clinical Data for the Study Patients

Variables	Total (n = 1507)	Tertile 1 (n = 502)	Tertile 2 (n = 509)	Tertile 3 (n = 496)	*P*
No. of C1/C2/C3/C4	316/794/36/361	124/249/9/120	130/249/7/123	62/296/20/118	< .001
No. of Men/Women	855/652	262/240	275/234	318/178	< .001
NLR	3.4 (2.5, 4.5)	2.2 (1.8, 2.5)	3.4 (3.0, 3.8)	5.4 (4.6,7.0)	< .001
Demographics					
Age, y	51 (41, 62)	49 (39, 61)	50 (41, 61)	53 (43,63)	< .001
BMI, kg/m^2	22.1 (20.0, 24.3)	22.0 (20.0, 24.2)	22.0 (20.1, 24.4)	22.1 (20.0,24.3)	> .05
Comorbid					
Systolic BP, mmHg	149 (132, 164)	146 (130, 160)	149 (134, 161)	150 (134,170)	> .05
Diastolic BP, mmHg	87 (78, 95)	87 (78, 95)	87 (80,95)	87 (78,96)	< .001
Hypertension, n (%)	990 (65.7)	312 (62.2)	321 (63.1)	357 (72.0)	< .05
Diabetes Mellitus, n (%)	346 (23.0)	106 (21.1)	116 (22.8)	124 (25.0)	> .05
Cardiovascular Disease, n (%)	136 (9.0)	47 (9.4)	44 (8.6)	45 (9.1)	> .05
Stroke Disease, n (%)	80 (5.3)	22 (4.4)	26 (5.1)	32 (6.5)	> .05
Laboratory Variables					
Hemoglobin, g/L	87 (74, 100)	89 (76, 103)	88 (76, 101)	82 (70,95)	< .05
Albumin, g/L	34.5 (31.1, 37.9)	34.9 (31.3, 38.4)	34.7 (31.3, 37.5)	34.2 (30.5,37.3)	< .001
Creatinine, μmol/L	710.0 (544.0, 933.4)	699.5 (543.8, 899.5)	711.0 (541.9, 931.1)	729.5 (546.0,950.0)	< .001
Urea nitrogen, mmol/L	20.47 (15.50, 26.85)	19.4 (15.1, 25.4)	20.2 (15.3, 26.4)	22.0 (16.4,29.3)	< .001
Uric acid, mmol/L	428 (355, 509)	430 (356, 512)	427 (354, 504)	426 (358,502)	< .05
FBG, mmol/L	4.7 (4.1, 5.5)	4.6 (4.1, 5.4)	4.7 (4.1, 5.5)	4.8 (4.1,5.7)	< .001
Total Cholesterol, mmol/L	4.2 (3.5, 5.0)	4.3 (3.6, 5.1)	4.2 (3.5, 5.0)	4.1 (3.4,4.9)	> .05
Total Triglycerides, mmol/L	1.3 (1.0, 1.8)	1.4 (1.0, 1.9)	1.4 (1.0, 1.9)	1.3 (0.9,1.7)	> .05
Sodium, mmol/L	140.0 (138.0, 142.3)	140.7 (138.1, 142.5)	140.0 (138.0, 142.9)	140.0 (137.6,142.0)	> .05
Chlorine, mmol/L	103.0 (99.3, 107.0)	103.2 (100.0, 107.0)	103.0 (99.3, 106.8)	102.8 (99.0,107.0)	> .05
Calcium, mmol/L	2.0 (1.9, 2.2)	2.1 (1.9, 2.2)	2.1 (1.9, 2.2)	2.0 (1.8,2.1)	> .05
Potassium, mmol/L	4.1 (3.6, 4.7)	4.2 (3.7, 4.8)	4.1 (3.6, 4.7)	4.1 (3.6,4.8)	< .05
Phosphorus, mmol/L	1.7 (1.4, 2.0)	1.6 (1.4, 2.0)	1.6 (1.3, 2.0)	1.8 (1.4,2.1)	< .001
Alkaline Phosphatase, U/L	73 (58, 94)	71 (56, 91)	73 (59, 91)	76 (60,99)	< .001
Total Kt/V	2.3 (1.8, 2.6)	2.3 (1.8, 2.7)	2.4 (1.7, 2.6)	2.2 (1.8,2.6)	> .05
RRF, mL/min	4.6 (2.1, 15.0)	4.4 (2.0, 13.4)	4.6 (2.2, 15.1)	4.7 (2.0,16.5)	> .05
Treatments					
CCB, n (%)	1108 (73.5)	371 (73.9)	371 (72.9)	366 (73.8)	> .05
ACEI/ARB, n (%)	554 (36.8)	186 (37.1)	199 (39.1)	169 (34.1)	> .05
Loop Diuretic, n (%)	101 (6.7)	33 (6.6)	30 (5.9)	38 (7.7)	> .05
Insulin, n (%)	232 (15.4)	75 (14.9)	71 (13.9)	86 (17.3)	> .05
Aspirin, n (%)	135 (9.0)	42 (8.4)	44 (8.6)	49 (9.9)	> .05
Time, mo	30.6 (18.9, 46.9)	33.0 (20.1, 50.8)	32.6 (21.4, 47.3)	27.9 (16.3,41.5)	< .001

Note: All continuous variables are skewed distribution, the values for continuous variables are given as median (P25, P75). Time refer to time from the onset of peritoneal dialysis to the first occurrence of stroke events.
Abbreviations: C1,center 1; C2,center 2; C3,center 3; C4,center 4; NLR, neutrophil lymphocyte ratio; BMI, body mass index; FBG, fasting blood-glucose; Kt/V, K (dialyzer clearance of urea), t (dialysis time), V (volume of distribution of urea); RRF, residual renal function; CCB, calcium channel blocker; ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blocker.

importance of clinical concern. Moreover, the interaction between subgroups variable of interest including sex, age, history of diabetes mellitus and NLR group were examined by performing a formal test of interaction. Forest plot was used to represent the relationship between NLR and new-onset stroke events in each subgroup. In Cox regression models, time at risk was from study entry until death, transferring to hemodialysis therapy, kidney transplantation, transferring care from our center, or the end of study on May 31, 2017. For primary effects, $P < .05$ was considered statistically significant. Statistical analyses were performed using SPSS version 23 and R software (version R-3.6.1, www.r-project.org).

RESULTS

Participants

Baseline demographic and clinical characteristics of the cohort were given in Table 1, divided according to tertiles of NLR levels. A total of 1507 patients were enrolled in this study (median age, 51 (41, 62) years; 56.7% men; 23.0% with diabetes; 65.7% with hypertension), with a median follow-up of 30.6 (maximum, 89.4) months. Median NLR value was 3.4 (2.5, 4.5) for all patients. In the whole process, 78 (5.2%) patients underwent kidney transplantation after a median of 20 months, 199 (13.2%) were transferred to hemodialysis therapy for any reason after a median of 28 months, 20 (1.3%) transferred to other center after a median of 36 months, and 25 (1.7%) lost to follow up. Stroke events were registered during follow-up. A total of 84 stroke events (5.6%) were recorded. (Figure 1).

NLR Associated with the First Occurrence of Stroke in PD Patients

The significant risk factors for new-onset stroke events were given in Table 2 by adjusting for covariates ($P < .05$ univariable logistic regression). The first occurrence of stroke was associated with male, history of hypertension, stroke and CVD as well as higher FBG. Associations of NLR with new-onset stroke events with defined models (with the group 1 as the reference group) are listed in Table 3. Regardless of the adjustment method used, the highest tertile of NLR level was associated significantly with the first occurrence of stroke compared to the lowest tertile.

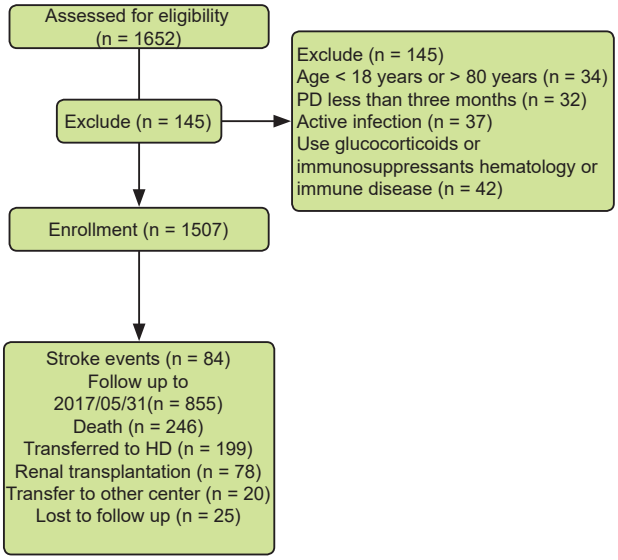

Figure 1. It shows study algorithm, including patient enrollment and outcomes. (PD, peritoneal dialysis; HD, hemodialysis).

Table 2. Significant Risk Factors for the First Occurrence of Stroke

Risk Factors	OR (95% CI)	*P*
Univariable Logistic Regression		
Sex (Female vs. Male)	0.45 (0.27 to 0.74)	< .05
Diabetes Mellitus (Yes vs. No)	2.17 (1.38 to 3.44)	< .05
Hypertension (Yes vs. No)	2.51 (1.42 to 4.43)	< .05
History of Stroke Disease (Yes vs. No)	3.32 (1.72 to 6.41)	< .001
Cardiovascular Disease (Yes vs. No)	2.78 (1.58 to 4.89)	< .001
FBG (> 1-mmol/L)	1.12 (1.05 to 1.20)	< .05
Use of ACEI/ARB (Yes vs. No)	1.69 (1.09 to 2.63)	< .05
Use of Insulin (Yes vs. No)	1.92 (1.14 to 3.21)	< .05
Multivariable Logistic Regression		
Sex (Female vs. Male)	0.43 (0.26 to 0.71)	< .05
Hypertension (Yes vs. No)	1.98 (1.10 to 3.56)	< .05
History of Stroke Disease (Yes vs. No)	2.53 (1.29 to 4.99)	< .05
Cardiovascular Disease (Yes vs. No)	1.95 (1.05 to 3.64)	< .05
FBG (> 1-mmol/L)	1.09 (1.01 to 1.17)	< .05

Note: Multivariable logistic regression adjusted for covariates ($P < .05$) list in univariable logistic regression. Abbreviations: OR, odds ratio; CI, confidence interval; FBG, fasting blood-glucose; ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blocker.

In crude analysis, the Kaplan-Meier cumulative incidence curves showed there were significant differences in the incidence of new-onset stroke events among the tertiles of NLR level (log-rank test: $P < .001$, Figure 2). In competing risk analysis, cumulative incidence curves for each tertile of NLR level are highly significant for the first occurrence

Table 3. Relationship Between Tertiles of NLR and the First Occurrence of Stroke

	Tertile 2 (n = 509)		Tertile 3 (n = 496)	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Unadjusted	1.09 (0.57 to 2.07)	> .05	3.15 (1.83 to 5.43)	< .001
Model 1	1.03 (0.54 to 1.97)	> .05	2.58 (1.49 to 4.46)	< .05
Model 2	1.00 (0.53 to 1.91)	> .05	2.55 (1.47 to 4.41)	< .05
Model 3	0.99 (0.52 to 1.88)	> .05	2.39 (1.37 to 4.15)	< .05

Note: Reference group is Tertile 1.
Model 1: sex, age, BMI
Model 2: Model 1 plus comorbid conditions (diabetes mellitus, hypertension, stroke disease, cardiovascular disease) and medical history (aspirin)
Model 3: Model 2 plus albumin, creatinine, urea nitrogen, uric acid, FBG, total cholesterol, total triglycerides, phosphorus, alkaline phosphatase
Abbreviations: NLR, neutrophil lymphocyte ratio; BMI, body mass index; FBG, fasting blood glucose; HR, hazard ratio; CI, confidence interval.

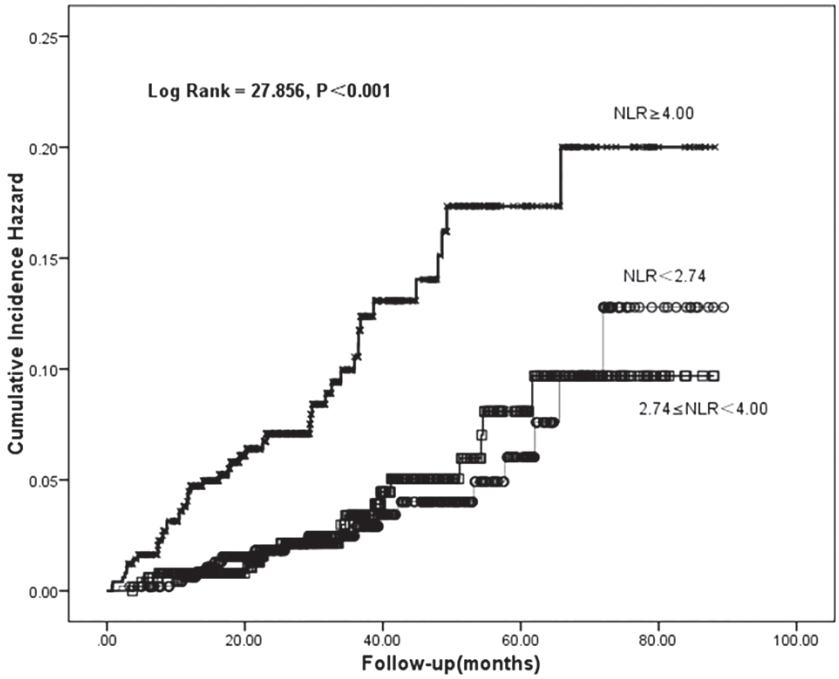

Figure 2. It demonstrates cumulative incidence of the first occurrence of stroke in 1507 peritoneal dialysis patients by NLR. The curves were constructed using the Kaplan–Meier method and compared using the Mantel–Cox log-rank test. Patients in the highest tertile of NLR level showed higher incidence of the first occurrence of stroke.

of stroke ($P < .001$), but they are not statistically different for transfer to hemodialysis therapy ($P > .05$), kidney transplantation ($P > .05$), transfer to other center ($P > .05$), being lost to follow up ($P > .05$), and death ($P > .05$) (Figure 3).

NLR Associated with the First Occurrence of Stroke in Different Subgroups

We investigated the association between NLR and the first occurrence of stroke in different subgroups which we were interested in, including male or female, with or without diabetes, old age (≥ 60 years) or young age (< 60 years). These subgroups were analyzed by COX regression and represented as a forest plot. No interaction was found in all subgroups (Figure 4).

DISCUSSION

This retrospective cohort study indicated that the incidence of first occurrence of stroke was significantly higher in Tertile 3, while compared to Tertile 1. It indicated that elevated NLR was associated with new-onset stroke events risk in PD patients, after adjusting possibly related confounders.

As is well-known, the relationship between chronic inflammation and CVD has been studied widely.[15] Elevated WBC was reported to be related to CVD,[16] in which neutrophils played a significant predictive role.[17] NLR, as a simple ratio readily obtained from inexpensive blood routine examination, has been reported that its predictive value was higher than individual cell counts.[15]

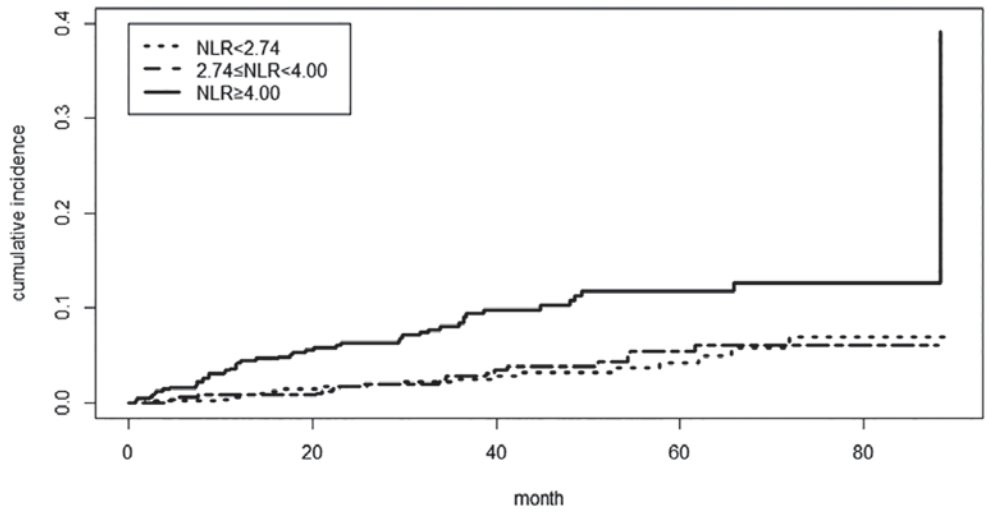

Figure 3. It shows estimated cumulative incidence curves with the first occurrence of stroke. The cumulative incidence curves for the tertiles of NLR level were highly significant for the first occurrence of stroke (*P* < .001), but they were not statistically different for transfer to hemodialysis therapy, kidney transplantation, transfer to other center, lost to follow up and death as competing events for each type of NLR level.

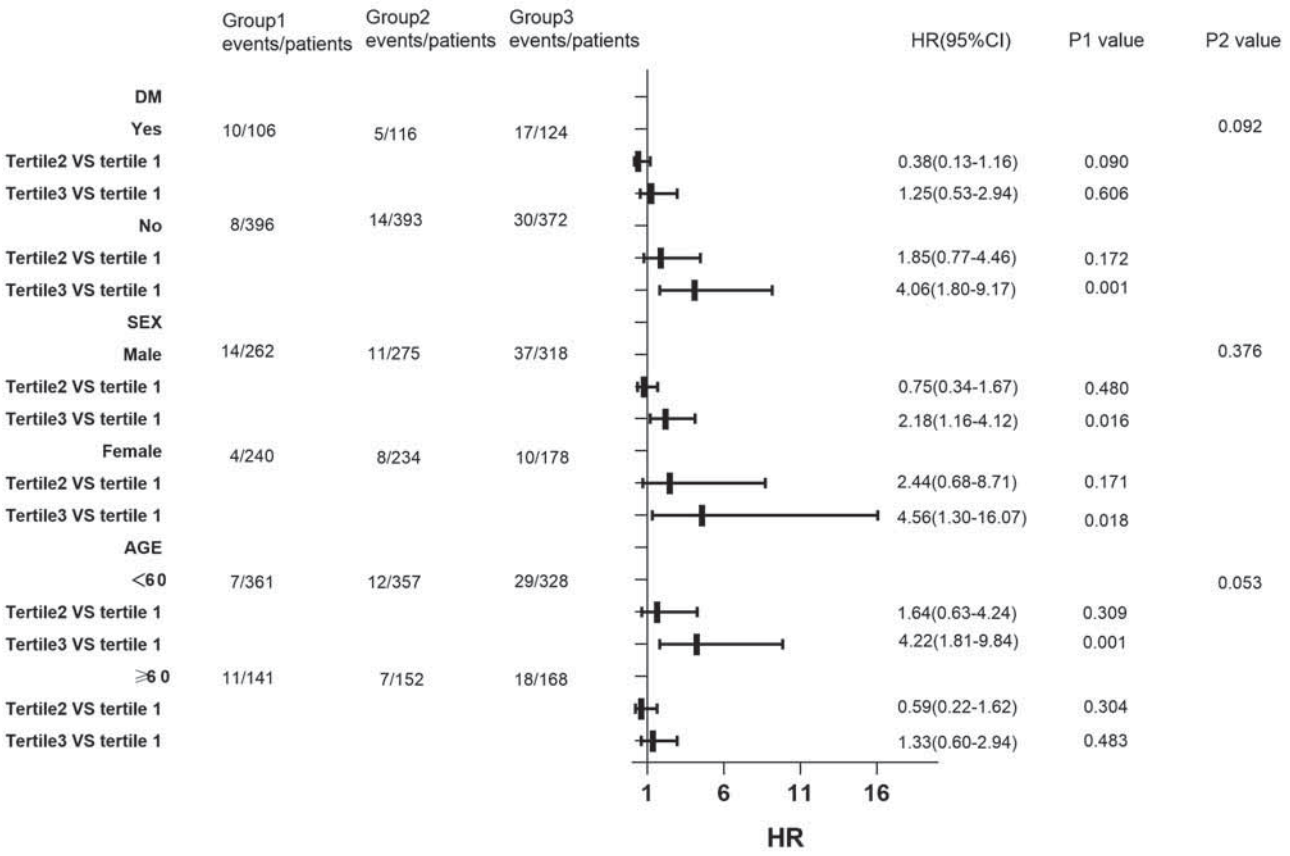

Figure 4. It demonstrates forest plot of relationship between NLR and the first occurrence of stroke in different subgroups.
Note: The *P*1 value corresponded to the relationship between NLR and the first occurrence of stroke in different subgroups. The *P*2 value corresponded to the interaction test between the NLR and the subgroups variable of interest. No interaction was observed for age, diabetes mellitus and sex in the first occurrence of stroke. Adjusted model: sex, age, BMI, history of diabetes mellitus, hypertension, stroke and cardiovascular disease, use of aspirin, albumin, creatinine, urea nitrogen, uric acid, FBG, total cholesterol, total triglycerides, Phosphorus, alkaline phosphatase (in particular, the adjustment model should exclude its own factors in different subgroups. For example, in the age subgroup, the adjustment model did not include age.) Abbreviations: NLR, Neutrophil / Lymphocyte ratio; HR, hazard ratio; CI, confidence interval.

Plenty of studies have indicated that the occurrence, development and mortality of coronary heart disease could be predicted by increased NLR level independently.[16,18-21] It has also been proved that NLR showed perfect predictive value in stroke,[11] which was relevant to the prognosis of cerebral hemorrhage and infarction.[13,22] A large-scale retrospective cohort study enrolled 24708 generally healthy screened people, it was demonstrated that subjects with increased NLR tended to have elevated risk for the incidence of ischemic stroke.[23] Luo P *et al*. reported that elevated NLR was positively related with cerebral hemorrhage incidence in T2DM patients.[24] These two studies have confirmed that NLR was associated with the incidence of stroke in non-dialysis population. However, few study investigated the relationship between NLR and the occurrence of stroke in dialysis patients.

So far, only one study[25] explored the relationship of NLR level and the occurrence of cardiovascular events in incident dialysis patients. The prospective cohort study, which, enrolled 86 PD patients with median of NLR equal to 3.72 showed that elevated NLR was associated with increased risk of CVD events (3.02, 95% CI: 1.32 to 8.00; $P < .05$). However, the association between NLR and stroke events has not been investigated independently. And some problems in the study should be pointed out: Firstly, the number of patients they enrolled was small; Secondly, they did not exclude patients who suffered from those diseases which altered NLR; Thirdly, they included patients receiving different dialysis treatment, which might be influential in CVD events. Yet the potential role of NLR as a simple and easily obtained indicator needs to be confirmed by multicenter prospective studies with relatively scientific grouping methods in the future.

In our study, a total of 1507 PD patients were included and stratified into tertiles of NLR levels. Multivariable Cox regression analysis showed that NLR was significantly associated with the first occurrence of stroke. This conclusion was consistent with the previous study.[23] Moreover, we investigated whether NLR independently predicted the new-onset stroke events in different subgroups. However, the result was negative.

Some strength could be found in our study. First, the number of patients we enrolled from multicenter was relatively large. Second, the association between NLR levels and the first occurrence of stroke were investigated independently for the first time in PD patients, instead of exploring the relationship between NLR and CVD events. Third, we finished a detailed evaluation and adjustment for stroke risk factors.

There were several limitations in this study. Firstly, because the patients were from four centers, some data was lack and not considered, such as CRP, smoking history and other confounding factors, which may influence the NLR value and statistical results. We should try to fill up previous flaw data through available information of patients and pay more attention to collecting new data carefully in the future. Secondly, our study was a retrospective cohort study rather than a prospective study. So, it is necessary to initiate a prospective study about the relationship between NLR and the new-onset stroke events in PD patients. Thirdly, all the parameters were measured on a single occasion at baseline and did not take into account changes over time. Some dynamic data of those patients should be included to strength the conclusion. Fourthly, in this study, we cannot analyze the association of NLR and the first occurrence of every type of stroke respectively, for not paying attention to registering the types of stroke when collecting data. In that case, the pathogenic hypothesis about NLR and the first occurrence of stroke in PD patients was not stated in this study.

CONCLUSION

In conclusion, our study demonstrated that high NLR is an independent risk factor for the first occurrence of stroke in PD patients. Although further study is needed, NLR could be considered as a useful and inexpensive marker for identifying higher risk for stroke in PD patients.

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DISCLOSURE

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

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The Effects of Nano-curcumin on Metabolic Status in Patients With Diabetes on Hemodialysis, a Randomized, Double Blind, Placebo-controlled Trial

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Original Paper

Introduction. This study evaluated the effects of nano-curcumin intake on metabolic status in patients with diabetes on hemodialysis (HD).
Methods. This randomized, double-blind, placebo-controlled clinical trial was performed on 60 patients with diabetes on HD. Participants were randomly divided into two groups to take either 80 mg/d nano-curcumin (n = 30) or placebo (n = 30) for 12 weeks.
Results. Nano-curcumin significantly decreased fasting plasma glucose (β = -19.68 mg/dL, 95% CI: -33.48 to -5.88; $P < .05$) and serum insulin levels (β = -1.70 μIU/mL, 95% CI: -2.96 to -0.44; $P < .05$) when compared with patients who received placebo. Nano-curcumin treatment was associated with a significant reduction in triglycerides (β = -16.13 mg/dL, 95% CI: -31.51 to -0.75; $P < .05$), VLDL-cholesterol (β = -3.22 mg/dL, 95% CI: -6.30 to -0.15; $P < .05$), total cholesterol (β = -17.83 mg/dL, 95% CI: -29.22 to -6.45; $P < .05$), LDL-cholesterol (β = -15.20 mg/dL, 95% CI: -25.53 to -4.87; $P < .05$), and total-cholesterol/HDL-cholesterol ratio (β = -1.15, 95% CI: -0.2.10 to -0.21; $P < .05$) when compared with the placebo. Nano-curcumin also resulted in a significant reduction of serum high sensitivity CRP (β = -0.78 mg/L, 95% CI: -1.41 to -0.15; $P < .05$), and plasma malondialdehyde (β = -0.25 μmol/L, 95% CI: -0.45 to -0.04; $P < .05$); but also with a significant increase in plasma total antioxidant capacity (β = 52.43 mmol/L; 95% CI: 4.52 to 100.35; $P < .05$) and total nitrite levels (β = 3.62 μmol/L, 95% CI: 2.17 to 5.08; $P < .001$) when compared with placebo.
Conclusion. Nano-curcumin intake for 12 weeks had beneficial effects on metabolic profile in patients with diabetes on HD.

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INTRODUCTION

Diabetes mellitus is one of the leading causes of chronic kidney disease (CKD) worldwide. Diabetic nephropathy (DN) is the most common risk factor for developing end-stage renal disease (ESRD). Approximately 95% of these patients are treated with hemodialysis (HD).[1] Hyperglycemia is the primary cause for developing DN because it increases generation of reactive oxygen species and causes oxidative damage, which are more expressed in HD patients. In addition to oxidative stress, other factors such as malnutrition, inflammation, and the reduced production of nitric oxide (NO) promote endothelial dysfunction and atherosclerosis in these patients.[2-4] Moreover, changes of lipoproteins, both quantitative and qualitative, are often found in

CKD patients and are more pronounced in end-stage of the disease.[5-7] However, diabetes per se, particularly type 2 diabetes mellitus (T2DM), is very often associated with atherogenic dyslipidemia which is characterized by hypertriglyceridemia, low HDL-cholesterol and moderately elevated or even normal LDL-cholesterol but LDL particles are small, dense and more atherogenic.[8-10] CKD is associated in patients with diabetes with higher levels of plasma triglycerides and lower levels of HDL-cholesterol even among patients with good control of LDL- cholesterol.[11]

Curcumin is the active compound of the traditional dietary and medicine plant named turmeric.[12,13] Curcumin has a wide variety of pharmacological and biomedical effects in various conditions such as inflammatory diseases, metabolic syndrome, obesity, dyslipidemia, cardiovascular diseases, and cancer.[14-16] This natural compound has attracted attention because of its beneficial properties in treatment of diabetes and its complications due to its hypoglycemic, lipid-lowering, anti-inflammatory and antioxidant effects.[17,18] Curcumin improves insulin resistance and glucose homeostasis by enhancing β-cells function and insulin secretion affecting glycolysis, glyconeogenesis and lipids metabolism in liver.[19] Lipid-lowering effects of curcumin are due to its ability to increase the activity of lipoprotein lipase, to reduce lipid peroxidation, plasma total cholesterol and triglycerides concentrations and to elevate HDL-cholesterol levels.[20,21] There are indications that curcumin can modulate the expression of some genes related to glucose and lipid metabolism such as peroxisome proliferator-activated receptor (PPAR-γ) and LDL receptor (LDLR).[22,23]

Despite of potential positive effects of curcumin, its oral bioavailability is low. Nano formulated curcumin is a novel way to improve its bioavailability.[24] Therefore, based upon reported beneficial effects of curcumin, we tried to evaluate the effects of Nano-curcumin intake on metabolic status in patients with diabetes mellitus on hemodialysis (HD).

MATERIALS AND METHODS

Trial Design and Participants

This study, registered in the Iranian website for clinical trials (http://www.irct.ir: IRCT20150606022562N6), was a randomized, double-blind, placebo-controlled clinical trial performed on 60 patients with diabetes on HD; 18 to 80 years old, which were referred to the Akhavan Clinic in Kashan, Iran, between December 2018 and April 2019. All participants fulfilled The Declaration of Helsinki requirements and signed an informed consent. The ethics committee of Kashan University of Medical Sciences (KAUMS) approved this study. Patients involved with infectious, inflammatory and malignant diseases, those who were taking curcumin supplements, antioxidant and/or anti-inflammatory supplements within 3 months before participation in the study, and subjects who were receiving immunosuppressive and antibiotics medications were not included in the study.

Study Design

Patients were asked to continue their routine physical activity, and not to take any anti-inflammatory and antioxidant medications or supplements that might affect their nutritional status during the 12-week intervention. By asking participants to give back the medication containers we checked administration of curcumin and placebo during the study. All participants were reminded to take the supplement (or placebo) by sending a short SMS message every day. All partients completed both 3-day food records and physical activity records at weeks 0, 6, and 12 of the intervention. To obtain macro- and micro-nutrient intake composition of participants based on these 3-day food records, Nutritionist IV software (First Databank, San Bruno, CA) modified for Iranian foods was used.

Intervention

Patients were randomized into two groups to take either nano-curcumin capsule (80 mg/d) or placebo (n = 30, each group) for 12 weeks. Nano-curcumin and placebo capsules were purchased from Exir Nano Sina Company (Tehran, Iran). Nano-curcumin and placebo were similar in shape and package.

Assessment of Anthropometric Measures

Body weight and height were assessed after overnight fasting using the same digital scale (Seca, Hamburg, Germany) at baseline and after the 12-week of intervention. Body mass index (BMI) was

calculated by weight and height measurements [weight (kg) / height (m^2)].

Clinical Measurements

Assessment of Outcomes. Insulin resistance and insulin levels were considered as primary outcomes while serum lipoproteins, and biomarkers of inflammation and oxidative stress were considered as secondary outcomes. A 15 mL fasting blood sample was collected at baseline and at week 12 after the intervention at Kashan reference laboratory and samples were centrifuged to separate serum. Then, the samples were stored at -80°C until analysis. Serum insulin and hs-CRP levels were quantified by using ELISA kit (DiaMetra, Milano, Italy and LDN, Nordhorn, Germany) with inter- and intra-assay coefficient variances (CVs) lower than 7%. The homeostasis model of assessment-insulin resistance (HOMA-IR), and the quantitative insulin sensitivity check index (QUICKI) were determined according to the standard formula.[25] Enzymatic kits (Pars Azmun, Tehran, Iran) were used to quantify fasting plasma glucose (FPG), serum lipoproteins, creatinine and blood urea nitrogen (BUN) with inter- and intra-assay CVs less than 5%. Total nitrite was estimated using Griess method,[26] total antioxidant capacity (TAC) by the method of ferric reducing antioxidant power developed by Benzie and Strain,[27] total glutathione (GSH) using the method of Beutler *et al*.[28] and malondialdehyde (MDA) concentrations were determined by the thiobarbituric acid reactive substances spectrophotometric test[29] with inter- and intra-assay CVs lower than 5%. Systolic (SBP) and diastolic blood pressure (DBP) was measured using the same sphygmomanometer (ALPK2, Zhejiang, China). Blood pressure was measured between 08:00 and 09:00 AM by the same investigator each time.

Isolation of Lymphocytes

Lymphocytes were extracted from blood samples using 50% percoll (Sigma-Aldrich, Dorset, UK). Cell count and viability test were conducted using trypan blue, RNA and DNA extraction.

RNA Extraction and Real-time PCR (RT-PCR)

Gene expressions of PPAR-γ, LDLR and transforming growth factor beta (TGF-β) were assessed by quantitative RT-PCR in peripheral blood mononuclear cells (PBMCs), using the LightCycler technology (Roche Diagnostics, Rotkreuz, Switzerland) with SYBR green detection and Amplicon Kit (Table 1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers were used as a housekeeping gene. Primer Express Software (Applied Biosystems, Foster City, USA) and Beacon designer software (Takaposizt, Tehran, Iran) were used to design primers. Relative transcription levels were calculated using the method of Pffafi.

Sample Size

In this study, we used a randomized clinical trial sample size calculation formula where type one (α) and type two errors (β) were 0.05, and 0.20 (power = 80%); respectively. According to our previously published trial,[30] we used 0.170 as the SD and 0.135 as the change in mean (d) of HOMA-IR as a primary outcome. Based on the formula, we needed 25 participants in each group. After allowing for 5 dropouts in each group, the final sample size was 30 persons in each group.

Table 1. Specific Primers Used for Real-time Quantitative PCR

Gene	Primer	Product Size (bp)	Annealing Temperature (°C)
GAPDH	F: AAGCTCATTTCCTGGTATGACAACG	126	61.3
	R: TCTTCCTCTTGTGCTCTTGCTGG		
PPAR-γ	F: ATGACAGACCTCAGACAGATTG	210	54
	R: AATGTTGGCAGTGGCTCAG		
LDLR	F: ACTTACGGACAGACAGACAG	223	57
	R: GGCCACACATCCCATGATTC		
TGF-β	F: TTGAGACTTTTCCGTTGCCG	227	56
	R: CGAGGTCTGGGGAAAAGTCT		

GAPDH, glyceraldehyde-3-Phosphate dehydrogenase; LDLR, low-density lipoprotein receptor; PPAR-γ, peroxisome proliferator-activated receptor gamma; TGF-β, transforming growth factor beta.

Randomization

Computer-generated random numbers were used for randomization. The researchers and patients were not aware of randomization details until the final analyses were completed. The enrolling of participants, randomized, and allocating them to treatment or placebo were performed by trained staff at the dialysis clinic.

Statistical Methods

The Kolmogorov-Smirnov test was done to determine the normality of data. To detect the differences in anthropometric parameters, dietary intakes and gene expression between two groups, we used the independent-samples *t*-test. Paired-samples *t*-test was used to detect within-group changes. Multiple linear regression models were used to assess treatment effects on study outcomes. The effect sizes were presented as the mean differences with 95% confidence intervals. *P* values < .05 were considered statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Four patients in the Nano-curcumin group and 3 in the placebo group withdraw from the trial, due to personal reasons; thus 53 patients [nano-curcumin (n = 26) and placebo (n = 27)] completed the study (Figure 1). The compliance rate was high, more than 90% of capsules were taken during the course of the trial in both groups. No side effects were reported following the consumption of Nano-curcumin in patients with diabetes on HD during the study.

Distribution of gender, mean age, height, baseline weight and BMI were not statistically different between the two groups (Table 2).

Based on the 3-day dietary records obtained during the treatment period, we found no significant change in dietary macro- and micro-nutrient intake (data not shown).

Nano-curcumin significantly decreased FPG (β = -19.68 mg/dL, 95% CI: -33.48 to -5.88; *P* < .05) and serum insulin levels (β = -1.70 μIU/mL, 95% CI:

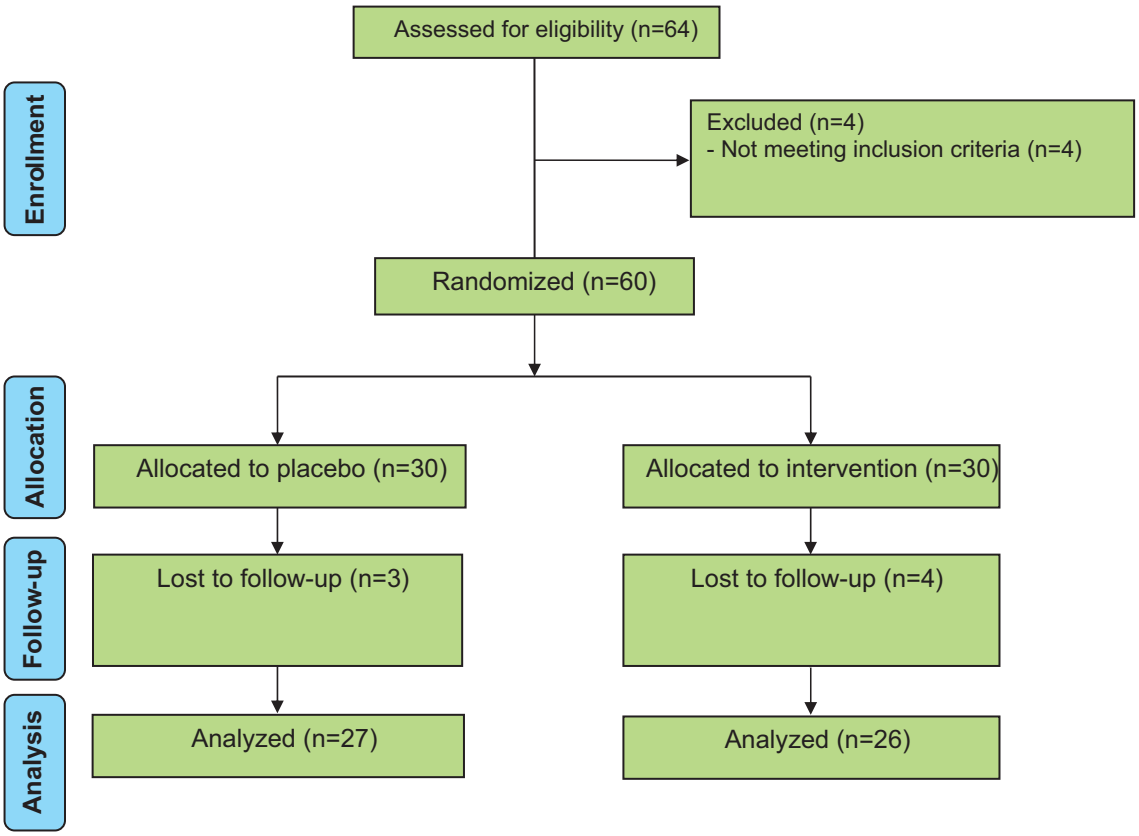

Figure 1. It shows summary of patients' flow diagram.

Table 2. General Characteristics of Study Participants

	Placebo Group (n = 27)	Nano-curcumin Group (n = 26)	*P*[1]
Gender (%)			
Males	15 (55.6)	17 (65.4)	> .05†
Females	12 (44.4)	9 (34.6)	
Age, years	56.2 ± 9.8	58.3 ± 9.4	> .05
Height, cm	165.5 ± 7.2	167.5 ± 7.3	> .05
Weight at Baseline, kg	73.9 ± 10.7	78.6 ± 15.9	> .05
Weight at the End of Trial, kg	74.9 ± 10.8	77.8 ± 15.4	> .05
Weight Change, kg	0.1 ± 1.4	-0.8 ± 1.5	< .05
BMI at Baseline, kg/m^2	27.1 ± 4.2	27.9 ± 4.9	> .05
BMI at the End of Trial, kg/m^2	27.1 ± 4.3	27.6 ± 4.7	> .05
BMI Change, kg/m^2	0.03 ± 0.5	-0.3 ± 0.5	< .05

Data are means ± SD.
[1]Obtained from independent *t*-test.
†Obtained from Pearson Chi-square test.

-2.96 to -0.44; $P < .05$) when compared with placebo (Table 3). Nano-curcumin was also associated with a significant reduction in triglycerides (β = -16.13 mg/dL, 95% CI: -31.51 to -0.75; $P < .05$), VLDL-cholesterol (β = -3.22 mg/dL, 95% CI: -6.30 to -0.15; $P < .05$), total cholesterol (β = -17.83 mg/dL, 95% CI: -29.22 to -6.45; $P < .05$), LDL-cholesterol (β = -15.20 mg/dL, 95% CI: -25.53 to -4.87; $P < .05$), and total-/HDL-cholesterol ratio (β = -1.15, 95% CI: -0.2.10 to -0.21; $P < .05$) when compared with placebo. Nano-curcumin significantly reduced serum hs-CRP (β = -0.78 mg/L, 95% CI: -1.41 to -0.15; $P < .05$) and plasma MDA (β = -0.25 µmol/L, 95% CI: -0.45 to -0.04; $P < .05$); and significantly increased plasma TAC (β = 52.43 mmol/L, 95% CI: 4.52 to 100.35; $P < .05$), and total nitrite levels (β = 3.62 µmol/L, 95% CI: 2.17 to 5.08; $P < .001$) when were compared with the placebo. Nano-curcumin intake did not change other metabolic parameters.

Baseline levels of HDL-cholesterol ($P < .05$), total-/HDL-cholesterol ratio ($P < .05$), and creatinine ($P < .05$) were significantly different between the two groups. Therefore, we adjusted the analyses for the baseline levels. However, after this adjustment no significant changes in our findings occurred (data not shown).

Nano-curcumin intake upregulated gene expression of PPAR-γ ($P < .05$) and LDLR ($P < .05$) in PBMCs of patients with diabetes on HD, when compared with placebo. Nano-curcumin did not affect gene expression of TGF-β (Figure 2).

DISCUSSION

In this study, we analyzed the effects of Nano-curcumin intake on metabolic profiles in patients with diabetes on HD. We found that Nano-curcumin supplementation during 12 weeks in these patients had beneficial effects on FPG, insulin levels, HOMA-IR, triglycerides, VLDL-cholesterol, total cholesterol, LDL-cholesterol, total-/HDL-cholesterol ratio, hs-CRP, total nitrite levels, TAC and MDA, and gene expression of PPAR-γ and LDLR, but did not affect other metabolic parameters and gene expression of TGF-β.

Effects on Glycemic Control and Serum Lipids

Our findings indicated that nano-curcumin intake during 12 weeks significantly reduced FPG, insulin

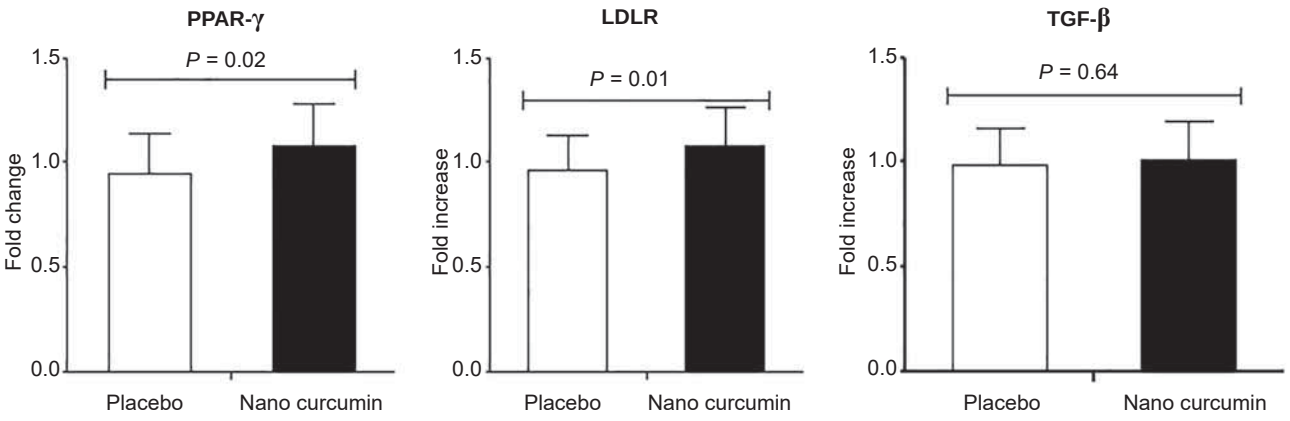

Figure 2. It determined effect of the 12-week supplementation with nano-curcumin or placebo on expression ratio of PPAR-γ, LDLR, and TGF-β gene in PBMCs of patients with diabetes on HD
(LDLR, low-density lipoprotein receptor; HD, hemodialysis; PBMCs, peripheral blood mononuclear cells; PPAR-γ, peroxisome proliferator-activated receptor gamma; TGF-β, transforming growth factor beta).

Table 3. Metabolic Profiles, Biomarkers of Inflammation and Oxidative Stress at Baseline and After the 12-week Intervention in Patients with Diabetes on Hemodialysis Who Received Either Nano-curcumin or Placebo

Variables	Placebo Group (n = 27)			Nano-curcumin Group (n = 26)			Difference in Outcome Parameters Between Nano-curcumin and Placebo Groups[1]	
	Baseline	Week 12	*P*[2]	Baseline	Week 12	*P*[2]	β (95% CI)	*P*[3]
FPG, mg/dL	126.2 ± 39.4	128.6 ± 39.2	> .05	139.8 ± 40.2	119.1 ± 38.0	< .05	-19.68 (-33.48 to -5.88)	< .05
Insulin, μIU/mL	10.4 ± 3.7	10.9 ± 3.6	> .05	11.4 ± 4.8	10.0 ± 4.2	< .05	-1.70 (-2.96 to -0.44)	< .05
HOMA-IR	3.2 ± 1.7	3.4 ± 1.6	> .05	3.8 ± 1.8	3.5 ± 1.8	> .05	-0.41 (-0.830 to -0.006)	> .05
QUICKI	0.33 ± 0.04	0.32 ± 0.02	> .05	0.32 ± 0.03	0.33 ± 0.03	> .05	0.008 (-0.002 to 0.0100)	> .05
Triglycerides, mg/dL	142.1 ± 77.8	138.9 ± 76.7	> .05	153.3 ± 72.1	132.3 ± 61.7	< .05	-16.13 (-31.51 to -0.75)	< .05
VLDL-cholesterol, mg/dL	28.4 ± 15.6	27.8 ± 15.3	> .05	30.7 ± 14.4	26.4 ± 12.3	< .05	-3.22 (-6.30 to -0.15)	< .05
Total Cholesterol, mg/dL	138.6 ± 37.5	146.1 ± 37.0	> .05	147.8 ± 40.3	133.9 ± 24.4	< .05	-17.83 (-29.22 to -6.45)	< .05
LDL-cholesterol, mg/dL	79.6 ± 29.9	86.8 ± 32.3	> .05	91.2 ± 34.5	78.4 ± 18.6	< .05	-15.20 (-25.53 to -4.87)	< .05
HDL-cholesterol, mg/dL	30.6 ± 6.1	31.6 ± 6.5	> .05	26.0 ± 6.2	29.1 ± 5.9	< .05	0.59 (-2.15 to 3.33)	> .05
Total-/HDL-cholesterol Ratio	4.7 ± 1.4	4.9 ± 2.6	> .05	5.9 ± 2.0	4.7 ± 1.0	< .001	-1.15 (-2.10 to -0.21)	< .05
hs-CRP, mg/L	6.3 ± 3.6	6.1 ± 3.5	> .05	5.4 ± 2.5	4.5 ± 2.0	< .05	-0.78 (-1.41 to -0.15)	< .05
Total Nitrite Level, μmol/L	40.8 ± 6.3	41.1 ± 6.0	> .05	39.6 ± 5.0	43.6 ± 5.2	< .001	3.62 (2.17 to 5.08)	< .001
TAC, mmol/L	1088.8 ± 203.7	1076.9 ± 225.9	> .05	1140.6 ± 187.7	1176.1 ± 159.6	> .05	52.43 (4.52 to 100.35)	< .05
GSH, μmol/L	493.7 ± 107.1	497.1 ± 120.7	> .05	408.8 ± 88.5	451.5 ± 88.6	< .05	28.21 (-10.00 to 66.43)	> .05
MDA, μmol/L	2.9 ± 0.8	2.9 ± 0.9	> .05	2.6 ± 0.4	2.4 ± 0.4	< .05	-0.25 (-0.45 to -0.04)	< .05
AGEs, AU	357.6 ± 124.5	364.6 ± 135.0	> .05	400.1 ± 106.2	379.8 ± 76.0	> .05	-21.20 (-48.49 to 6.09)	> .05
Creatinine, mg/dL	7.3 ± 2.5	6.7 ± 2.1	< .05	8.6 ± 1.9	7.5 ± 1.8	< .001	-0.24 (-0.84 to 0.35)	> .05
BUN, mg/dL	51.5 ± 13.7	48.8 ± 12.8	> .05	58.4 ± 12.1	48.7 ± 11.3	< .001	-3.92 (-9.53 to 1.67)	> .05
SBP, mmHg	133.9 ± 14.1	132.2 ± 11.5	> .05	138.8 ± 13.6	133.5 ± 14.4	< .05	-3.08 (-7.70 to 1.53)	> .05
DBP, mmHg	82.6 ± 10.2	81.3 ± 8.3	> .05	79.2 ± 6.9	76.5 ± 6.3	< .05	-2.74 (-5.68 to 0.19)	> .05

Data are mean ± SD.

[1]"Outcome measures" refers to the change in values of measures of interest between baseline and week 12. β shows difference in the mean outcome's measures between treatment groups (nano curcumin group = 1 and placebo group = 0).

[2]Obtained from paired-samples t-tests.

[3]Obtained from multiple regression model (adjusted for baseline values of each biochemical variables).

AGEs, advanced glycation end products; BUN, blood urea nitrogen; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GSH, total glutathione; HOMA-IR, homeostasis model of assessment-insulin resistance; HDL-cholesterol, high density lipoprotein-cholesterol; Hs-CRP, high sensitivity C-reactive protein; LDL-cholesterol, low density lipoprotein-cholesterol; MDA, malondialdehyde; QUICKI, quantitative insulin sensitivity check index; VLDL-cholesterol, very low density lipoprotein-cholesterol; SBP, systolic blood pressure; TAC, total antioxidant capacity.

levels, HOMA-IR, triglycerides, VLDL-cholesterol, total cholesterol, LDL-cholesterol, and total-/HDL-cholesterol ratio. A meta-analysis showed that curcumin intake in subjects with prediabetes and T2DM can reduce FPG, but did not change HOMA-IR.[31] Another study reported that 300 mg curcumin administration during 3 months to patients with (T2DM) significantly decreased FPG and HOMA-IR.[32] However, Kocher *et al*.[33] reported that 294 mg micellar curcumin intake for 6 weeks in moderately hyperlipidemic individuals did not show any glucose-lowering effect. A recent meta-analysis suggested that turmeric and curcumin in patients with cardiovascular risk factors was associated with a significant reduction in total cholesterol, LDL-cholesterol, and triglycerides; but did not affect HDL-cholesterol levels.[34] In a clinical study, 1,000 mg curcumin plus 10 mg piperine intake during 12 weeks reduced total cholesterol; non-HDL-cholesterol, lipoprotein A and elevated HDL-cholesterol levels, but failed to change LDL-cholesterol and triglycerides levels in T2DM patients.[35] In contrast to these results, an earlier meta-analysis on a heterogeneous population reported that curcumin administration did not significantly change any lipoproteins.[36] On the other hand, 40 mg nano-curcumin during 3 months in overweight/obese subjects with non-alcoholic fatty liver disease (NAFLD) resulted in a significant reduction in FPG, HOMA-IR, total- and LDL-cholesterol, and triglycerides levels as well as an elevation in HDL-cholesterol concentrations.[30] Several factors including the type of the study, curcumin dosage and intervention duration may account for these conflicting results of different studies. Hyperglycemia is the most important factor in the development of DN because it increases oxidative stress and inflammation. Insulin resistance is an independent predictor for cardiovascular disease and mortality in patients with CKD. Besides changed glucose, changes in plasma lipoproteins are frequent in early stages of kidney disease and are more severe in end stages.[37,38] Curcumin improves hyperglycemia by lowering oxidative stress.[39] Curcumin can also affect β-cells function increasing production and secretion of insulin.[40] The beneficial effect of curcumin on insulin resistance is mediated by stimulation of glycolysis and inhibition of glyconeogenesis in the liver.[19] The results of many studies suggested that curcumin can influence cholesterol absorption and excretion by the bile as well as to decrease lipid peroxides.[41] Nano-curcumin supplementation increased gene expression of PPAR-γ and LDLR. LDLR is involved in LDL-cholesterol catabolism and therefore its increased expression decreases plasma LDL-cholesterol levels. Furthermore, PPAR-γ induction is one of the main mechanisms by which glucose-lowering effect of curcumin can be explained.[42] Since curcumin can upregulate PPAR-γ and LDLR this might explain the improvement of lipoproteins and glucose metabolism.

Effects on Inflammation and Oxidative Stress Biomarkers

The results of our study suggest that Nano-curcumin during 12 weeks significantly reduced hs-CRP and MDA, and increased total nitrite and TAC levels, but did not affect GSH levels and gene expression of TGF-β in patients with diabetes on HD. In a meta-analysis, we have previously documented that taking curcumin-containing supplements could have anti-inflammatory and antioxidant effects which are achieved by a significant decrease in IL-6, hs-CRP, and MDA concentrations.[43] A significant reduction in hs-CRP, IL-6, and TNF-α concentrations following the intake of 1,500 mg/d turmeric for 12 weeks in HD patients was seen in another study, but there was no significant difference between intervention and control groups.[44] Short-term therapy with curcuminoids (500 mg/d for 4 weeks) resulted in suppressing systemic inflammation in subjects suffering from sulfur mustard-induced chronic pulmonary complications.[45] In another study on patients with T2DM, 2 g/d turmeric treatment for 4 weeks significantly reduced MDA concentrations.[46] However, in a meta-analysis; turmeric or curcumin intake did not reduce inflammatory cytokines in subjects with chronic inflammatory diseases.[47] Discrepancies in might be because of different characteristics of study populations, because of differences in study design, dosage and kind of curcumin-containing supplements used, quality of curcumin used and duration of the intervention. Earlier studies suggested that different factors, including dialysis clearance inflammation and oxidative damage are associated with morbidity and mortality in HD patients.[48,49] High rate of morbidity has been correlated with high concentrations of

CRP and other inflammatory markers such as IL-1 or IL-6 in these patients.[48,50] Curcumin is a natural antioxidant that has protective effects due to both increasing biological antioxidant defense system and free radical scavenging.[51] Curcumin intake may also reduce oxidative damage by chelating the redox-active metals and suppressing chain reactions producing metal ion-induced radicals.[52]

This study has some limitations. Due to budget restrictions, we did not check complience to Nano-curcumin intake by a biomarker. We were also unable to determine the effects of Nano-curcumin administration on other biomarkers of oxidative stress and inflammation.

CONCLUSION

We found that nano-curcumin supplementation for 12 weeks to patients with diabetes on HD had beneficial effects on FPG, insulin levels, HOMA-IR, triglycerides, VLDL-cholesterol, total cholesterol, LDL-cholesterol, total-/HDL-cholesterol ratio, hs-CRP, total nitrite, TAC and MDA, and gene expression of PPAR-γ and LDLR; but did not affect other metabolic parameters and gene expression of TGF-β.

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Not applicable.

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CONFLICT OF INTEREST

No conflicted.

AUTHOR CONTRIBUTIONS

FB and ZA contributed in conception, design, statistical analysis, and manuscript drafting. RS, ZR, AS, and EA contributed in data collection and manuscript drafting. All authors approved the paper for submission

CLINICAL REGISTRATION

http://www.irct.ir: IRCT20150606022562N6.

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The Added Value of Trabecular Bone Score in Fracture Risk Assessment of Kidney Transplant Recipients

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Introduction. Trabecular Bone Score (TBS) is an index of bone microarchitecture independent of Bone Mineral Density (BMD). Recently, TBS data has been used to optimize the predictive value of the Fracture Risk Assessment Tool (FRAX). The aim of this study was to evaluate the clinical value of FRAX adjustment with TBS in kidney transplant recipients.
Methods. Seventy post-transplant Iranian kidney recipients were included in this study. After the evaluation of BMD and TBS, the risk of major osteoporotic fracture (MOF) and hip fracture (HF) was assessed once with and once without TBS adjustment. The proportion of patients who needed a therapeutic intervention was compared before and after TBS adjustment. The association between TBS and BMD data was also evaluated.
Results. The mean age of the patients was 54 ± 8.8 years (range: 40 to 77). The mean TBS of the patients was 1.30 ± 0.12. In multivariate analysis, the TBS was significantly associated with the age ($P < .05$) and dialysis period ($P < .05$). A strong correlation was found between the spine BMD and TBS data ($r = 0.612$, $P < .001$). A significant correlation was found between the MOF and HF of the patients before and after adjustment for TBS. The proportion of patients needed a therapeutic intervention significantly increased from 17.1% to 25.7% after TBS adjustment of FRAX.
Conclusion. Adjustment of FRAX with TBS will reclassify the treatment decision in a considerable number of kidney transplant recipients. This clinical value warrants the adjustment of FRAX data with TBS in future workouts.

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INTRODUCTION

Osteoporosis is a major public health problem and a leading cause of fragility fracture.[1] Kidney transplant recipients are at increased risk of osteoporosis as well as fragility fracture.[2] It has been revealed that bone mineral density (BMD) declines by 4% to 10% in the first six months after transplantation by several mechanisms such as immunosuppression, alterations in the parathyroid hormone, changes in mineral metabolism, and glucocorticoid administration post-transplant.[3] This bone loss contributes to an increased risk of fragility fractures so that nearly 22.5% of kidney transplant recipients experience a fracture in the first five years after transplantation, an incidence that is four times greater than in the general population.[4] Considering the severe mortality and morbidity of fragility fracture and its remarkable

health and economic impact, the development of new diagnostic techniques for the prevention of osteoporotic fragility fractures is of significant importance.[5]

In the general population, low BMD strongly reflects the presence of osteoporosis and the risk of fragility fracture. However, conflicting results are reported in the kidney transplant recipients as BMD may be falsely elevated in these patients due to aortic calcification, particularly in long-term dialysis patients.[6-8] Accordingly, the bone quality may also be adversely affected besides bone density, and if not considered, the fracture risk of kidney transplant recipients might be underestimated.[9]

Although bone biopsy provides adequate information about bone quality, it is an invasive test and not suitable for routine workouts.[10] Thus, more practical approaches are needed for the evaluation of bone quality in kidney transplant recipients.

Trabecular bone score (TBS) is a novel, noninvasive measure of bone quality derived from lumbar spine dual-energy X-ray absorptiometry (DXA) images. It is a texture measurement that quantifies local variations in gray level distribution from the DXA image and is significantly correlated with 3-dimensional parameters of bone microarchitecture, independently of BMD. In this regard, a higher TBS value is indicative of better bone structure, vice versa.[11-5] Hence, attempts are being made to include TBS data in the fracture risk assessment.

Fracture risk assessment tool (FRAX) is a supportive software in osteoporosis management that provides a 10-year percentage of the risk of hip fracture (HF) and major osteoporotic fracture (MOF). Before the introduction of TBS, the FRAX assessment was based on the BMD information. Recently, FRAX data are adjustable with TBS information, providing a fracture risk assessment based on a combination of TBS and BMD.

The main goal of this study was to assess the association between BMD and TBS data in Iranian kidney transplant population as well as to evaluate the added value of TBS-adjusted FRAX in the reclassification of treatment threshold in these patients.

MATERIALS AND METHODS

This study was approved by the review board of our institute under the code of 9511402001, and written informed consent was obtained from the patients before their participation in the research. In a cross-sectional study, Iranian kidney transplant recipients were recruited from the nephrology clinic of Shahid Hashemi Nejad nephrology Hospital, Tehran, Iran. The patients were referred to the densitometry department for BMD and TBS examination providing that they were identified as eligible for the study. Inclusion criteria were the age of more than 40 years, at least six months past the date of transplantation, and a glomerular filtration rate (GFR) of more than 30 mL/min. Exclusion criteria included the patients undergoing osteoporosis treatment within the past two years, a history of Cushing's syndrome, malabsorption syndrome, liver failure, or any chronic disorders affecting the mineral metabolism. Since TBS can solely be computed for patients with a Body Mass Index (BMI) ranging from 15 to 37 kg/m^2, patients with a BMI of less than 15 or higher than 37 were also excluded.

BMD of the spine (L1 to L4) and femoral neck were assessed by a DXA machine (Hologic Horizon WI). The region with the lowest T-score was used for the evaluation of osteoporosis. According, the patients were categorized into osteoporotic (T-score < -2.5), osteopenic (-1 < T-score < -2.5), and normal (T-score > -1).

TBS measurement was performed at the same time with BMD evaluation using TBS software version 3.0.2.0, which determines the variogram of the trabecular bone projected image concerning the sum of the squared gray level differences between pixels at a specific distance and angle.[16] TBS results were considered degraded if < 1.2, partially degraded if 1.2 to 1.35, and normal if > 1.35.

MOF and HF risks were calculated using the FRAX calculator defined for the Iranian population. According to the guideline of the National Osteoporosis Foundation, cutoff values of 20% and 3% were considered as high absolute ten years risk of fracture for MOF and HF risk, respectively.[17] The FRAX calculation was done once without TBS adjustment and once with TBS adjustment (TBS-adjusted FRAX).

Statistical Analysis

SPSS version 16 was used for the statistical analysis of the data. Paired data were compared

using a paired t-test or its nonparametric counterpart (Kruskal-Wallis Test). A comparison of the mean value of two independent groups was made using an independent t-test or its nonparametric equivalent (Mann–Whitney U test). A multiple linear regression analysis was used to evaluate the association of TBS value with independent variables. A chi-square test was used for the evaluation of the difference between categorical variables. Pearson's correlation coefficient test was used for the evaluation of potential correlations. The proportion of patients needing a therapeutic intervention before and after TBS adjustment was compared using a McNemar's test. A *P* value of fewer than .05 was considered as statistically significant.

RESULTS

A total of 70 kidney transplant recipients were identified as eligible for the study. The patient's population included 30 (42.9%) females and 40 (57.1%) males with the mean age of 54 ± 8.8 years (range: 40 to 77 years). The mean glomerular filtration rate (GFR) of the patients was 66.3 ± 21.7 mL/min (range: 30 to 112.1 mL/min). The mean dialysis period before transplantation was 26.9 ± 31.2 months (range: 0 to 204 months). The mean time passed the transplantation date was 5.1 ± 5.7 years (range: 0.5 to 31 years). The mean parathyroid hormone (PTH) of the patients was 75.8 ± 63.2 pg/mL (range: 13.5 to 356 pg/mL). The mean serum vitamin D level was 20.1 ± 13.1 ng/mL (range: 3 to 75 ng/mL). In 44 (62.8%) patients, the kidney was transplanted from a living donor, while in 26 (37.2%) cases; it was transplanted from a deceased donor. Based on the routine protocol of our center, all the patients were under prednisolone, mycophenolate mofetil, and CN inhibitor medications. Eleven (15.7%) patients also were receiving mTOR inhibitors. None of the patients were receiving osteoporosis treatment. The clinical and demographic characteristics of the patients are demonstrated in more detail in Table 1.

The mean femoral neck and spine T-score of the patients were -1.49 ± 1.09 and -1.56 ± 1.3, respectively. According to the results of BMD evaluation, 19 (27.1%) patients were identified as osteoporotic, 36 (51.4%) patients were characterized as osteopenic, and 15 (21.4%) patients were normal. The mean TBS of the patients was 1.296 ± 0.123 (range: 0.93 to 1.56). Based on the TBS results, degraded, partially degraded, and normal bone quality was identified in 15 (21.4%), 24 (34.3%), and 31 (44.3%) patients, respectively. The densitometric data of the patients are demonstrated in Table 2.

In bivariate analysis, the mean TBS was significantly different in two dialysis groups (≤ 12 months and > 12 months dialysis; $P < .05$).

Table 1. The Demographic, Clinical, and Laboratory Data of Kidney Transplant Patients

Variables	Mean ± SD Number (%)
Age, year	54 ± 8.8
Gender	
Male	40 (57.1)
Female	30 (42.9)
Body Mass Index, k/m^2	25.7 ± 3
Etiology of ESKD	
Unknown	25 (35.7)
Glomerulonephritis	14 (20)
Type 2 Diabetes	13 (18.5)
ADPKD	9 (12.8)
Infection	3 (4.2)
Hypertension	2 (2.8)
Reflux Nephropathy	1 (1.4)
Urate Nephropathy	1 (1.4)
mTOR Inhibitors Medication	
Yes	11 (15.7)
No	59 (84.3)
Glomerular Filtration Rate, mL/min	66.3 ± 21.7
Pre-transplant Dialysis Period, mo	26.9 ± 31.2
Time Past the Transplant, year	5.1 ± 5.7
Time Past the Transplant	
≤ 5	49 (70)
> 5	21 (30)
Donor	
Living	44 (62.8%)
Deceased	26 (37.2%)
Parathyroid Hormone, pg/mL	75.8 ± 63.2
Serum Vitamin D, ng/mL	20.1 ± 13.1

ESKD, end-stage kidney disease; ADPKD, autosomal dominant polycystic kidney disease.

Table 2. The Densitometric Characteristics of the Kidney Transplant Patients

Variables	Mean ± SD
Femoral Neck BMD, g/cm^2	0.71 ± 0.14
Femoral Neck T-score	-1.49 ± 1.09
L1-L4 Spinal BMD, g/cm^2)	0.90 ± 0.15
L1-L4 Spinal T-score	-1.56 ± 1.3
TBS	1.30 ± 0.12
BMD-based MOF	6.03 ± 4.06
BMD-based HF	2.05 ± 2.89
TBS-adjusted MOF	6.98 ± 7.73
TBS-adjusted HF	2.53 ± 4.32

MOF, major osteoporotic fracture; HF, Hip fracture.

Besides, the mean TBS was significantly lower in diabetic patients compared with non-diabetic patients ($P < .05$). However, the mean TBS was not significantly different in two GFR groups (30 to 60 mL/min and > 60 mL/min, $P > .05$). In addition, the mean TBS was not significantly different between patients who had been transplanted for more than five years and those who had been transplanted for less than five years ($P > .05$). Also, the mean TBS was not significantly different in patients who received mTOR inhibitors and those who did not ($P > .05$). A significant negative correlation was also found between the age and TBS of the patients (r = -0.381, $P < .05$).

In multivariate analysis, TBS was still significantly associated with the age ($P < .05$, 95% CI: -0.008 to -0.001) and dialysis period ($P < .05$, 95% CI: -114 to -0.005) but not with the GFR ($P > .05$) and diabetic status ($P > .05$).

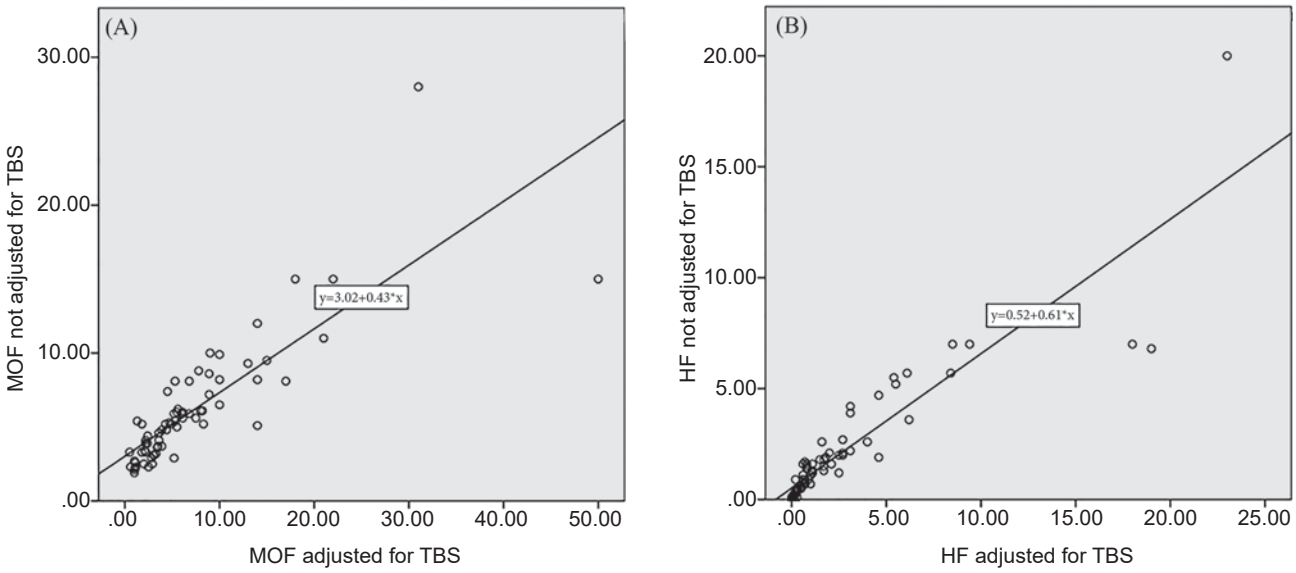
A significant correlation was found between the femoral neck BMD and TBS (r = 0.38, $P < .05$) as well as spine BMD and TBS (r = 0.61, $P < .001$). Moreover, a significant association was found between the BMD status (osteoporotic, osteopenic, and normal) and TBS status (degraded, partially degraded, and normal) of the patients ($P < .001$). In this respect, almost half of patients with an osteoporotic BMD had a degraded TBS, while the majority of patients with a normal BMD also had a normal TBS (Table 3).

The mean MOF of the patients was 6.03 ± 4.06 before the adjustment with TBS and 6.98 ± 7.73 after the adjustment with TBS. This difference was not statistically significant ($P > .05$). A significant positive correlation was found between the MOF of the patients before and after adjustment with TBS (r = 0.82, $P < .001$; Figure A).

The mean HF of the patients was 2.05 ± 2.89 before the adjustment with TBS and 2.53 ± 4.32 after the adjustment with TBS. This difference was not statistically significant, as well ($P > .05$). A significant positive correlation was also found between the HF of the patients before and after adjustment with TBS (r = 0.90, $P < .001$, Figure B).

Table 3. The Association Between BMD and TBS Status of the Kidney Transplant Patients

BMD Status	TBS Status			Total	*P*
	Degraded	Partially Degraded	Normal		
Osteoporosis	9 (47.4)	9 (47.4)	1 (5.3)	19 (27.1)	< .001
Osteopenia	5 (13.9)	14 (38.9)	17 (47.2)	36 (51.4)	
Normal	1 (6.7)	1 (6.7)	13 (86.7)	15 (21.4)	
Total	15 (21.4)	24 (34.3)	31 (44.3)	70 (100)	



It shows scatter plots showing the strong correlation of major osteoporotic fracture (A) and hip fracture risk (B) of the kidney transplant patients before and after adjustment with TBS.

Before TBS adjustment, MOF risk of only one patient passed the treatment threshold (> 20%). After TBS adjustment, the MOF of three other patients passed the treatment threshold. Before the TBS adjustment, HF of 11 patients was above the treatment threshold (> 3%). After TBS adjustment, The HF of three additional patients passed the treatment threshold. These patients were not the same patients who their MOF was reclassified after TBS adjustment. Overall, before the TBS adjustment, 12 (17.1%) patients needed a therapeutic intervention, while after TBS adjustment, 18 (25.7%) patients were required therapeutic intervention. This difference was statistically significant ($P < .05$). TBS adjustment did not result in the reduction of fracture risk below the treatment threshold in any patient.

DISCUSSION

In this study, we evaluated the BMD and TBS in a series of Iranian kidney transplant recipients.

The effect of TBS adjustment of FRAX on the MOF and HF of the patients and the treatment strategy was evaluated as well. Based on the result of multivariate analysis, TBS was significantly lower in patients with dialysis history of ≥ 12 months. Besides, TBS was negatively correlated with the age of patients. However, TBS was not associated with the GFR and diabetic status of the patients. A significant positive correlation was also found between the TBS and femoral neck/spine BMD of the patients. The FRAX score of the patients revealed a significantly strong correlation before and after adjustment with TBS as well. Despite this correlation, the treatment decision was reclassified in six patients after TBS adjustment (three patients based on MOF and three patients based on HF). In other words, the FRAX score of these six patients crossed the treatment threshold after TBS adjustment, indicating a pharmacologic osteoporosis treatment.

Naylor *et al*. compared the TBS in the kidney transplant population with the age and sex-matched general population from Manitoba, Canada. Based on their results, TBS was significantly lower in kidney transplant recipients when compared with the general population (1.37 vs. 1.41). Moreover, TBS was associated with a fracture rate independent of BMD.[9] Lower mean TBS was also noticed in kidney transplant recipients of the study of Pasquali *et al*. when compared with age-matched normal control Italian population (1.32 vs. 1.40).[18] Similarly, Bonani *et al*. observed a lower mean TBS in kidney transplant recipients (1.31) in comparison with the published reference value in a normal control Italian population (range: 1.36 to 1.47).[19] We did not find any previous study evaluating the TBS value in either Iranian kidney transplant recipient or the general population. The mean TBS of the patients in the current series was 1.30 that was considerably lower than the TBS of the general population in the study of Naylor *et al*., as expected. The mean TBS of the present series was also remarkably lower than the TBS of kidney transplant recipients in the earlier investigations.[9,18-9] This difference could be attributed to the different characteristics of patients. According to the results of the present study, factors such as age and dialysis period might affect the TBS of the patients. The negative correlation between age and TBS has been reported in earlier studies.[20,21] Lower TBS level in diabetic patients has been reported in other investigations.[22-3] In the present study, the diabetic status of the patients was significantly associated with TBS in the bivariate analysis but not in multivariate analysis, suggesting that this association could be confounded by other variables. The present study revealed a significant negative association between the dialysis period and TBS in both bivariate and multivariate analyses. By contrast to the present study, the study of Shevroja *et al*. revealed no effect of the pre-transplant dialysis period on post-transplant TBS.[24] Naylor *et al*. evaluated the association between TBS and incident fractures in adults with reduced kidney function. Based on their results, mean TBS was significantly lower in adults with reduced kidney function compared with those with normal kidney function (n: 1.28 vs. 1.30).[24] The TBS was not significantly associated with GFR of the patients in the current series, either in bivariate or in univariate analysis. However, it should be noted that the patients' number was markedly higher in the study of Naylor *et al*.

The value of TBS in the kidney transplant population has been acknowledged in other investigations as well.[25-7] As a new field of interest in osteoporosis, the number of studies on the potential optimizing effect of TBS on the predictive value of FRAX for fracture (MOF and HF) is increasing.

Couraud *et al*. compared the proportion of patients at high fracture risk before and after adjustment with TBS in 413 patients hospitalized for a non-vertebral fracture. Based on their results, the proportion of patients with a risk of MOF ≥ 20% before the fracture was similar before and after TBS adjustment (24.7% vs. 25.4%). The proportion of patients with a risk of MOF above the threshold of the therapeutic intervention was significantly higher after TBS adjustment for the age categories of 60-70years (38.3% vs. 30.9%) and 70 to 80 years (31.2% vs. 26.6%).[28]

Mirzaei *et al*. evaluated the effect of TBS adjustment on the FRAX algorithm in 358 postmenopausal Iranian women. Based on their results, the proportion of the women requiring a therapeutic intervention remained unchanged after FRAX adjustment with TBS. They reported no clinical benefit for FRAX-adjustment with TBS in postmenopausal women.[14]

Tamaki *et al*. aimed to find if TBS improves the predictive ability of FRAX for MOF in the Japanese population-based osteoporosis cohort study. They compared the predictive ability of the FRAX model before and after combination with TBS in 1541 women aged ≥ 40 at baseline. They identified 67 events of MOF in their cohort during a 10-year follow-up period. Based on their results, the model incorporating FRAX with TBS demonstrated a better fit compared to a model consisting of FRAX alone.[29]

To the best of our knowledge, no study has been performed to evaluate the effect of TBS adjustment on the MOF and HF risk of the kidney transplant population. Based on the results of the current study, the proportion of patients needing a therapeutic intervention significantly increased from 17.1% to 25.7% after TBS adjustment of FRAX. These findings reveal that TBS adjustment of FRAX contains valuable clinical utility in kidney transplant recipients.

One patient of the current series had normal BMD despite fully degraded TBS (Table 3). In reviewing her documents, we noticed aortic calcification along L1 to L4 lumbar vertebra, which could be responsible for misleading normal BMD. Aortic calcification in renal transplant patients is considered an important predisposing factor for falsely elevated bone density in the lumbar spine, and adding TBS to the bone evaluation partly resolves this problem. This point is highlighted in the study of Aleksova *et al.*, which aimed to evaluate the association of the TBS with abdominal aortic calcification in patients with chronic kidney disorders receiving dialysis. They evaluated 146 patients, of whom 49% had prevalent calcification and found an inverse association between TBS to vascular calcification.[30]

The value of TBS in fracture risk assessment has also been reported in other diseases such as Ankylosing Spondylitis, in which BMD results could be falsely elevated by the presence of typical syndesmophytes.[13]

Although mTOR inhibitors have revealed on the bone quality,[31] no significant association was found between the mTOR inhibitors medication and TBS of the patients in the present study. However, this results could have been adversely affected by the small number of patients who were taking mTOR inhibitors in the current series. Therefore, further studies are required to evaluate the effect of mTOR inhibitors on TBS.

The present study was not without weakness. The main weakness of this study was the small number of patients that could have affected the power of statistical analysis. Therefore, future investigations with a larger sample size will provide valuable complementary information regarding the value of TBS in kidney transplant recipients.

CONCLUSION

TBS was impaired in Iranian kidney transplant recipients. Factors such as age and duration of dialysis are associated with TBS. Despite a significant correlation between MOF and HF risk before and after adjustment with TBS, the proportion of patients who needed a therapeutic intervention significantly increased after FRAX adjustment with TBS. These findings highlight the complementary role of TBS in kidney transplant recipients and suggest TBS adjustment of FRAX in future workouts evaluating the bone quality of patients after kidney transplant. Moreover, the evaluation of TBS beside BMD provides awareness regarding the misleading BMD results caused by aortic calcification in the kidney transplant recipient.

CONFLICT OF INTEREST

The authors of this article declare no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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The Prevalence of Renal Scars Among Infants Under One Year Old With a First UTI With or Without VUR in Qom, Iran, 2017

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Keywords. urinary tract infection, vesicoureteral reflux, pediatrica, renal damage, prevalance

Renal scarring with reflux develops renal nephropathy. The risk is higher when it is associated with urinary tract infection (UTI). Hence, we investigated the prevalence of renal scars among children under one-year-old with the first UTI in Qom, Iran. We conducted this retrospective study on 140 infants divided in two reflux (n = 70) and non-reflux (n = 70) groups. Participant's records had been analyzed by descriptive and analytic statistics. The prevalence of renal scar was 32.12% among all 140 infants. The prevalence of renal scars among children with and without reflux, was 33 (47.1%), and 12 (17.1%) out of 70 ($P < .001$); respectively. The rate of renal defects increased with higher grades of reflux. We found that the rate of renal scar is high in Qom. Therefore, we recommend screening susceptible children in order to prevent renal damage.

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INTRODUCTION

Renal scarring induced by reflux can progress to reflux nephropathy. This risk would be higher, if vesicoureteral reflux (VUR) is associated with urinary tract infection (UTI).[1] UTI is one of the most common infections among children.[2-4] It is the first sign in 30% of children with urinary tract anomalies.[5] Although renal scar can be developed by even a single urinary tract infection, but repeated infections more probably cause reflux nephropathy.[1]

VUR is the retrograde urinary flow from the bladder to the ureter or kidney.[6] It may be familial or secondary to distal obstacle of the bladder or any other urinary tract anomalies.[7] Reflux can lead to incomplete urinary evacuation.[1] This defect may prepare children's renal infection.[8] Although VUR is often diagnosed following a UTI, the routine ultrasonographic imaging in pregnancy may reveal this defect before UTI represented. There is no reliable clinical sign to differentiate UTI in patients with or without reflux.[1]

Diagnostic techniques used for urinary tract assessment included: Renal ultrasound (RUS), voiding cystourethrogram (VCUG), and nuclear cystogram (NCG).[1,9] Dimercaptosuccinic Acid (DMSA) scan is the best way to identify renal scars.[10]

Any delay in treatment of UTI predisposes the children to kidney injuries.[4] The long-term complications of renal scarring include hypertension, renal dysfunction and end-stage renal disease (ESRD).[3,8] VUR is divided into 5 grades I–V.[7] Surgical intervention is rarely used for under one-year-old infants with reflux. It is indicated in some cases such as a high-grade reflux, and renal

impairment induced by renal nephropathy.[11]

As mentioned above, children are more likely to develop kidney damage following UTI. But the risk is highest in young children since ureteral bladder reflux is more common in this group. On the other hand, one more influencing factor on renal scar is genetic predisposibility.[3] Although several researches had been performed on renal scar prevalence, we did not find any study focused on children in the first year of life in Qom city. Thus, we investigated the rate of renal scars in under one-year-old infants with the first UTI affected to reflux or not in Qom, Iran in 2017.

MATERIALS AND METHODS

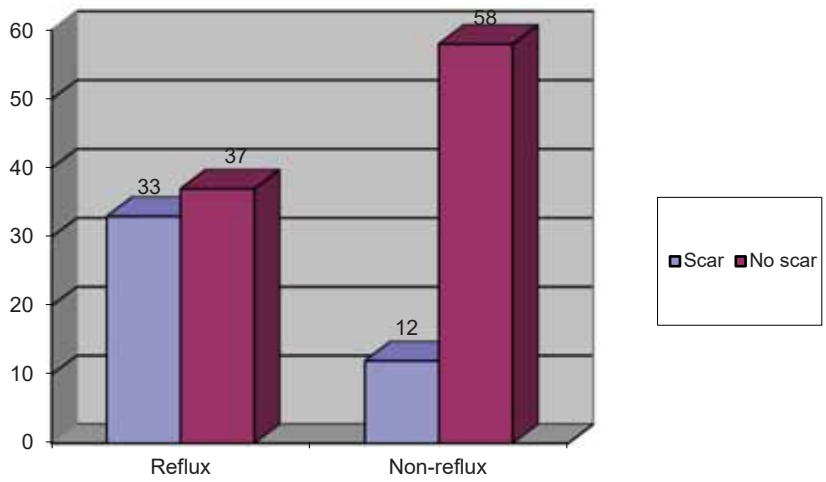
This historical cohort study was conducted in Hazrat Masoume hospital, Qom, Iran. This hospital has a referral nephrology ward for patients with urinary infection. The appropriate sample size was 139 children. It was calculated based on the statistical indices from previous studies and considering the probability of type *I* error 5%, power 80%, patients with VUR and abnormal DMSA equal to 75.9%. Data were gathered randomly from records of the patients admitted in nephrology ward anytime in the past till accomplishing the required sample size. So, we included all under one-year-old infants suffering from UTI. We diagnosed whether each infant affected with reflux or not and its severity by using VCUG data. The patients with reflux were allocated to exposed group, and those in unexposed group had no VUR. Both groups had been evaluated to detect renal scars by DMSA at intervals of 6 months. Some variables including age and gender were extracted, too.

The Research Committee of Qom University of Medical Sciences (IR.MUQ.REC.1396.124) approved this study. All data were gathered, coded, entered into a computer, and analyzed. Statistical analysis was performed using the STATA version 14. We analyzed data by using descriptive statistics and Pearson, chi squared tests, as well as logistic regression model, ordinal logistic regression model. The significance level used was a *P* value of less than .05.

RESULTS

Records of 140 infants affected with the first UTI were examined. In each group, 70 children were assessed. The average age of the children was 10 months. The mean age was 5 and 8 months, respectively, in the exposed and unexposed groups.

Of 140, number of 45 patients had abnormal DMSA in favor of scars. Thus the prevalence of renal scar was 32.12% among infants under one-year-old with the first urinary tract infection. Among them, 8.6% (n = 12) belongs to unexposed group; and 23.6% (n = 33) belongs to exposed group. The prevalence of renal scars in children with and without reflux was 33 (47.1%), and 12 (17.1%) out of 70; respectively. The chi - square test showed a significant relationship between kidney defects and the presence or absence of reflux ($P < .001$, Figure). Odds ratio for scar prevalence in the exposed group was 4.31 (95% CI: 1.98 to 9.39) folds comparing the unexposed group. The number of girls affected



It shows frequency distribution of children affected to scar according to exposed and non-exposed groups.

The Assessment of the Relationhip Between Renal Scars and VUR Grade

VUR Grade	Renal Scar				Total		*P*
	Yes		No				
	n	%	n	%	n	%	
Mild	18	34.6	34	65.4	52	100	< .001
Moderate and Sever	15	83.3	3	16.7	18	100	
Total	33	47.1	37	52.9	70	100	

with scar (n = 9) was higher than the boys (n = 3) in the unexposed group. Similar findings were observed in the exposed group (n = 25 (girls) vs. n = 8 (boys)). Chi-square test showed no significant relationship between gender and VUR.

In the exposed group, the number of 33 infants affected with scar. Of which, 18 babies had moderate and severe reflux (Grade 3, 4, 5), and 15 had mild reflux (Grade 1, 2). There was a positive relationship between renal scarring and VUR grade ($P < .001$, Table).

DISCUSSION

The present study investigates the prevalence of renal scars among infants under one-year-old with the first UTI in two groups with and without VUR. Of 140 children, 32.12% had renal scar. The prevalence of renal scars reported by Warren *et al* was 15.5%.[12] This prevalence is nearly half of our study. Faust et, al reported the rate of renal defects in patients following acute pyelonephritis varied from 26.5% (Australia) to 49.0% (Asia).[13] It shows renal defect in Iran has a better situation compared with other countries in the Middle East region.

According to the present findings, 47.1% of the babies with reflux and 17.1% of ones without reflux had kidney scars. Our findings are nearly consistent with this study. Based on Lee *et al.*, the first DMSA showed renal damage in 34 (70.8%) out of 48 refluxing units and in 13 (27.1%) out of 48 without reflux ($P < .01$, OR = 6.54).[14] Although similar results concluded from these two studies based on higher prevalence of scars in patients with reflux, the rate of renal scars in our study ($P < .01$, OR = 4.31) is lower than Lee's study.

Shaikh *et al.* reported the prevalence of renal scarring 15% in the follow up by DMSA in children with the first UTI. This rate is half of our study. Since Shaikh's study was a systematic review, which assessed 325 worldwide articles, it could be considered as a serious warning for us. Since it demonstrates the rate of renal scar in the present society is catastrophic in comparison with the other places. They also concluded that children with reflux are more likely to affect to renal defects other than the other group (RR = 2.6 [95% CI: 1.7 to 3.9]).[15] This finding is in consistence with ours.

According to the present study, the rate of kidney defects was 83.3% and 34.6%, respectively in high and low grades of reflux. According to Nelson, the prevalence of renal scars in patients with low-grade reflux is 15%, and in patients with high-grade reflux is 65%.[1] However, according to the present and previous studies, the higher the severity of VUR, the greater the risk of renal scarring, but the rate of our study is more than the others.

Wide variations of renal scars also reported as 15 to 60% in different studies. Some reasons for controversial findings in the mentioned above researches can be confounding factors such as different sample sizes, race, geographical regions, genetic context, age, gender, the presence or absence of reflux, and any human mistake in accurate diagnosis.[3]

Some limitations of the present study included: hard achievement to data due to lack of electronic records, and incomplete records of patients' information. These conditions may increase the likelihood of human errors. So, we recommend similar studies in different communities for a more precise assessment with an experimental, etc. methods, esp. with larger and a multi-center sample size, and assessing more confounding factors influencing on the patients' prognosis. As we know that the prevalence of renal scar in patients with vesicoureteral reflux clearly increased, it is suggested to conduct studies to evaluate appropriate screening tests for UTI in the exposed children, too.

CONCLUSION

According to the findings, the rate of renal defects in our society is high in comparison with the worldwide statistics. We observed all children

with UTI, even those without VUR, are at risk of renal scar. As we mentioned before, renal scars can lead to irreversible renal injuries. So, we must prevent urinary infections in children esp. infants under one-year-old. Also, we suggest to apply screening methods for early detection of UTI in infants to prevent such these complications.

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Proteinuria in Two Sisters with Beaulieu-Boycott-Innes Syndrome, A Case Report

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Keywords. developmental delay, intellectual disability, dysmorphic facial feature

We report two sisters (13- and 4-year-old) presenting with moderate intellectual disability, dysmorphic facial features, intermittent hematuria, proteinuria, and dental caries. Their parents and other family members were not affected. Whole-exome sequencing was performed to screen the underlying genetic cause. These patients have been analyzed using Next-Generation Sequencing (NGS) method and homozygote variant (c.890delC) has been detected in the THOC6 gene. Direct Sanger sequencing confirmed that they are homozygote for the pathogenic variant mutations in the THOC6 gene, which is associated with Beaulieu-Boycott-Innes syndrome (BBIS). These patients also had proteinuria and subsequently developed hematuria. This is the first report of BBIS in association with proteinuria and hematuria without renal defects. Core clinical features include low birth weight with subsequent growth failure, short stature, and intellectual disability with language delay, characteristic faces, cardiac defects, and renal anomalies. The possible pathophysiological mechanisms associated with proteinuria and transient hematuria without renal defects are discussed.

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Case Report

INTRODUCTION

Beaulieu-Boycott-Innes syndrome (BBIS) is an autosomal recessive neurodevelopmental disorder characterized by delayed development, moderate to severe intellectual disability, and dysmorphic facial features.[1]

Core clinical features include low birth weight with subsequent growth failure, short stature, mild microcephaly, intellectual disability with language delay, characteristic facies and cardiac and renal defects. Cryptorchidism in males, submucous cleft palate, and corpus callosum dysgenesis, may also be present.[2] All patients show characteristic dysmorphic facial features including a tall forehead with high anterior hairline, short and upslanting palpebral fissures, deep-set eyes, flat philtrum, and dental malocclusion with caries.[3-7] The prognosis of this syndrome is unknown.[5] Anatomic anomalies include malformations of the genitourinary system (absent and duplicated kidneys), and cardiac defects such as ventricular septal defects and persistent ductus arteriosus.[5] These patients are the first report of BBIS in association with transient proteinuria and hematuria.

CASE REPORT

Here, we describe two sisters (13- and 4-year-old) with BBIS presented with delayed development, severe intellectual disability, and dysmorphic facial features (Figure 1 and 2).

No neonatal problems have been described except for low birth weight and small head circumference, and subsequent growth was slow. Language and learning was delayed. On presentation to the pediatric nephrology clinic, they weighed 27 kg (25th to 50th percentile) and 14 kg (< 25th percentile),

Figure 1. This photo is related to older sister

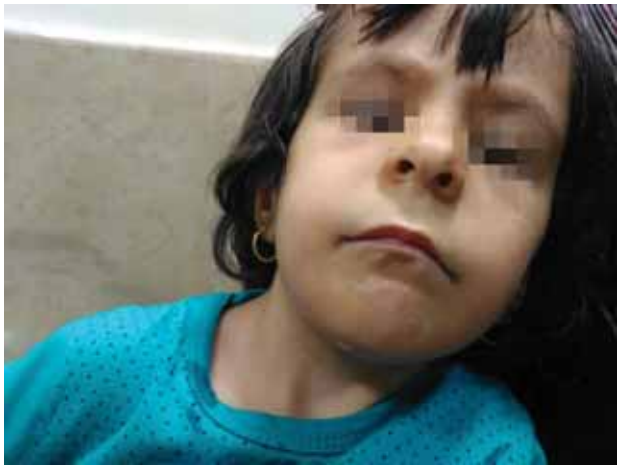

Figure 2. This photo is belonging to younger sister.

respectively. Patients were given a blood sample; urine collection cup, a urine container, and the parents grasp a written instruction for random and 24-hour urine sample collection. They also had mild proteinuria and hematuria and venous blood gases including PH and bicarbonate levels were normal. Figure 3 shows familial pedigree.

Case 1 (Older Sister)

Hemoglobin was 12.7 g/dL, total leucocyte count was 6.5 × 10^9/L (70% neutrophils, 29% lymphocytes, and 1% eosinophil) and platelet count was 198 × 10^9/L. Blood urea nitrogen was 18.5 mg/L, serum creatinine was 19.2 mg/L, serum sodium and potassium were 137 mg/dL and 4.5 mg/dL; respectively. Our patient had normal levels of lipid profile and serum albumin was normal (3.5 g/dL). Venous blood gases including PH and bicarbonate

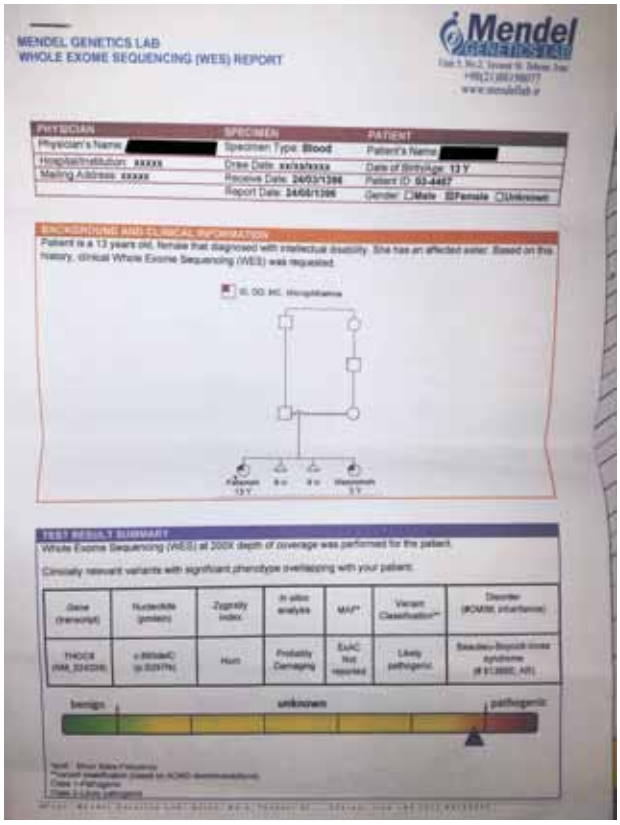

Figure 3. It shows familial pedigree.

levels were in normal range.

She also had normal vital signs but further investigation of urine sediment demonstrated mild proteinuria and hematuria. Laboratory urine tests include the following: urine analysis (PH: 5, WBC: 1-2, RBC: 8-10, SG: 1.005), urine culture: negative, 24-hour urine test (protein: 295 mg/dL, Cr: 450 mg/dL), random urine (Cr: 36 mg/dL, Na: 75 mg/dL, K: 25 mg/dL).

Case 2 (Younger Sister)

Hemoglobin was 11.5 g/dL, total leucocyte count was 5.5 × 10^9/L (60% neutrophils, 36% lymphocytes, and 1% eosinophil) and platelet count was 198 × 10^9, blood urea nitrogen was 19.8 mg/L, serum creatinine was 0.52 mg/L, serum sodium and potassium were 135 mg/dL and 3.5 mg/dL; respectively. Venous blood gases including PH and bicarbonate levels were in normal range. Our patient had normal levels of lipid profile and her serum albumin was normal (3.5 g/dL). She also had normal vital signs but further investigation of urine sediment demonstrated mild proteinuria and transient hematuria. Laboratory urine tests include

the following: urine analysis (PH: 6, WBC: 1-2, RBC: 14-16, SG: 1.025), urine culture: negative, 24-hour urine test (protein: 195 mg/dL, Cr: 350 mg/dL), random urine (Na: 75 mg/dL, K: 25 mg/dL, Cr: 56.5 mg/dL). The ultrasonographic evaluation of kidneys and urinary system was normal. There were no signs of fever, edema, lymphadenopathy or organomegaly. Results of cardiovascular, respiratory and other physical examinations were normal. There was no family history of BBIS or other syndromic disorders.

DISCUSSION

To date, some patients with BBIS and renal defect have been reported. These cases are unusual for two reasons. The development of urine sediment proteinuria and transient hematuria without renal defects is rare in BBIS and there are no similar reports in the literature.

Secondly, the patients developed intermittent dysuria during the course of this illness; the clinical feature was a discomfort in urination for more than 5 days. The dysuria rapidly resolved after administration of acetaminophen. This convincing response to therapy has been considered to represent a major diagnostic test for UTI but urine culture was negative. These patients have proteinuria and subsequently developed hematuria. Although all children with proteinuria need laboratory examination; treatment is not required in most cases.[8,9]

This is the first report of Beaulieu-Boycott-Innes syndrome (BBIS) in association with proteinuria and hematuria without renal defects. The possible pathophysiological mechanisms are not defined but more research is needed to find the reason. Regarding to BBIS in association with proteinuria and transient hematuria, our study requires kidney biopsy and no result was similar to those in other parts of the world. We recommended more studies on this syndrome especially larger and multi-center investigation.

AUTHORS' CONTRIBUTION

MAS and MHA were the principal investigators of the study. MAS, MHA, and FA participated in preparing the concept, design, and revision of the manuscript and critically evaluated the intellectual contents. The authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

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Peritoneal Dialysis in Extremely Obese Patient From Palestine, A Case Report

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Introduction. The incidence of ESRD is increasing dramatically and the majority of patients are commenced to hemodialysis (HD) or peritoneal dialysis (PD) due to the long waiting time for renal transplantation. PD has comparable outcomes with HD but many barriers limit its utilization. Obesity is considered among the barriers and this was attributed to its related complications.
Case Report. A 50-year-old male patient with ESRD presented to our hospital for PD. He was extremely obese (BMI = 44.2 kg/m^2). The case was discussed between the nephrology, surgical and nursing team, and the decision was made to proceed towards PD.
Conclusion. Obesity should not impede the beneficial effects of PD. The obstacles of obesity, which we faced; could be overcome with the collaboration between a highly qualified multidisciplinary team.

Keywords. peritoneal dialysis, obesity, end-stage renal disease

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Case Report

ABBREVIATIONS

ESRD, end-Stage Renal Disease; CDC, the centers for disease control and prevention; PD, peritoneal dialysis; HD, hemodialysis; BMI, body mass index; PO, per OS; IP, intra-peritoneally; US, united states.

INTRODUCTION

The incidence of end-Stage renal disease (ESRD) has increased dramatically, in 1996; the incidence in the US was 77,003 (compared with 124,675 in 2016).[1] Hemodialysis (HD) and peritoneal dialysis (PD) vary significantly in terms of patient lifestyle, employment, and interaction with the healthcare system.[2] The principles of peritoneal dialysis were first described by Popovich and his colleagues in 1976.[4,5] Patients on PD have comparable clinical outcomes, and even better; than patients on HD and their survival rate is equivalent to the center-based HD.[4,6,7] However, many barriers limit the utilization of PD and Obesity is considered among them.[8,9] One study published in 2003 showed that the most nephrologists in the US do not recommend PD for ESRD Patients weighing 200 pounds or more.[2]

This case report presents a patient with ESRD with a BMI of 44.2 kg/m^2 who underwent successful treatment with PD, by collaboration between a multidisciplinary team.

CASE REPORT

A 50-year-old man presented to our hospital for renal replacement therapy via peritoneal dialysis. His past medical history includes type 2 DM and HTN. The patient was tolerating moderate exercise until 4 months before admission, when he started experiencing progressively increasing lower limb swelling, nausea, exercise-induced dyspnea as well as orthopnea.

Laboratory results at admission: hemoglobin = 8 g/dL, K = 3.7 mEq/L, PH = 7.10, PCO_2 = 26.2 mmHg, PO_2 = 85 mmHg, HCO_3^- = 11.3 mEq/L, albumin = 2.9 g/dL, BUN = 85.8 g/dL, and Cr = 12.4 mg/dL.

The case was discussed between the nephrologists, surgeons, and nurse staff; and the decision was made to proceed towards doing PD as a life-saving

procedure given that the patient refused HD initially. Under local anesthesia, Tenckhoff-swan neck curl peritoneal catheter, 62.5 cm in length, double cuff was inserted smoothly. Two days following the PD catheter insertion, the uremic symptoms worsened dramatically and the patient became more distressed so the decision was to do an urgent session of hemodialysis. After four sessions of hemodialysis, patient improved and was kept in the peritoneal ward for the whole day for educational purposes.

The patient experienced two episodes of peritoneal leak and the PD was discontinued for 14 days. After 14 days, the leak stopped and the exchanges went smoothly when the patient suddenly started complaining of abdominal pain and a cloudy fluid coming out with exchanges. A diagnosis of peritonitis was established and the patient was treated with IP antibiotics for 14 days.

His dry weight was set to 119 kg after 3 months with 4 exchanges/d (two with 2.27% dextrose and two with 4.5% dextrose). Dwell time was considered 4 hours.

DISCUSSION

Peritoneal dialysis is one of two major modalities to treat ESRD patients waiting or not amenable for transplantation.[2] Despite the wide variety of PD use over the world (72% in Hong Kong, 9.7% in the US, and 4% in Sudan)[4]. Contraindications for PD include: obesity, severe protein malnutrition, polycystic kidney disease, lack of the integrity of the abdominal wall, and massive adhesions.[8,11] Obesity is our main concern. It is thought that obesity and increased BMI are associated with increased risk of catheter leak, inadequate clearance, infectious processes, and peritonitis.[11] Many studies have reported the paradoxical relationship between obesity and mortality among dialysis patients, a term referred to as "Obesity Paradox" or "reverse epidemiology".[12] According to the CDC, BMI of more than 40 Kg/m^2 is defined as extreme or severe obesity.[14]

The arguments about the possibility to proceed towards PD among obese patients are diverse as some studies assumed obesity as a relative contraindication to PD,[11,16] a barrier to PD[8] or not a contraindication.[15] We have faced most of the complications related to obesity but we were able to deal with them.

CONCLUSION

Peritoneal dialysis is a highly valuable modality of treatment for end-stage renal disease patients. Being overweight should not impede the beneficial effects of PD for patients who are willing to do so, as it confers them the ability to be engaged deeper in the community. The previous recommendations that considered obesity as a contraindication for PD are attributed to technical problems related to catheter insertion and possible future complications that can be handled if the patient was transferred to a highly qualified center.

DECLARATIONS

Consent for Publication

No images or other personal data that might compromise the anonymity of the patient. Written consent was obtained from the patient for publication of this Case report.

Ethical Approval and Consent to Participate

Full verbal and written consent has been obtained from patient himself.

Competing Interests

The authors report no conflict of interest.

Availability of Data and Materials

Data are all contained within the case report. The raw data are available by the corresponding author when requested.

FUNDING

No funding was received for conducting the study.

AUTHORS' CONTRIBUTIONS

ZH, MT, EK, EA, and OS designed the study and its protocol.

OS, HN, and KJ collected the data.

All authors managed follow-up of the patient.

All authors reviewed the manuscript critically for important intellectual content.

All authors read and approved the final manuscript for submission.

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Late Acute Cellular Rejection After Anakinra Treatment in a Kidney Transplant Patient, Is It a Coincidence?

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Keywords. anakinra, anti-drug-antibody, hapten, kidney transplantation, rejection

Familial mediterranean fever (FMF) is an autosomal recessive auto-inflammatory disorder, which could lead to secondary (AA) amyloidosis. Anakinra is an IL-1 receptor blocker and a treatment option for patients with FMF. There is no reported rejection episode associated with the use of Anakinra in the literature. A forty-nine years old woman with a history of kidney transplantation is described here. Anakinra was initiated in the patients whose FMF attacks were exacerbated, and the inflammation could not be controlled under the colchicine treatment. After eight months of follow up under Anakinra treatment, a moderate but persistent increase in serum creatinine level was observed. Allograft biopsy was compatible with acute T cell-mediated rejection with BANFF type 2A. Data on the use of Anakinra in KTRs is limited. Anti-drug-antibodies or hapten induced T cell activation may facilitate late-onset acute T cell-mediated rejection in the patient who used Anakinra.

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Case Report

INTRODUCTION

Familial mediterranean fever (FMF) is an autosomal recessive auto-inflammatory disorder which is characterized by lifelong recurrent self-limiting attacks of fever and systemic inflammation.[1] Progressive secondary (AA) amyloidosis is the primary cause of mortality and morbidity in patients with FMF. Renal amyloidosis leads to proteinuria, and end-stage kidney disease develops 2 to 13 years after the onset of proteinuria.[2] Anakinra (Kineret;r-metHuIL-1ra) is a recombinant human interleukin-1(IL-1) receptor antagonist that inhibits the activity of both IL-1α and IL-1β and seems to be safe and effective alternative treatment option for patients with FMF who do not respond to colchicine.[1,3]

Here, we report acute T cell-mediated rejection (ACR) episode that occurs after Anakinra use in a 49-year-old woman with kidney transplantation.

CASE REPORT

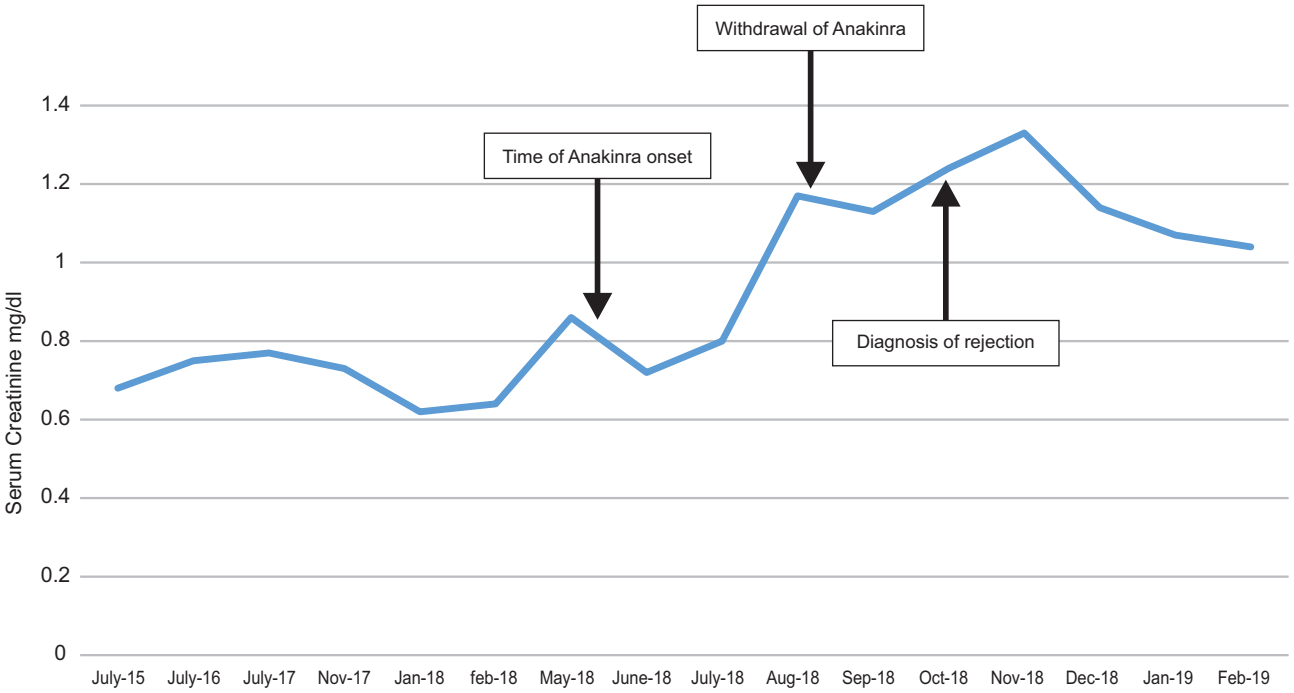
We present a 49-year-old woman who was diagnosed with AA type amyloidosis secondary to FMF at the age of twenty. Ten years after diagnosis, she started peritoneal dialysis as maintenance renal replacement therapy, and two years after the first transplantation was done from a living related donor. The patient lost his allograft due to hyper acute rejection. Then, she continued with peritoneal dialysis for seven years until the second kidney transplantation was done from a deceased donor. The patient and donor were one haplotype matched and class 1 and class 2 panel reactive antibodies were negative. Her allograft function was stable ten years after transplantation, and serum creatinine levels were between 0.6 to 0.8 mg/dL (Table). Anakinra treatment was started due to the resistant disease to colchicine. After eight months follow up there was no more attack of FMF, but a

Laboratory Parameters of the Patient Before and After Anakinra Treatment

Variables	Before Anakinra	After Anakinra (8th Month)	After Rejection Treatment
Hb, g/dL	14.9	12.6	12.3
WBC, 103 u/L	11.16	14.16	10.8
PLT, 109 u/L	364	422	378
BUN, mg/dL	14	38	27
Creatinine, mg/dL	0.64	1.36	1.04
eGFR, mL/m/ 1.73m^2	104.8	45.6	63
Sodium, mmol/L	139	139	138
Potassium, mmol/L	4.13	4.41	4.36
CRP, mg/L	23	7.8	1.18
24-h Urine Protein, mg/d	135	134	
Tacrolimus Level, ng/mL	5.6	5.7	7.5
BKV PCR	-	Negative	Negative
CMV PCR	-	Negative	Negative
Human Leukocyte Antigen			
A		24, 32	
B		52, 55	
DR		4, 11	

Hb, Hemoglobin; WBC, white blood cell; PLT, platelet; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein.

moderate and persistent increase in serum creatinine level was observed (Figure). As the patient's creatinine level elevated simultaneously with the onset of Anakinra, treatment was discontinued, and other possible causes of allograft dysfunction were examined. Allograft biopsy was performed because renal doppler ultrasonography was within normal ranges and urine BK PCR, and CMV PCR were negative. Allograft biopsy was reported as acute T cell-mediated rejection compatible with BANFF type 2A. Methylprednisolone was started 500 mg/d for six days. Despite high dose glucocorticoid therapy, the creatinine level of the patient did not decrease; therefore anti-thymocyte



It shows serum Cr monitoring of the patient over time.

globulin (ATG) was initiated 2 mg/kg per dose for total dose of 10 mg/kg. After ATG treatment serum creatinine level decreased and stabilized to 1.04 mg/dL. Prednisolone dose was reduced to 5 mg gradually and the patient continued to use mycophenolate mofetil and tacrolimus as maintenance immunosuppressive therapy. The patient is still in follow up, and she is using colchicine for FMF. She experienced no FMF attack until now.

DISCUSSION

IL-1 blockade is an effective treatment option in patients with colchicine resistant FMF. To the best of our knowledge, there is no reported rejection episode associated with the use of Anakinra in the literature. Although, it is challenging to relate ACR episode directly to Anakinra use; the deterioration of allograft function, which has been stable for ten years until Anakinra usage and relatively late period for ACR development, makes the case interesting.

Two possible mechanisms could cause this association. One of these is that antibodies that develop against biological agents named as "anti-drug antibody" (ADA) may have triggered ACR. These antibodies are well defined and responsible for the non-response to biological agents and hypersensitivity reactions.[4] ADA development was defined against Infliximab, Etanercept, Canakinumab, Tocilizumab, and Anakinra in the literature.[5] ADA against biological drugs is strongly associated with T cell-dependent reaction lymphoid tissue, which requires CD40 and CD154 interaction. Therefore, antibodies against IL-1 receptor may facilitate the development of ACR by causing activation of T cells.

The other possible mechanism is the hapten induced rejection process. Haptens are small non-protein chemical groups, which could not cause antibody stimulation alone but gain antigenic structure when coupled to a carrier protein.[6] After binding to the carrier protein, they become immunogenic and can cross-link B cell receptors and activate T cells.[6] Also, there are immunogenetic factors defined that could facilitate hapten reactions such as HLA-B57, -B15, -B58, -DR4, and -DR2 alleles.[7,8] It has been shown that the risk of drug-related lupus development increases in the presence of these alleles.[7] Our patient may also be susceptible to hapten related reactions because of the presence of the HLA DR4 allele.

CONCLUSION

In conclusion, the possible role of Anakinra should be considered in cases of acute rejection of renal transplantation during the late period. Data about the possible side effects of biological agents in literature is growing. We informed a situation where we observed a possible relationship with the use of a biological agent in this report.

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Surveillance and Isolation Based Strategies to Prevent COVID-19 in a Dialysis Center of Tehran, a Customized Approach

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The possibility of rapid spread of SARS-Cov-2 infection (COVID-19) via hemodialysis facilities should not be ignored. Unavoidable clustering of hemodialysis patients and close contact with nursing staff would increase the risk of viral transmission. In addition, these patients are more likely to be old and fragile and have multiple comorbidities. Therefore, patients on maintenance hemodialysis are more susceptible to COVID-19 and the infection might be more severe and complicated.[1]

Imam Khomeini Hospital Complex (IKHC) is a governmental hospital affiliated to Tehran University of Medical Sciences. Soon after epidemic of COVID-19, IKHC was selected as one of the

IKHC Guideline for COVID-19 Prevention in Patients and Healthcare Workers of Dialysis Center

General Recommendations for Patients	
Lockdown Principles	• Abstain from unnecessary personal contacts or public events • Stay at home while off dialysis • Transport by individual vehicle to and from dialysis center • For patients with acceptable residual renal function, two rather than three times per week hemodialysis temporarily • At least 6 feet distance between the patients in the dialysis hall • Prohibiting the presence of accompanying personnel in the dialysis ward
Screening	• Body temperature measurement before entrance to waiting room • Asking about respiratory symptoms upon arrival
Hygienic Measures	• Providing alcohol dispenser and surgical masks at the waiting room • Instructing the patients about: ○ appropriate hand hygiene ○ use of face mask ○ disposal of contaminated tissues and materials. • Discourage eating and drinking at the ward
Suspicious Cases of COVID-19	• Early referral of suspicious and known cases to the wards allocated for COVID-19 patients (Corona Wards) • Complete isolation of Corona Wards from general wards of hospital and the dialysis center • Prohibiting the entrance of suspicious or known cases of COVID-19 to dialysis ward • In cases of acute or chronic kidney diseases who requires renal replacement therapy, performing hemodialysis bedside the patient in the Corona Ward • Assigning another dialysis center for hemodialysis patients who discharged from Corona Ward
Healthcare Workers	• Providing online educational materials and pamphlets about: ○ governmental guidelines regarding COVID-19 ○ hand hygiene ○ appropriate personal protection • Requesting the staff members to inform the head of ward immediately, if they developed suggestive symptoms of COVID-19 • Standard protection protocol for: ○ healthcare workers of the dialysis center: ■ surgical facemask ■ non-sterile rubber glove ■ water-repellent gown with long sleeves ○ Nurses who run hemodialysis in Corona Ward: ■ N95 mask ■ face shield visor ■ waterproof disposable gown • Wearing new gloves for each patient

three referral hospitals in Tehran for COVID-19 admission. Thus, IKHC played a dual role during epidemic of COVID-19; it should manage referral cases of COVID-19 while providing routine medical services for other patients.

Maintaining the activity of dialysis center of IKHC during the outbreak of COVID-19 necessitates adopting appropriate preventive strategies to protect medical staff and patients.[2] Previous experiences from MERS-CoV revealed that strict patient surveillance and proper isolation practice would prevent secondary viral transmission.[3]

To provide a comprehensive guideline for COVID-19 prevention in dialysis center of IKHC, we reviewed interim additional guidance released by Center for Disease Control and Prevention and other guidelines and recommendations.[4,5] Considering our limitations and resources, we customized an appropriate guideline for COVID-19 prevention in healthcare workers and dialysis patients. Our recommendations were oriented in three main categories: general recommendations for hemodialysis patients, instructions for suspicious and known cases of COVID-19 and recommendations to protect healthcare workers. Table 1 represents our guideline briefly.

In conclusion, preventive managements would play a key role in breaking the chain of viral transmission and containment of COVID-19 pandemic in hemodialysis centers. Our strategy for COVID-19 prevention in the dialysis center of IKHC was mainly based on surveillance and isolation of otherwise healthy hemodialysis patients from suspicious cases of COVID-19. By adopting such strategies, we encountered very few cases of COVID-19 in healthcare workers and hemodialysis patients of our center. So, it seems that strategies based on surveillance and isolation would be very effective in prevention of COVID-19, as it was shown in similar setting of MERS-CoV outbreak previously.[3]

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Comment: Newly Diagnosed Glomerulonephritis During COVID-19 Infection Undergoing Immunosuppression Therapy, a Case Report

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We read with great interest the case report article by Moeinzadeh *et al*, entitled "newly diagnosed glomerulonephritis during COVID-19 infection undergoing immunosuppression therapy" published in a recent issue of IJKD.[1] The authors presented a case of a 25-year-old male with no known co-morbidities who presented with weakness and arthralgia to coronavirus clinic. Initial work up revealed severe anemia (hemoglobin [Hb] of 5.2 g/dL) and renal impairment (serum creatinine [SCr] of 3.7 mg/dL). He was admitted to the hospital, where further investigations showed worsening of renal function (SCr 4.2 mg/dL) with active urinary sediment and a further decline in Hb concentration (4.5 g/dL). High-resolution computed tomography (HRCT) of the chest demonstrated ground glass opacities (GGO) with a differential of diffuse alveolar hemorrhage and possible coronavirus infection. Patient received three days pulse of steroid [1 gram of Methylprednisolone/day] with presumed diagnosis of rapidly progressive Glomerulonephritis (RPGN). Serology and secondary work up were sent out and renal biopsy was obtained. The patient subsequently underwent plasmapheresis and three doses of intravenous immunoglobulin (IVIG), 20 g each time for alveolar hemorrhage. Renal biopsy was later reported as diffuse crescentic GN. Meanwhile, his coronavirus test was found to be positive and hydroxychloroquine in addition of levofloxacin was initiated.

The above case adds yet another dimension to the expanding spectrum of renal pathological lesions seen in patients of COVID-19 disease, particularly one based on renal histopathology. We would like to take this opportunity to highlight some points pertaining to kidney involvement in COVID-19, in general, and in this particular case. Clarification of the following items by the authors will further improve the understanding of the kidney pathology in this disease.

1- The data of kidney involvement in COVID-19 infection has started to accumulate but the results are conflicting.[2] Renal involvement in COVID-19 is common and has been shown to correlate with in-hospital deaths.[3] However, biopsy based studies are scarce.[4] Most reports suggest tubulointerstitial involvement in this disease, but more recently, a number of case reports have reported glomerular involvement, particularly, collapsing glomerulopathy (CG), especially in persons of African ancestry.[5-7] This suggests a possible role of APOL1 high risk alleles in predisposing these patients to the development of CG in COVID-19 infection, and the viral illness serving as second-hit in its pathogenesis.[8-10]

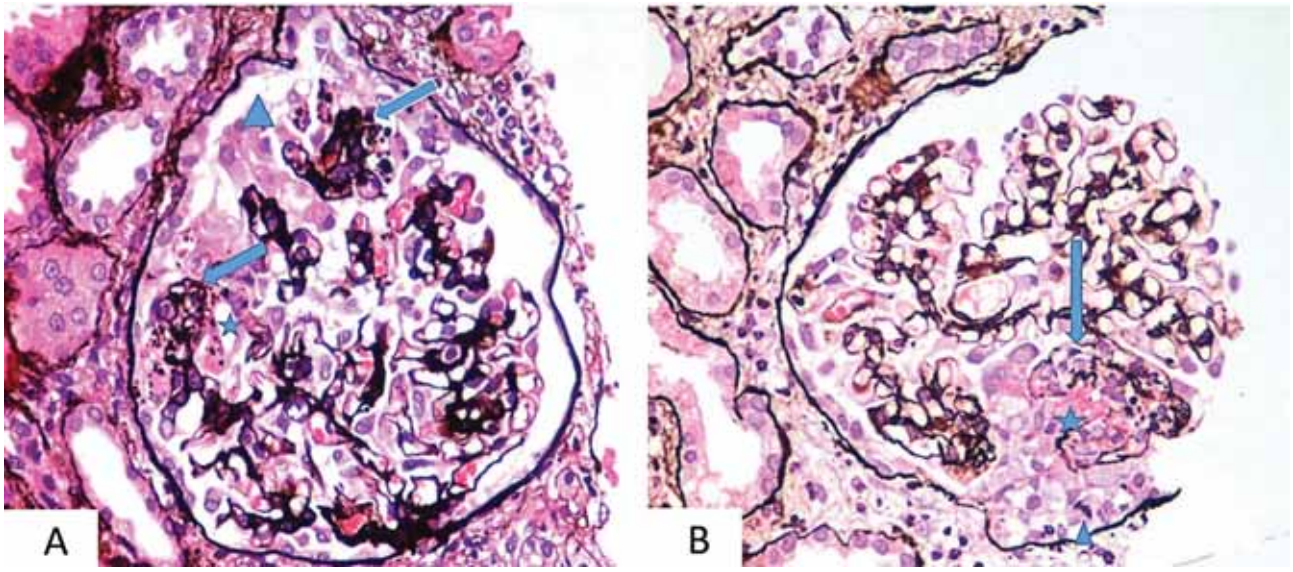
2- The case presented by Moeinzadeh *et al* was diagnosed and treated in the lines of primary GN coexisting with COVID-19 infection. The patient was treated for both conditions and there is also some overlap in the treatment of COVID-19 disease and primary GN. The major cause of morbidity in this case, according to authors, seems to be autoantibody-mediated vasculitis, manifesting as pulmonary renal syndrome. However, lack of any respiratory symptoms with this degree of involvement in vasculitis is unusual. Regarding renal biopsy findings, it is important to note that renal biopsy was obtained on completion of induction treatment of RPGN and no detailed pathology description was given. The authors only provided representative images of one or two abnormal glomeruli. All the three images show segmental obliteration of capillary lumina with segmental collapse of capillary loops and associated florid extracapillary proliferation of cells, which appear to be visceral epithelial cells (podocytes). These cells either surround the segmentally sclerosed

tuft (with a cleft-like space between the parietal epithelial cell layer) or fill the urinary space resembling a cellular crescent (pseudo-crescent). There is focal vacuolization and a few hyaline droplets in the cytoplasm of some of these proliferating cells. No evidence of glomerular necrosis, such as influx of leukocytes, necrotic cell debris or accumulation of fibrin is visible. No immunofluorescence or electron microscopy findings are given. A right approach would have been to consider both pseudo-crescents and true crescents in the differential diagnosis. All the features noted above, in our view, favor pseudo-crescents and hence CG should have been considered in the differential. It is well known that sometimes it is difficult to differentiate among these two forms of extracapillary proliferative GN and this case represents one of those examples. We take the liberty to illustrate this point in Figure 1, where preliminary view shows striking homology between the two conditions; however, more critical review shows tangible differences between these. The authors could utilize some immunohistochemical markers or electron microscopic study to differentiate between the two. Additionally, other known causes of CG, such as HIV status, parvovirus B19 infection, and others should be considered and clinically ruled out. If it turns out to be CG, this will be a significant finding as almost all previous cases of CG in association with COVID-19 infection have been reported in African Americans.

3- The results of the primary and many subsequent investigations were not correctly presented. His hemoglobin (Hb) is stated to be 5.2 g/L at presentation, which should be in g/dL. Similarly, SCr is given as 3.7 g/dL, which should be 3.7 mg/dL. C-reactive protein (CRP) is given as 2+, which is not a correct presentation of this result. Titer of c-ANCA given as 1/50 (positive) is incomprehensible. What method of ANCA testing was used?

4- The authors state that they discharged the patient with stable creatinine value of 5.5 mg/dl. With just one value of SCr, how can they claim that the function was stable, when all previous readings showed a continued rise?

5- The authors also did not establish definitive recovery from COVID-19 in this case according to Iran Ministry of Health and Medical Education COVID-19 guidelines. The reason put forward was the critical condition of the patient. This is



It shows morphological features of true crescent and pseudo-crescent. A) Medium-power view of a glomerulus showing collapse of two tufts with overlying podocyte hyperplasia and hypertrophy forming focal pseudocrescents over the involved tufts (arrows). Note: There are numerous protein resorption droplets in the cytoplasm of podocytes. These also show cytoplasmic vacuolization (asterisk). There is no fibrin or capillary wall rupture. Moreover, an irregular cleft-like space (arrowhead) separates this mass of proliferating podocytes from parietal epithelial layer (Jones silver stain, ×200). B) A glomerulus with focal true crescent formation (arrow). Note the rupture of capillary walls of the tuft at 5 O'clock position with exudation of fibrin into the Bowman's space and nuclear debris (asterisk). Note that the proliferating cells in the extracapillary space are originating from the parietal epithelium, which is showing a mitotic figure (arrowhead). There is no space between true crescent and parietal epithelium in this case. (Jones silver stain, ×200).

contradictory with their subsequent statement, in which, they claim that the patient was discharged healthy.

In summary, the authors need commendation on presenting the above case for increasing the awareness of nephrology and pathology community regarding expanding spectrum of pathological lesions in COVID-19 disease. We think this critique will further improve the understanding of many aspects of this interesting case.

Competing interests

The authors declare that they have no competing interests.

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Response to Comment on; Newly Diagnosed Glomerulonephritis During COVID-19 Infection Undergoing Immunosuppression Therapy, a Case Report

IJKD 2020;14:326-8
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Dear Editor,

I have read the article entitled "Comment on; Newly Diagnosed Glomerulonephritis During COVID-19 Infection Undergoing Immunosuppression Therapy, a Case Report." By Mubarak *et al.*, I want to congratulate the authors for this successful editorial letter, and make some contributions.

In this comment Mubarak *et al.* have been noted some point that we clarify them in the following:

1- Mubarak *et al.* mentioned that our case did not have any respiratory symptoms related to glomerulonephritis (GN), but it is notable that our case had diffuse alveolar hemorrhage in his computed tomography report and it could related to systemic vasculitis.[1]

2- It is a reality that differentiation between these two entities (crescent & pseudocrescent) can be very hard and challenging, but not in our case which reveals clear crescentic features of gloms in figures.

The term crescent is used for a lesion consisting of extracapillary hypercellularity, composed of a variable mixture of cells. Fibrin and fibrous matrix may be present; 10% or more of the circumference of Bowman's capsule should be involved.[2] In our case parietal epithelial cells show proliferation and make cellular crescent, although in some glom's podocyte hyperplasia is seen also and it's not in conflict with the diagnosis of crescentic GN.

True crescents and pseudocrescents even may coexist in the same glomerulus.[3]

The presence of fibrinoid necrosis, karyorrhexis, glomerular basement membrane rupture and red blood cell casts to be helpful indicators of crescent formation while the absence of these findings with the presence of protein resorption droplets admixed with the hypertrophied and hyperplastic podocytes, significant tubular intracytoplasmic protein resorption drops, microcystic tubular dilatation, thyroid type tubular atrophy and a predominance of solidified or disappearing-type global glomerulosclerosis suggests collapsing glomerulopathy.[4]

In contrast with your comment, there were no protein resorption droplets in our pictures and also cellular vacuolation was not specific for pseudocrescent formation.

The glomeruli in the case also show capsular rapture (Figure 1A), fibrinoid necrosis (Figure 1B), and karyorrhexis (Figure 1C); which define the diagnosis of crescentic GN.

Collapsing lesions are more commonly global than segmental and are often accompanied by severe tubulointerstitial injury with microcysts and hypertrophic tubular epithelial cells swollen.[5]

Many various IHC markers like CD68, CK, Nestin, CD44, WT1, and ki67 can be helpful in challenging case for differentiation between crescent and pseudo crescent,[3,4,6] but in this case the diagnosis was clear by morphology and IHC study just achieved for responding your comment and as expected, confirmed our diagnosis.

In collapsing glomerulopathy, hyperplastic podocytes showed complete loss of normal podocyte phenotype utilizing known markers of podocytes (CALLA, GLEPP1, Podocalyxin, Synaptopodin, WT1, P27, and p57) were decreased while Cyclin D1, Cyclin E, Cyclin A, Ki-67, Desmin, Cytokeratin, and CD68 were increased.[4,7]

We use the markers of cytokeratin, CD68, and Ki67 (Figure 2) and no accentuated staining compatible for hyperplastic podocytes was seen. Usually in true crescents, no cell expresses cytokeratin and numerous CD68-positive hyperplastic dysregulated podocytes in a glomerulus showing a pattern of collapsing GN.[3]

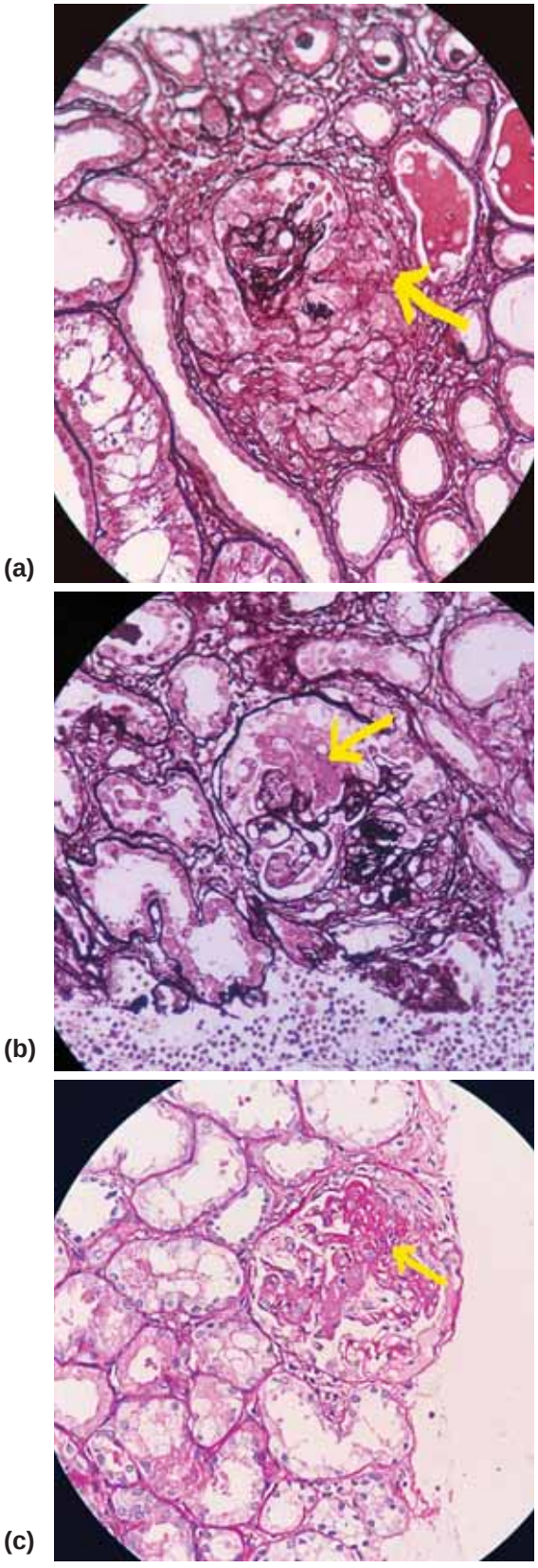

Figure 1. It shows true crescents with a) capsular rupture (Jones staining × 400); b) fibrinoid necrosis (Jones staining × 400); and c) karyorrhexis (PAS staining × 400).

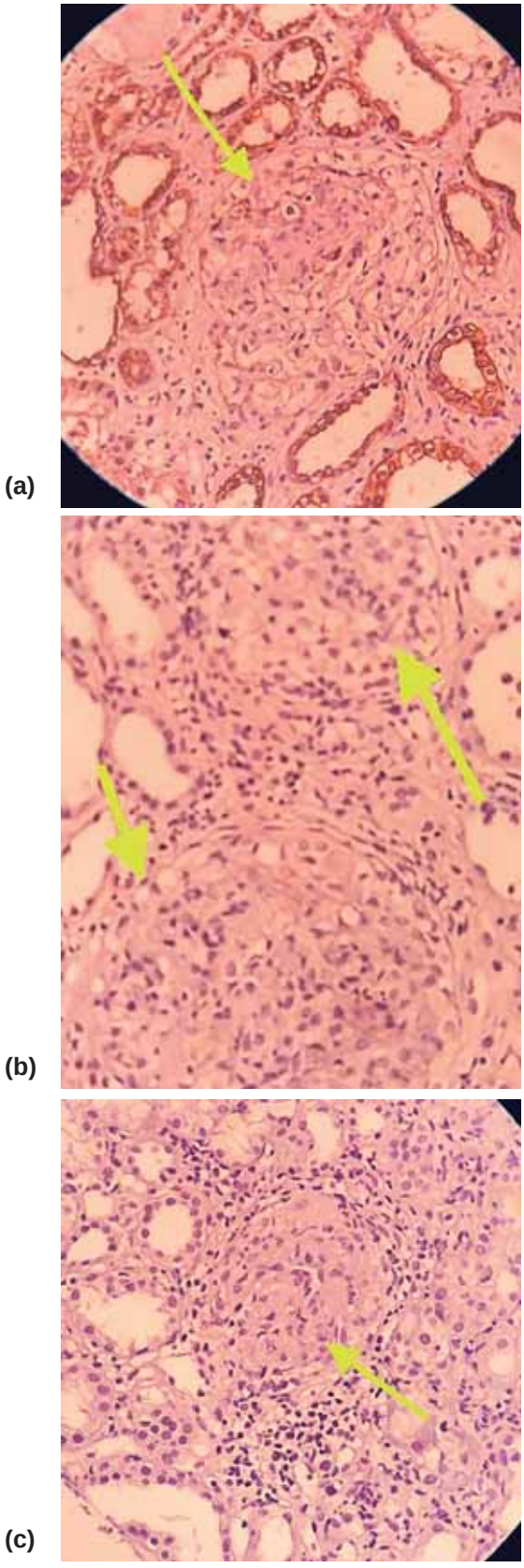

Figure 2. It demonstrates true crescents; IHC staining, a) Cytokeratin (× 400); b) CD68 (× 400); c) Ki67 (× 400).

It should be mentioned that contrary to previous reports, podocytes are indeed involved in human crescentic GN too and therefore interpretation of IHC study for differentiating crescent from pseudocrescent should be done by cautious.[3] Unfortunately, we don't have access to electron microscopy at our center. Immunofluorescence study was negative in the case, but didn't prepare photos and as you know IF staining is not stable for long time and now, we can't send you the IF photos.

3- It was noted that renal biopsy was given on completion of induction treatment of rapidly progressive glomerulonephritis (RPGN), but in our case; renal biopsy was given before intravenous immunoglobulin or cyclophosphamide administration.

4- It was correctly mentioned that hemoglobin should have been reported in g/dL. It was a mistake. It also noted that C-reactive protein (CRP) is given in qualitative form, which is not a correct presentation of this result. We should mention that In this case CRP is reported as qualitative result in that situation.

5- We tested antineutrophil cytoplasmic antibodies (ANCA) with ELISA and the titration was 50.

6- It is notable that the creatinine level of our case was stable around 5.5 mg/dL during hospitalization. There was no significant change in creatinine to report in our article.

7- In this case, the importance of receiving a strong immunosuppressive drug despite COVID-19 was considered. Despite receiving immunosuppressive agents, the patient did not progress respiratory failure caused by COVID-19. Therefore, this condition considered as health during COVID-19 infection. His renal disease condition will be determined over time.

In the last several weeks, there have been numerous concerns not just from patients but also from other nephrologists on the most effective way to treat immunosuppression in today's environment. Will patients with GN could their doses of immunosuppression or avoid the treatment altogether?[8] When evaluating the effect of immunosuppression on COVID-19 outcomes, nephrologists must take into consideration the possible influence of avoiding immunosuppression on the kidney outcomes at the same time. It still recommended that patients who are at high risk of progression to kidney disease without prompt treatment, initiate regular immunosuppression regimens.[8,9] There is evidence that cyclophosphamide-based regimens is an important immunosuppressive drug for induction therapy in these patients.

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Erratum

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Erratum information (IJKD_V14_No3):
On page 184, the name of Elahe Sanei should be added as 5th author with following affiliation "Mashhad University of Medical Sciences, Mashhad, Iran".

On Page 215, Table 2; Alpha Koloto must be changed to Alpha Klotho.
On Page 215, Figure explanation must be read as " It shows comparison of alpha klotho levels in exercise and control patients (alpha 1: before, alpha 2: after study, darman: exercise)".