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REVIEW

Role of gastroesophageal reflux disease in lung transplantation

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Abstract

Lung transplantation is one of the highest risk solid organ transplant modalities. Recent studies have demonstrated a relationship between gastroesophageal reflux disease (GERD) and lung transplant outcomes, including acute and chronic rejection. The aim of this review is to discuss the pathophysiology, evaluation, and management of GERD in lung transplantation, as informed by the most recent publications in the field. The pathophysiology of reflux-induced lung injury includes the effects of aspiration and local immunomodulation in the development of pulmonary decline and histologic rejection, as reflective of allograft injury. Modalities of reflux and esophageal assessment, including ambulatory pH testing, impedance, and esophageal manometry, are discussed, as well as timing of these evaluations relative to transplantation. Finally, antireflux treatments are reviewed, including medical acid suppression and surgical fundoplication, as well as the safety, efficacy, and timing of such treatments relative to transplantation. Our review of the data supports an association between GERD and allograft injury, encouraging a strategy of early diagnosis and aggressive reflux management in lung transplant recipients to improve transplant outcomes. Further studies are needed to explore additional objective measures of reflux and aspiration, better compare medical and surgical antireflux treatment options, extend follow-up times to capture longer-term clinical outcomes, and investigate newer interventions including minimally invasive surgery and advanced endoscopic techniques.

Key words: Lung transplant; Reflux; Aspiration; Rejection; Bronchiolitis obliterans syndrome; Fundoplication

Core tip: Gastroesophageal reflux disease (GERD) has been associated with increased morbidity in lung transplant patients through a proposed pathway of reflux, aspiration, immunomodulation, and allograft injury, culminating in functional decline and rejection. This paper reviews the mechanisms of GERD-induced

injury, describes outcome measures important in post-transplant assessment, and discusses the timing and modalities of diagnostic evaluation and management, including medical and surgical antireflux treatment, in optimizing post-transplant outcomes. A greater awareness of the harmful effects of GERD in the lung transplant population is important in the early diagnosis and management of such patients to minimize allograft injury and improve outcomes.

Hathorn KE, Chan WW, Lo WK. Role of gastroesophageal reflux disease in lung transplantation. *World J Transplant* 2017; 7(2): 103-116 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i2/103.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i2.103

INTRODUCTION

Lung transplantation has proven to be an effective therapeutic option for the treatment of different end-stage pulmonary disorders, improving the quality of life and extending survival[1] for the recipients. Since the first human lung transplant in 1963[2], we have seen improvements in surgical technique, lung preservation, immunosuppression, and the treatment of ischemic reperfusion injury and infection. However, it remains one of the highest risk solid-organ transplant modalities, with 5-year survival rates of 53%[3], compared to 75% for heart transplantation[4], and 71% for liver transplantation[5].

Over time, transplanted lungs may become susceptible to injury manifesting as acute or chronic rejection, diagnosed clinically and histologically using established guidelines of the International Society of Heart and Lung Transplantation (ISHLT)[6]. Acute rejection is an early manifestation of allograft injury occurring usually within the first year after transplantation, impacting up to 55% of patients[7,8], and includes acute cellular rejection (grade A rejection), and lymphocytic bronchiolitis (grade B rejection). Both are independently associated with later development of chronic rejection[7-9].

Chronic rejection traditionally encompassed the spectrum of bronchiolitis obliterans (BO) and bronchiolitis obliterans syndrome (BOS). Bronchiolitis obliterans is a type of progressive airway obstruction occurring as a result of macrophage and myofibroblast infiltration, which induces fibrous obliteration and scar formation[10-12]. The diagnosis is made histologically, requiring surgical biopsy which can be invasive, and may present additional challenges given the patchy involvement of disease[10,13]. Therefore, the clinical correlate of BOS is often applied. BOS was originally defined as a persistent drop in forced expiratory volume in 1 s (FEV1) by 20% in the absence of other identifiable causes[14]. However, given the significance of BOS in predicting poor long-term outcomes, the criteria were adjusted to include an early BOS stage (BOS 0-p) in which an FEV1 of 81%-90% and/or a drop in mid-expiratory flow rate (FEF 25-75) may alert physicians to a need for closer functional monitoring and in-depth assessment[15]. BOS has a variable course, with some patients experiencing rapid decline in lung function, while others develop a slower and more gradual loss of function[16]. Regardless of the speed of progression, BOS remains one of the greatest impediments to long-term survival after lung transplantation, as it ultimately affects up to 80% of transplant recipients by five years[17-19], and most transplant deaths beyond the first year occur directly or indirectly as a result of BOS[7,14].

Recently, a new restrictive form of chronic rejection has been described, termed restrictive allograft syndrome (RAS). RAS manifests as progressive, restrictive physiology with an appearance of increasing fibrosis on imaging studies[20,21], and is defined as a persistent decline in total lung capacity alongside a decline in FEV1[22]. RAS is histologically characterized by diffuse alveolar damage and extensive fibrosis in the alveolar interstitium, visceral pleura, and interlobular septa, and may also contain scattered obliterative bronchiolitis lesions[21-24]. Recent research using immunofluorescence labeling for α-smooth muscle actin has demonstrated massive infiltration of myofibroblasts in the peripheral lung tissue of RAS patients; whereas in BOS, myofibroblasts were observed predominantly in the small airway obliterative bronchiolitis lesions and not in the peripheral lung[21], affording a potential method to differentiate the two types of chronic allograft rejection.

As a consequence of these findings, a new descriptor of the effects of chronic rejection, termed chronic lung allograft dysfunction (CLAD), has been created to cover obstructive, restrictive, and all other manifestations of chronic rejection, including those as yet undetermined, with resulting clinical decline[25]. This review will focus on the chronic rejection syndromes of BO and BOS, which have been studied more extensively in the setting of gastroesophageal reflux disease (GERD).

Immune-mediated lung injury, including cellular and humoral rejection, has been recognized as the leading cause of BOS[7,26-28] and chronic rejection; however, non-immune mechanisms, such as infection, ischemic reperfusion injury, brain death, chronic aspiration, and GERD may also contribute[14,15,19,26,29-32]. GERD, in particular, has been identified as a potential risk factor for both early allograft injury[27], including acute rejection and lymphocytic bronchiolitis, and chronic airway rejection associated with BOS[28,29]. Although no clear causal link has yet been demonstrated, many studies have proposed that GERD is a risk factor in the development of BOS through silent aspiration of stomach contents, leading to direct airway injury and/or upregulation of the inflammatory response in the lung[29,33-38]. Given the significant commonality between GERD and chronic respiratory diseases, the high prevalence of GERD in the lung transplant population[33,39-41], and the more rapid progression to BOS in transplant recipients with objective evidence

of aspiration[34,40,42,43], many groups have begun investigating the impact of diagnosis and treatment of reflux on pulmonary outcomes in this population.

GERD AND LUNG DISEASE: SIGNIFICANCE OF THE PROBLEM

Population-based studies have demonstrated that as many as 11% of Americans experience typical symptoms of reflux daily, and 33% experience symptoms during a 72-h period[44]. It is well known that there may be a higher prevalence of GERD in patients with end-stage lung disease[33,34,45-48]. For example, D' Ovidio *et al*[47] described a 63% (49 of 78 patients) prevalence of gastroesophageal reflux-related symptoms in end-stage lung disease, 38% with documented significant acid reflux on objective testing, which was often asymptomatic[47,49]. Additionally, in patients with idiopathic pulmonary fibrosis (IPF), GERD has been shown to have increased prevalence in comparison to other chronic lung diseases[46,50,51]. Gavini *et al*[52] demonstrated that patients with IPF undergoing pre-lung transplant evaluation have a significantly higher prevalence of abnormal reflux compared to those with COPD, after controlling for potential confounders such as underlying disease severity. Savarino *et al*[53] demonstrated that IPF patients had a higher total reflux episodes and total proximal reflux episodes compared to both non-IPF chronic lung disease patients and healthy volunteers. These findings support the theory that GERD may increase microaspiration episodes, resulting in activation of an inflammatory cascade in lung tissue, which over time, induces fibrotic changes that characterize IPF[42,54,55].

In addition to its higher prevalence in patients with underlying lung disease prior to transplantation, numerous studies have also documented that GERD is increased following transplantation. Young *et al*[56] have shown that the incidence of GERD rose from 35% pre-transplant to 65% post-transplant in their cohort of patients. Similarly, other groups have demonstrated a prevalence of reflux as high as 51-69% in patients after transplant[33,48]. D'Ovidio *et al*[57] have investigated the prevalence of reflux at 3- and 12-mo post-transplant, and found that it increased from 32% to 53%, suggesting that transplantation may itself induce worsened reflux[56,57]. Fisichella *et al*[58] have demonstrated that distal and proximal reflux were more prevalent in patients with bilateral lung transplant or re-transplant, and less prevalent in patients after unilateral transplant, regardless of the cause of their lung disease, suggesting not only the importance of screening for reflux in the post-transplant population, but also the necessity for higher vigilance in patients following double lung transplantation. Various factors have been implicated, including intraoperative vagal nerve damage, loss of cough reflex, impaired mucociliary clearance, and development of gastroparesis as a side effect of calcineurin inhibitors, steroids, mycophenolate mofetil, and other post-transplant immunosuppression treatments[16,39,56,57,59-70].

BACKGROUND AND PATHOPHYSIOLOGY

The association between reflux and rejection post-lung transplant has been investigated in both animal and human studies (Table 1). Stovold *et al*[35] demonstrated that in rats, exposure of the lung allograft to gastric juice leads to high grade acute rejection, which is characterized by monocyte infiltration, fibrosis, and lung destruction. Aspiration has also been shown to increase allograft CD8+ T cells, which are involved in acute rejection[71], and chronic aspiration has been associated with bronchiolitis obliterans[72]. Meltzer *et al*[73] demonstrated similar results in a miniature swine study where chronic aspiration was associated with increased shedding of allograft alloantigens and increased activity of the indirect alloimmune response, which may contribute to fibrosis, obliterative bronchiolitis, and infection.

The central belief is that BOS is a chronic inflammatory and fibrotic process of the small airways, marked by recurrent injury, remodeling, and repair, ultimately resulting in allograft failure typified by obliterative fibrosis[74,75]. Multiple studies supporting this claim have shown that aspiration of gastroduodenal contents is linked to immunomodulation, including increased local levels of IL-1α, IL-1B, IL-6, IL-10, TNF-α, TNF-β[72], increased alveolar neutrophils[37,76,77], increased IL-8[37,76], increased IL-15, IL-17, basic-FGF, TNF-α, and MPO and reduced alpha-1-antitrypsin[42], augmented indirect allorecognition[73], and reduced levels of surfactant proteins SP-A and SP-D[57].

Additionally, numerous studies have investigated the specific role of bile acids and pepsin in the association between reflux and BOS. Bile acids and pepsin, used as markers of aspiration and reflux, have been demonstrated in bronchoalveolar (BAL) fluid of post-lung transplant patients[35,37,57,78,79]. Bile aspiration is cytotoxic, disrupts cellular membranes, and damages type II pneumocytes[80], which are responsible for surfactant protein and phospholipid production and homeostasis[37,57,81,82]. D'Ovidio *et al*[37] investigated 120 post-transplant patients, and found that 20 (17%) had high concentrations of bile acids in BAL. They also noted an association between the presence of bile acids and decreased surfactant proteins and phospholipids, suggesting that aspiration of bile acids may have impaired the innate immunity of the allograft[37]. Importantly, they demonstrated that the highest concentrations of bile acids were found in 70% of patients with early onset (< 1 year post-transplant) and most severe manifestation of BOS, suggesting a temporal and dose-related relationship[37,57]. Blondeau *et al*[78] found that 50% of the lung transplant patients in their study demonstrated elevated levels of bile acids, and 70% of

Table 1 Papers summarizing effects of gastroesophageal reflux disease on transplant outcomes

Ref.	Population	Definition GERD and/or aspiration	Outcomes evaluated	Adjunctive therapy
King *et al*[29], 2009	59 pts. Post-LTx	Abnormal acid and non-acid reflux on esophageal impedance monitoring	Effect of reflux on time to development of BOS *via* hazard ratio	
Hadjiliadis *et al*[33], 2003	43 pts. Post-LTx, survived > 6 mo, and underwent pH and manometry testing	Abnormal acid exposure time on 24-h pH testing	Effect of reflux on FEV1 (*via* Pearson correlation coefficient for time of study, *via* multivariable linear regression to assess overall effect)	PPI d/c'ed > 5 d prior to testing, H2 blockers and pro-motility agents > 1 d prior to testing
Stovold *et al*[35], 2007	36 asymptomatic pts. Post-LTx *vs* 4 healthy volunteers *vs* 17 patients with chronic cough	Increased levels of pepsin in BALF	Presence of pepsin, association between level of pepsin and acute rejection	30 LTx patients on antireflux therapy
Blondeau *et al*[36], 2009	24 pts. Post-LTx	Abnormal reflux on 24-h impedance-pH testing, bile acids in BALF	Relationship between acid exposure, volume exposure, or reflux events and bile acids in BALF	PPI d/c'ed 1 wk prior to testing
D'Ovidio *et al*[37], 2005	120 pts. Post-LTx	Increased levels of bile acids in BALF	Relationship between increased levels of bile acids, IL-8, neutrophils on development of BOS	
Benden *et al*[41], 2005	10 pts. Post-LTx	Abnormal reflux on 24-h pH testing	Prevalence of GERD in population	
Fisichella *et al*[42], 2013	105 pts. Post-LTx with 257 BALF samples	24-h pH testing and DeMeester score calculation, Increased levels of pepsin in BALF	Association between aspiration and patterns of dysregulation of immune mediator concentrations and BOS	PPI d/c'ed 2 wk prior to testing, H2 blocker d/c'ed 3 d prior to testing
Young *et al*[56], 2003	23 pts. evaluated pre- and post-LTx	Total, upright, and supine acid exposure time on 24-h pH testing, esophageal manometry, gastric-emptying study	Paired comparison between pre-transplant and post-transplant results (paired *t* test)	Acid suppression and gastric motility meds discontinued before testing
D'Ovidio *et al*[57], 2006	70 pts. Post-LTx	Esophageal manometry, 24-h pH-testing (DeMeester score calculation, Castell's method) and gastric emptying study; BALF analysis	Actuarial freedom from BOS, impact of aspiration on pulmonary surfactant collectin proteins	PPI d/c'ed 7 d prior, H2-blockers d/c'ed 2 d prior
Fisichella *et al*[58], 2012	61 pts. Post-LTx	Esophageal impedance-manometry, 24-h pH testing (DeMeester score calculation), EGD, barium swallow, gastric emptying study	Relationship between prevalence and extent of GERD and type of transplant (unilateral *vs* bilateral *vs* retransplant)	PPI d/c'ed 14 d prior to pH testing, H2 blockers stopped 3 d prior to pH testing
Fisichella *et al*[74], 2012	8 pts. Post-LARS and LTx in whom BALF had been collected	Esophageal 24-h impedance-pH testing (DeMeester score calculation), gastric emptying study	Comparison of BALF concentrations of leukocytes, immune mediators, and pepsin pre- and post-LARS and post-LTx	PPI d/c'ed 14 d prior to pH testing, H2 blockers stopped 3 d prior to pH testing
Blondeau *et al*[78], 2008	45 pts. Post-LTx off PPI, 18 pts. Post-LTx on PPI	Esophageal 24-h impedance-pH catheter, BALF analysis for pepsin and bile acids	Association between the prevalence and type of reflux and gastric aspiration in pts. with and without BOS	Antacids and promotility agents d/c'ed > 14 d prior to testing *vs* remained on for testing
Griffin *et al*[45], 2013	18 pts. Post-LTx	RSI, esophageal manometry and 24-h impedance-pH monitoring, BALF analysis	Quantification of reflux, aspiration, and allograft injury immediately post-operatively	Testing performed on PPI
Davis *et al*[84], 2013	100 pts Post-LTx with 252 BALF samples	BALF pepsin concentration, esophageal manometry, esophageal 24-h pH catheter (DeMeester score calculation), gastric emptying study	Association between concentration of pepsin in BALF and results of esophageal function testing, barium swallow and gastric emptying to identify risk factors for GERD	PPI d/c'ed 14 d prior to pH testing, H2 blockers d/c'ed 3 d prior to pH testing
Hartwig *et al*[71], 2006	7 models of rat lung transplantation	Weekly injection of gastric contents for 4-8 wk	Degree of pulmonary allograft dysfunction reflective of chronic aspiration	N/A
Li *et al*[72], 2008	9 models of rat lung transplantation	Weekly injection of gastric contents for 8 wk	Association between chronic aspiration and development of OB	N/A
Meltzer *et al*[73], 2008	3 models of swine lung transplantation	Daily injection of gastric contents for 50 d	Effect on chronic aspiration on the direct and indirect pathways of allorecognition	N/A

BALF: Bronchoalveolar lavage fluid; BOS: Bronchiolitis obliterans syndrome; OB: Obliterative bronchiolitis; RSI: Reflux severity index; GERD: Gastroesophageal reflux disease; N/A: Not available.

those with BOS had elevated bile acids, compared to 31% without BOS, indicating that bile acid may be a specific marker for allograft injury.

Pepsin is a proteolytic enzyme, active at acidic pH, which is increasingly reported as a marker of inflammation in asthma, COPD, bronchiectasis, CF, and following cardiothoracic surgery[83]. Numerous studies have documented increased levels of pepsin in BAL of patients following lung-transplantation[35,78,79,84]. In a small study by Ward *et al*[79], pepsin was present in the BAL of all lung allografts, while not detected in the control group. In a later follow-up study of 36 post-transplant patients, 4 normal volunteers, and 1 patient with unexplained chronic cough, it was shown that pepsin levels were significantly higher in the transplant cohort; among these patients, pepsin levels were highest in those with acute rejection, a risk factor for the progression to BOS[85,86]. Stovold *et al*[35] also demonstrated consistently elevated levels of pepsin in the BAL fluid of lung transplant patients, again with the highest levels in association with acute rejection. Davis *et al*[84] have even specifically compared patients with IPF to those with alpha-1-antitrypsin deficiency, cystic fibrosis, or COPD, and have found that patients with IPF had higher pepsin concentrations and greater frequency of acute rejection than those with other diseases. Interestingly, despite higher pepsin concentrations and rates of acute rejection, IPF patients did not have a significantly greater incidence of BOS compared with other indications for lung transplantation[84], though the short follow-up time was a significant limitation that likely reduced development of the BOS outcome.

Furthermore, as previously mentioned, both acute cellular rejection[7-9] and lymphocytic bronchiolitis[9] are independently associated with bronchiolitis obliterans. Acute cellular rejection may represent an earlier endpoint in the model of chronic lung injury, supporting the relationship between early allograft injury and eventual development of BOS. Lymphocytic bronchiolitis not only represents an independent risk factor for bronchiolitis obliterans[9], but also has been associated with the occurrence and severity of acute cellular rejection[10]. While no causal relationship between lymphocytic bronchiolitis and BOS has been identified, a prior study has documented the presence of lymphocytic infiltration and esophageal inflammation in association with GERD in the upper gastrointestinal tract, which improves with acid suppression therapy[87]. Therefore, GERD and aspiration may play a role in early development of both lymphocytic bronchiolitis and acute cellular rejection, which in turn, independently predict onset of BOS[7-9].

EVALUATION AND DIAGNOSIS

There is mounting evidence that patients with reflux have a higher risk of poor outcomes post-transplant. For example, King *et al*[29] have demonstrated that increased reflux is associated with BOS, even after controlling for the graft ischemic time, type of surgery, recipient age, underlying pathology, CMV mismatch, or HLA mismatches, concluding that reflux is a prevalent and modifiable risk factor[29]. Hadjiliadis *et al*[33] have even demonstrated a negative correlation between measurements of FEV1 and pH test results in a post-transplant population. These and other studies highlight the importance of identifying patients at risk for allograft injury relating to GERD. Typical GI symptoms, such as heartburn and regurgitation symptoms, have not been predictive of respiratory symptoms attributed to GERD, and are an unreliable correlate between reflux and airway disease[16,29,47,49-51,88-92]. Sweet *et al*[49] have demonstrated that in patients with IPF, 67% had pathologic reflux, which frequently extended into the proximal esophagus, and that heartburn symptoms were unreliable means of patient detection, demonstrating sensitivity of 65% and specificity of 71%. This again emphasizes the importance of screening transplant candidates for GERD to identifying those at increased risk of poor outcomes.

In the past, gastric transit studies[62], esophagoscopy[93], and radiologic swallow studies[93] were used as tenuous proxies for reflux. Recently, a variety of more sophisticated techniques have been utilized to characterize reflux in the lung transplant population, including 24-h ambulatory pH monitoring, multichannel intraluminal impedance and pH (MII-pH) testing, and bronchoscopy with BAL evaluation. Collection of exhaled breath condensate for pH and other chemical assays has been used with limited accuracy and poor availability, and is primarily a research tool[87-89]. While ambulatory pH testing is the most universally advocated, the optimal testing modality remains undefined.

Ambulatory pH testing has the longest history of use in the assessment of transplant patients. Hadjiliadis *et al*[33] used 24-h pH monitoring to demonstrate that 69.8% of patients in their post-transplant group had abnormal total acid exposure times, and that there was an inverse correlation between total or upright acid reflux and FEV1 at the time of the ambulatory pH study. Similarly, Young *et al*[56] have also used pH monitoring to demonstrate that 65% of their patients had abnormal acid exposure times post-transplant. However, ambulatory pH monitoring has had variable sensitivity for reflux detection in this population, ranging from 50%-80%[41,84,90]. One possible reason for this limitation may be that the test underestimates the amount and frequency of reflux, as it is not capable of detecting nonacidic or bolus reflux. Other modalities for evaluation of acid reflux, such as BRAVO capsule-based pH monitoring (Given Imaging, Yoqneam, Israel)[94] have not been assessed in the transplant population, but may offer few benefits over catheter-based testing as it requires endoscopic evaluation prior to placement.

To better assess potential contributions from nonacid and bolus reflux, impedance testing was developed to sensitively detect the presence of liquid bolus, its direction of movement, and the proximal extent of reflux, independent of pH[29,95,96]. Through this minimally

invasive outpatient procedure, patients at risk of reflux and aspiration can be identified[29]. In one study, impedance detected 96% of reflux events compared with 28% detected by ambulatory pH study alone[97], highlighting that a significant portion of reflux events may be nonacidic or weakly acidic events not detectable by pH testing, but still potentially contributing to the pathophysiology of post-transplant reflux-induced allograft injury. Similarly, our group has demonstrated that impedance data, specifically the additional information regarding nonacid reflux, offers statistically significant advantages over their corresponding pH-only parameters in predicting lung transplant outcomes[98]. It is our general belief that impedance is being under-utilized, and our data suggests a role for more routine use of impedance as a standard part of pre-transplant evaluation[98].

Although not specifically for reflux assessment, use of high resolution esophageal manometry (HREM) is also growing in the transplant population. Practically, HREM may help identify the lower esophageal sphincter to guide proper placement of the pH catheter. Additionally, esophageal motility disorders may present primarily with GERD symptoms and can impact GERD severity, including connective tissue diseases, so HREM may be helpful in the diagnosis of secondary reflux. Esophageal dysmotility may also impact candidacy for surgical antireflux treatment. Further studies are required to assess the relationship between HREM measures of esophageal function and pulmonary outcomes.

Oelschlager *et al*[89] have demonstrated that in 518 patients, the combination of symptoms, esophageal manometry, and ambulatory pH monitoring was insufficient to accurately identify reflux as the cause of aspiration. While this included only standard ambulatory pH monitoring rather than MII-pH, it raises the possibility that additional tests may be required to more directly assess reflux severity. Some groups have proposed that BAL fluid analysis may contribute additional information in the evaluation of these patients. For example, BAL may be used to quantify pepsin and bile acids as markers of aspiration, which have been associated with progression to BOS[75,79,99-101]. However, bronchoscopy sampling is relatively expensive, more invasive than other techniques, and time consuming[29]. Additionally, because only a single sample is taken at a moment in time[29,39], without standardization of results or a full understanding of temporal changes in bile acid or pepsin concentrations, this test may be exquisitely sensitive to provider technique[39]. In short, clinical feasibility remains a challenge.

In addition to poor consensus on the optimal mode of reflux testing among lung transplant candidates[98], there is no standard for timing of testing. Our group favors routine pre-transplant impedance testing, as we have previously shown that prolonged bolus clearance, increased total distal reflux episodes, and increased total proximal reflux episodes on pre-transplant MII-pH were associated with decreased time to early allograft injury after lung transplantation[102]. Researchers from Duke University have suggested the following approach based on available data, and previous experience at their center: Prior to transplant, all patients undergo esophageal manometry, 24-h ambulatory pH or MII-pH study (off anti-secretory therapy), and upper GI series[13]. However, not all groups have adopted this pre-transplant assessment approach, especially given the tenuous pulmonary status of some transplant candidates. It does seem, however, that if evaluation were to be performed post-transplant, the importance of early assessment should not be ignored. As mentioned previously in this review, there are several processes during and after transplant surgery that may result in worsening of reflux, and thus, it is imperative to screen for reflux in the early post-transplant period if not before. Griffin *et al*[45] recommended that all patients should be routinely assessed within 1 mo post-transplant given the high prevalence of reflux and aspiration in the immediate post-transplant period, despite use of proton-pump inhibitor (PPI). Additionally, as our group has demonstrated the benefits of timely antireflux surgery in improving transplant outcomes[103], earlier reflux assessment may be essential to guide management.

TREATMENT

Medical treatment of reflux consists of the conventional pharmacologic methods of histamine-2 receptor blockers and PPIs, and prokinetic agents to enhance esophageal and gastric clearance. These agents may ameliorate symptoms, diminish the acid component of gastric refluxate, and promote bolus clearance. Additionally, recent publications have suggested that antireflux therapies may prolong survival and decrease the incidence of acute disease exacerbation in patients with IPF (Table 2)[53,104-109]. Blondeau *et al*[78] demonstrated that PPI use did reduce acid exposure in lung transplant patients, but had minimal effect on pepsin as a surrogate marker of aspiration. Unfortunately, additional literature on the effects of medical acid suppression in the lung transplant population is sparse. Azithromycin has been used as a therapy for BOS with some success, possibly relating to its mild pro-kinetic effects, although the full mechanism of action is not clearly defined[32,110,111]. Mertens *et al*[112] used impedance and BAL testing to evaluate the effect of azithromycin on reflux and gastric aspiration parameters, and found that patients on azithromycin had significantly less reflux, including decreased number of reflux events, fewer proximal reflux episodes, and decreased esophageal acid exposure. In addition, bile acid levels in the BAL were significantly reduced after azithromycin treatment[112]. However, given the unclear mode of action and concern for antibiotic overuse, routine application of azithromycin has not been recommended.

While the aforementioned pharmacologic therapies may ameliorate symptoms, diminish the acid

Table 2 Papers on the effect of pharmacologic reflux treatment on transplant outcome

Ref.	*n*	Population	Treatment type	Adjunctive treatments	Outcomes assessed
Yates *et al*[32], 2005	20	Post-LTx with diagnosis of BOS (*n* = 18) or potential BOS (*n* = 2)	AZI 250 mg QOD from time of BOS diagnosis to time of manuscript writing (mean 6.25 mo)	Immunosuppressive regimen, no additional antireflux agents specified	Effect on FEV1
Verleden *et al*[110], 2004	8	Post-LTx with significant decrease in their FEV1 attributed to BOS	AZI 250 mg qd × 5 d then 250 mg po QOD	Immunosuppressive regimen, no additional antireflux agents specified	Effect on FEV1
Verleden *et al*[111], 2006	14	Post-LTx with BOS	AZI 250 mg po qd × 5 d then AZI 250 mg po 3 × /wk × 3 mo	Immunosuppressive regimen, no additional antireflux agents specified	Reduction in airway neutrophilia and IL-8 mRNA, effect on FEV1
Mertens *et al*[112], 2009	12	Post-LTx on AZI with pH monitoring	AZI 250 mg PO 3 ×/wk	Immunosuppressive regimen, held antireflux treatments × 1 wk prior to testing	Effect on impedance-pH monitoring, gastric aspiration *via* BAL analysis
Blondeau *et al*[78], 2008	18	Post-LTx on PPI *vs* off PPI at time of testing (secondary cohort)	Omeprazole 20 mg PO BID	Immunosuppressive regimen	Prevalence of reflux on objective testing, effect on aspiration in BAL

n: Patients in the study in the treatment arm; BOS: Bronchiolitis obliterans syndrome; LTx: Lung transplant; AZI: Azithromycin; QOD: Every other day; FEV1: Forced expiratory volume in 1 s; BID: Twice a day.

component of gastric refluxate, and improve clearance, the underlying mechanism provoking reflux often persists[29,39,78,113-116]. For example, Patti *et al*[114] demonstrated that while acid-reducing medications alter the pH of the refluxate, clinical symptoms may recur, suggesting persistence of pathology in spite of medical antireflux therapy, and that surgery may provide more definitive treatment of reflux and aspiration regardless of pH. Blondeau *et al*[78] demonstrated that 71% of lung transplant recipients taking PPIs had increased non-acid reflux, and that PPI use did not reduce the number of reflux events, non-acid reflux exposure, proximal reflux extent, or markers of aspiration on BAL.

Consequently, many groups are now turning to antireflux surgery as a more definitive approach to reflux management and for prevention of further complications. Previous studies have shown that antireflux surgery is a safe procedure in this patient population[34,40,75,117-122], and is associated with improved survival and stabilization of lung function (Table 3)[29,33,34,40,43,75,117,118,123-125]. For example, Robertson *et al*[75] demonstrated that post-lung transplant antireflux surgery resulted in no deaths or serious post-operative complications in all 16 patients undergoing surgery, although one patient required minor surgical revision for dysphagia. Fisichella *et al*[119] similarly demonstrated that post-lung transplant patients had perioperative morbidity and mortality rates similar to those of transplant-free controls undergoing laparoscopic antireflux surgery. However, these and other studies have been limited by single-center experiences and small patient numbers. Subsequently, Kilic *et al*[17] performed a study using the all-payer database in the United States to evaluate nationwide outcomes of antireflux surgery in transplant recipients *vs* transplant-free controls, confirming similar outcomes in both groups. The post-lung transplant group did not demonstrate an increased risk of respiratory complications, although they did have a longer median hospital stay, higher resource utilization, and higher median cost of inpatient care[17]. In congruence with these results, O'Halloran *et al*[121] demonstrated that while lung transplant patients in their study also required longer hospital stay and had higher rates of readmission compared to controls, no differences were detected with regard to operative time, estimated blood loss, or peri-operative complications. Furthermore, no intra- or peri-operative deaths were seen, and both transplant and control groups reported symptom resolution following surgery.

Additional studies have focused on the efficacy of antireflux surgical management with regard to transplant outcomes such as pulmonary function and allograft rejection. Halsey *et al*[124] published a case report on a post-transplant patient with progressive allograft dysfunction, associated with a significant decline in FEV1 and FVC, despite twice-daily use of PPI. Their patient underwent impedance testing, which demonstrated ongoing non-acid reflux, and proceeded to laparoscopic Nissen fundoplication. Post-operatively, the patient improved symptomatically and spirometry results returned to baseline[124]. Hoppo *et al*[16] demonstrated that antireflux surgery either improved or prolonged native lung or allograft function during the pre- or post-lung transplant period, respectively. One year after antireflux surgery, significant improvement in FEV1 was detected in 91% of the post-lung transplant patients ($P < 0.01$) and 85% of the pre-lung transplant patients ($P = 0.02$)[16]. Additionally, all patients in this study were using anti-secretory medications, which lends further credence to the observation that acid suppression alone may not be sufficient to prevent reflux in every case[16]. Hartwig *et al*[126] have similarly demonstrated that early fundoplication was associated with preservation of lung function, and Lau *et al*[118] reported that 67% of lung transplant recipients actually had improvement in

Table 3 Papers of surgical antireflux procedures and lung transplant outcomes

Ref.	*n*	Population undergoing surgery	Type of surgical intervention (Type Nissen: *n*)	Outcomes assessed
Davis *et al*[32], 2003	43	Post-LTx with abnormal pH study (*n* = 39), severe reflux with normal manometry (*n* = 2), repetitive aspiration events leading to retransplant (*n* = 1) or pneumonia (*n* = 1)	Laparoscopic: 36 Open: 3 Partial Toupet: 4	In-hospital or 30-d mortality, FEV1 pre- and post-procedure
Cantu *et al*[40], 2004	74	Post-LTx with abnormal pH studies	Laparoscopic: 71 Open: 5 Partial Toupet: 4 Other: 5[1]	In-hospital or 30 d mortality, freedom from BOS in early *vs* late fundoplication groups
Robertson *et al*[75], 2012	16	Post-LTx undergoing antireflux surgery	Laparoscopic: 16	Effect on quality of life, peri-operative mortality and complications, reduction in deterioration of lung function
Linden *et al*[117], 2006	19	Pre-LTx IPF with h/o reflux, symptoms, and severe reflux on pH and manometry testing	Laparoscopic: 19	Peri-operative complications, post-operative lung function
Lau *et al*[118], 2002	18	Post-LTx with documented GERD	Laparoscopic: 13 Open: 1 Partial Toupet: 4	Length of hospital stay, post-operative lung function, morbidity and mortality
Fisichella *et al*[119], 2011	29	Post-LTx with GERD dx on symptoms, BAL, or decreased lung function; with abnormal pH monitoring	Laparoscopic: 27	30-d morbidity and mortality, hospital readmissions
Fisichella *et al*[43], 2011	19	Post-LTx with GERD symptoms, aspiration on BAL, or unexplained decrease in lung function	Partial Toupet: 2 Laparoscopic: 19	decreased aspiration as defined by the presence of pepsin in the BALF
Fisichella *et al*[74], 2012	8	Post-LTx patients with GERD and evidence of reflux on ambulatory pH monitoring	Laparoscopic: 8	Quantification and comparison of pulm leukocyte differential and concentration of inflammatory mediators in BAL, freedom from BOS, effect on FEV1, and survival
Burton *et al*[120], 2009	21	Post-LTx with reflux confirmed on EGD, pH testing, or BALF	Laparoscopic: 5 Partial Toupet: 16	Patient satisfaction, symptom changes and side effects, effect on lung function, BMI, rate progression to BOS
O'Halloran *et al*[121], 2004	28	Post-LTx with reflux on pH testing and manometry	Laparoscopic: 28	Perioperative complications, length of stay, readmission rate, effect on lung function
Gasper *et al*[122], 2008	35	Pre-LTx in 15 patients, Post-LTx in 20 patients with GERD or delayed gastric emptying study	Laparoscopic: 27 Partial Toupet: 5 Other: 3[2]	Length of stay, perioperative complications pre- or post-LTx
Kilic *et al*[17], 2013	401	Post-LTx who pursued elective antireflux procedure	Laparoscopic: 338[3] Open: 23	Inpatient mortality, length of stay, perioperative complications, hospital costs
Hoppo *et al*[16], 2011	43	Pre-LTx in 19 patients, Post-LTx in 24 patients with documented symptoms or signs of GERD on EGD, barium, manometry, pH or impedance testing; or declining lung function	Laparoscopic: 24 Other: 17[4]	Effect on lung function, number cases of pneumonia and acute rejection episodes
Hartwig *et al*[126], 2011	157	Post-LTx with abnormal acid contact times before or early after transplantation	Laparoscopic: 157[3]	Effect on lung function
Lo *et al*[103], 2016	48	Pre-LTx or Post-LTx patients with persistent symptoms on maximal PPI and with objective evidence of reflux on pH testing	Laparoscopic = 48	Time to early allograft injury in pre-LTx *vs* early *vs* late post-LTx groups
Patti *et al*[114], 2000	39	Pt with GERD and respiratory symptoms on H2 agents *vs* PPI *vs* pro-kinetic agents, ± bronchodilators (*n* = 3) and bronchodilators/prednisone (*n* = 4)	Laparoscopic = 39	Outcome of surgery on GERD-induced respiratory symptoms

[1]Three cases Belsey-Mark IVs, 1 Toupet and 1 Nissen at OSH (without further information); [2]Two cases had pyloroplasty without fundoplication, 1 case had hypotension at induction and was discharged without operation; [3]Does not specify full Nissen *vs* partial toupet, only laparoscopic *vs* open approach; [4]Seventeen cases underwent laparoscopic Dor procedure. *n*: Study patients in the fundoplication group specifically; LTx: Lung transplant; BALF: Bronchoalveolar lavage fluid; BOS: Bronchiolitis obliterans syndrome; GERD: Gastroesophageal reflux disease; BMI: Body mass index; EGD: esophagogastroduodenoscopy.

their pulmonary function following antireflux surgery. Interestingly, Fisichella *et al*[119] investigated changes in BAL fluid analysis four weeks after antireflux surgery, and showed that in 8 lung transplant recipients, the percentages of neutrophils and lymphocytes in the BAL fluid were reduced, the concentration of myeloperoxide

and IL-1b tended to decrease, and the percentage of macrophages was increased. While this was a limited study given its small sample size, the findings suggest that antireflux surgery may restore the physiologic balance of pulmonary leukocyte populations with ensuing reduction in pro-inflammatory mediators[119]. Additionally, this same group detected decreased pepsin levels in transplant recipients with reflux that underwent antireflux surgery, compared to those that did not receive surgery. Both groups had higher pepsin levels compared against controls, whose levels were undetectable[43]. Notably, subjects with increased pepsin levels were noted to have more acute rejection episodes and faster progression to BOS[43], further underscoring the relevance and necessity of reflux and aspiration management in this patient population.

One important consideration surrounding antireflux surgery in this population is the appropriate timing of the procedure, not just before or after transplant, but also how soon after transplant would be of greatest benefit. Several groups argue that antireflux surgery should be considered in the pre-transplant period[50,117,122]. Linden *et al*[117] focused specifically on IPF patients, and demonstrated no perioperative complications or decrease in lung function over the 15-mo average follow-up. Importantly, patients treated with antireflux surgery had stable oxygen requirements, while control patients with IPF on the waiting list had a statistically significant deterioration[117]. Thus, in spite of theoretical risks in the setting of pre-transplant pulmonary compromise, the absence of serious complications in clinical practice led to the conclusions that pre-transplant antireflux surgery is safe, may ameliorate the progression of underlying disease while awaiting transplant, and provide early protection from reflux and aspiration upon transplantation[117]. Other groups similarly note that pre-transplant surgery may be performed safely, but acknowledge the high-risk nature of these patients given their limited pulmonary reserve. To accommodate these risks, the decision to operate should be made individually, based on objective measures of pulmonary function[16], and under the guidance of an experienced surgical team[122].

In patients that are unable to tolerate pre-transplant antireflux surgery, the timing of surgery post-transplant may be of great importance. Cantu *et al*[40] demonstrated that early fundoplication within 90 d of transplantation resulted in greater freedom from BOS and improved survival compared to later fundoplication, with post-transplant reflux incidence of 76%. Importantly, both BOS and survival were improved in the early post-transplant antireflux surgery group, compared to those with later surgery as well as those with reflux but without surgical intervention. Our group has similarly demonstrated the importance of early intervention. In a retrospective cohort study of 48 patients, we detected a significant increase in early allograft injury in late post-transplant antireflux surgery patients (mean time from transplant 1.8 years) compared to pre-transplant (mean time 3.5 years prior to transplant) and early post-transplant (mean time from transplant 118 d) antireflux surgical groups[103]. The surgeries were well tolerated in the pre- and early post-transplant groups. One death was reported in the late post-transplant group in a patient that had already developed BOS. The trend in this study supports the pathophysiologic model in which antireflux surgery reduces microaspiration events, as suggested by prior studies[16,34,74], and it is our speculation that the earlier antireflux surgery is performed, the greater the protection against reflux and aspiration events, which lowers the risk of pulmonary decline[103]. Interestingly, our study also highlights the lack of additional benefit to providing antireflux surgery pre-transplant compared to within 6 mo post-transplantation. Given the potentially elevated risks of pre-transplant surgery in this population, it may be reasonable to wait for the early post-transplant period to reduce peri-operative risks. Finally, although antireflux surgery performed concurrently with lung transplantation has been reported anecdotally, it has not been extensively studied and is not available at our institution. Over time, with the development of new and less invasive antireflux technologies such as the LYNX magnetic reflux management system (Torax, Shoreview, MN, USA), concurrent surgical antireflux management alongside transplantation may come under greater consideration.

CONCLUSION

This review has highlighted an abundance of research regarding the role of reflux in the pathophysiology of allograft injury following lung transplantation, along with options for diagnosis and management. Nevertheless, unanswered questions remain, and additional studies are needed to clarify the optimal modality and timing for reflux evaluation and management in these patients. As King *et al*[29] have previously discussed, there remains frustratingly no clear causal relationship between reflux and the development of BOS. Additionally, the absence of a gold standard to diagnose GERD, and the difficulties of defining and describing reflux severity continue to limit accuracy in patient stratification, given potential contributions from acid reflux, non-acid or bolus reflux, and aspiration[29]. Future studies should explore different objective measurements of reflux and aspiration parameters, better compare medical and surgical antireflux treatment options, extend follow-up times to capture longer-term clinical outcomes such as RAS or CLAD, and investigate newer antireflux interventions including minimally invasive surgery and advanced endoscopic techniques. However, it is clear that a definite association exists between reflux and lung disease, which represents a tangible and significant target to improve outcomes in the lung transplant population.

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REVIEW

Intra-islet endothelial cell and β-cell crosstalk: Implication for islet cell transplantation

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Abstract

The intra-islet microvasculature is a critical interface between the blood and islet endocrine cells governing a number of cellular and pathophysiological processes associated with the pancreatic tissue. A growing body of evidence indicates a strong functional and physical interdependency of β-cells with endothelial cells (ECs), the building blocks of islet microvasculature. Intra-islet ECs, actively regulate vascular permeability and appear to play a role in fine-tuning blood glucose sensing and regulation. These cells also tend to behave as "guardians", controlling the expression and movement of a number of important immune mediators, thereby strongly contributing to the physiology of islets. This review will focus on the molecular signalling and crosstalk between the intra-islet ECs and β-cells and how their relationship can be a potential target for intervention strategies in islet pathology and islet transplantation.

Key words: Islets; Endothelial cells; Islet cell transplantation; Beta-cells; Microvasculature; Paracrine signalling

Core tip: This review article summarizes recent developments in the cross-talk relationship between intra-islet endothelial cells and beta cells. The molecules involved in the signalling pathways can be potential targets for therapeutic strategies and islet transplantation.

Narayanan S, Loganathan G, Dhanasekaran M, Tucker W, Patel A, Subhashree V, Mokshagundam S, Hughes MG, Williams SK, Balamurugan AN. Intra-islet endothelial cell and β-cell crosstalk: Implication for islet cell transplantation. *World J Transplant* 2017; 7(2): 117-128 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i2/117.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i2.117

INTRODUCTION

Pancreatic islets represent endocrine "island" cell clusters, embedded and scattered throughout the pancreas within large amounts of exocrine acinar tissue[1]. Islets are perfused by a dense, specialized microcirculation and receive 10% of the pancreatic blood flow despite comprising only 1%-2% of the overall tissue mass[2]. Most islets are irregularly shaped spheroids with a size distribution ranging from 50-500 μm, each composed of 800-3000 individual cells. The islet microcirculation is characterized by pre islet arterioles that rapidly arcade to a dense population of capillaries[3].

The cellular components of the islet include β-cells, other endocrine cells, as well as endothelium, perivascular, and support cells such as pericytes[4-9]. The cellular composition of islets is not uniform across species. Rodent and rabbit islets are primarily composed of a β-cell core with other cell types in the periphery whereas human and primate islets exhibit endocrine cell types intermingled with each other[4,10,11]. Beta cells, the central regulator of glucose homeostasis, are the largest cellular component of islets in most species[9,10].

Studies using vascular corrosion casts have demonstrated that 1-3 arterioles feed larger islets[12]. The capillary network within islets is about five times denser in comparison with exocrine tissue[3]. The capillary wall is composed of a permeable layer of ECs and contains ten times more fenestrae than ECs present in the exocrine pancreas[13,14]. The islet endothelial fenestra are highly specialized and contain a diaphragm that regulates solute transport[15,16]. Typically, a microvessel consists of ECs arranged into a tube formation wrapped by one or more layers of perivascular cells. Vascular ECs represent a major cell type present in islets and these cells are organized into a highly regulated and morphologically unique microcirculation. In culture, islet ECs express the classic endothelial markers such as von Willebrand factor, CD31, CD105, CD146, uptake of acetylated LDL, expression of leucocyte adhesion molecules, contain Weibel-Palade bodies in the cytoplasm, and form tight junctions[17,18]. Other markers expressed within islet ECs include α-1 antitrypsin, a major proteinase inhibitor[17,19,20]; nephrin, a highly specific barrier protein[16]; platelet-activating factor receptor[21], and genes expressing angiogenic (vascular endothelial growth factor, VEGF) and angiostatic (endostatin, pigment epithelial-derived factor) molecules[22].

Islet ECs have a significant relationship with islet function. For example, islets grafts, when co-transplanted[23] with ECs in diabetes induced rats or coated[24] with ECs in diabetes induced mice, have better engraftment capacity and improved islet function. Donor islet ECs, immediately after transplantation, participate in neovascularization by increasing β-cell survival[25] and promote both pancreatic stem cell proliferation and islet regeneration after β-cell injury[26]. Research performed over the last two decades has evaluated the link between islets and the ECs, demonstrating how the molecular interplay between these two cell types can regulate many critical physiological processes associated with the islet.

THE SIGNALS FROM β-CELL TO ECS

In vitro studies demonstrate that conditioned medium derived from cultured rat islets induces liver and islet-derived EC proliferation and migration[27], suggesting presence and secretion of paracrine pro-angiogenic factors (Figure 1) which promote islet vascularization[28]. As a major soluble β-cell secreted product, insulin promotes β-cell survival. In addition, insulin causes the upregulation of endothelial nitric oxide synthase in ECs promoting intra-islet blood flow[29]. Post-natal beta mass is dynamic and can increase in function and mass to compensate for additional physiological requirements[30].

VEGFs

The family of VEGF ligands and their receptors are critical as they regulate a number of developmental processes and play major roles in wound healing and vessel homeostasis in adult organisms[31,32]. VEGF secretion is stimulated by tumor, hypoxia, low pH and many other factors. Beta-cells secrete large amounts of VEGF-A early in development and throughout adult life[33]. The VEGF binds to its receptor (VEGFR) located on the blood vessel ECs, which activates multiple signalling cascades eventually resulting in the production of enzymes and other specific molecules required for EC growth and proliferation. Other activation effects include mobilization of endothelial progenitor cells from bone marrow, increased vascular permeability and tissue factor induction[34]. The VEGF family comprises seven secreted glycoproteins that are designated VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental growth factor and VEGF-F[35-37]. VEGF family members interact with three main receptors, VEGFR-1 (FLt-1), VEGFR-2 (KDR in humans and Flk-1 in mice) and VEGFR-3 (Flt4), all tyrosine kinase receptors and members of the PGDF receptor family. VEGFR-2 appears to be the main receptor responsible for mediating the proangiogenic effects of VEGF-A[35,38,39]. The consequence of this specific ligand-receptor interaction facilitates EC proliferation *via* the PKC-Ras pathway (by inducing MAPK/ERK pathways)[40,41]; promotes cytoskeletal reorganization and cell migration *via* p38 and focal adhesion kinase

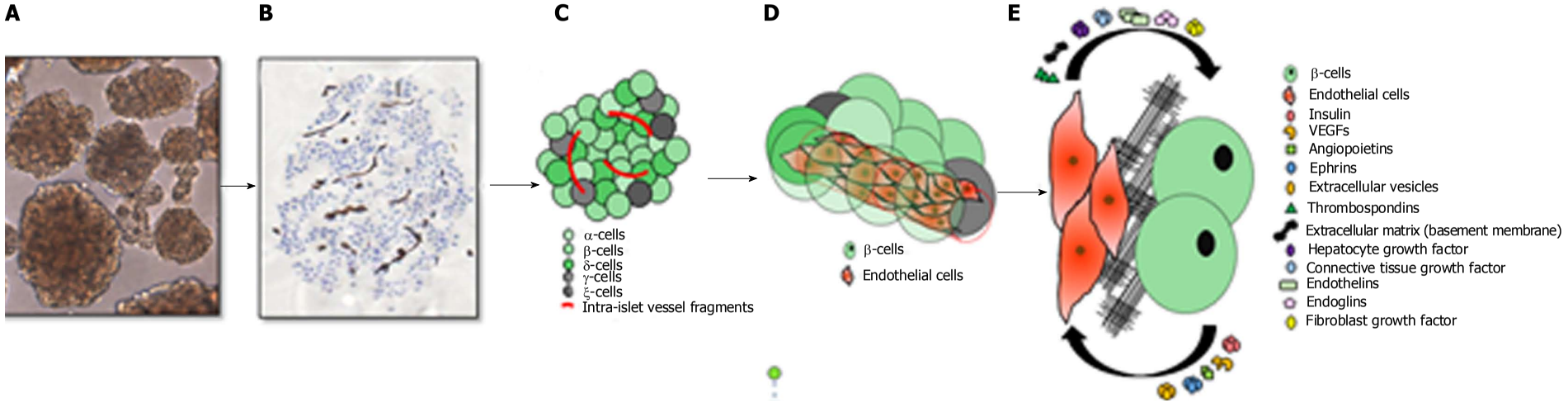

Figure 1 A model demonstrating the intra-islet endothelial cell and β-cell crosstalk. A: An image of freshly isolated human islets; B: Immunohistochemical staining of an islet demonstrating intra-islet vessels stained with CD31 (brown); C: Schematic representation of different cells within an islet along with intra-islet vessel fragments; D: A three dimensional (3D) depiction of islet cells and how these surround the intra-islet vessels, which are a group of endothelial cells arranged into a tube like structure; E: A model demonstrating a cross-talk relationship between endothelial cells and β-cells mediated by various endocrine factors/molecules. VEGFs, angiopoietins, insulin, cell surface molecules including ephrins mainly produced by the β-cell, are important factors for endothelial cell proliferation. Endothelium-derived factors such as hepatocyte growth factor, thrombospondins, basement membrane components (laminins, collagens) improve β-cell survival and promote insulin transcription and secretion. Other EC-derived factors include fibroblast growth factor and the vasoconstrictive endothelin-1. VEGF: Vascular endothelial growth factor; EC: Endothelial cell.

activation[42]; and supports EC cell survival and migration by activating the PI3K/Akt/PKB pathway[43,44].

VEGF-A is known to utilize the VEGFR-2 receptor on ECs[45], with the receptor highly expressed in intra-islet capillaries[46]. VEGF likely stimulates EC growth in neonatal pancreas; increased levels of VEGF-A correspond with islet growth in pregnant rats[47]. VEGF-A signaling is also essential in maintaining vascular beds in adult islets, this was validated using VEGF receptor antagonists[48]. VEGF-A expression is further upregulated in islets by hypoxia and glucose[49,50] and is important for the establishment of native intra-islet vasculature[51], maintenance of β-cell mass[52], and the revascularization of islets following transplantation[53].

Angiopoietins

Apart from VEGF-A, other known factors such as those within Ang/Tie family are known to contribute towards the survival and integrity of blood vessels[33,54,55]. These angiogenic factors consist of ligands Ang-1, Ang-2 and Ang-4 (its mouse orthologue, Ang-3) and the tyrosine kinase receptors Tie-1 and Tie-2. Ang-1 is expressed mainly by the perivascular cells and β-cells in mouse and human islets[33], and its agonist Tie-2 is expressed by the ECs. Ang-1 activates the pI3k/Akt pathway and prevents cytokine mediated apoptosis in ECs[56]. Moreover, β-cell specific overexpression of Ang-1 or Ang-2 only slightly impairs insulin secretion and glucose tolerance together with marginal altered vascularization, islet mass and morphology[57]. Reports also suggest that Ang-1/Tie-2 signaling promotes cell-cell contacts and contact to extracellular matrix (ECM)[58,59]. Ang-2 however, expressed by ECs, classically antagonizes Tie-2 signaling[60] and plays key roles in angiogenesis and inflammation.

Ephrins

Ephrin ligands and their tyrosine kinase receptors are involved in various aspects of cell communication[61,62]. Each ephrin ligand together with its specific receptor (Eph) is categorised either into the A or B subclass. Most EphA receptors bind to ephrin-A ligand, while most EphB receptors bind to ephrin-B ligands[63]. Transcriptome analyses

suggest that Eph-ephrin interaction between exocrine and endocrine cells contributes to pancreatic function[64]. Ephrin-A and its receptor EphA play a role in β-cell to β-cell communication; specifically, ephrin subtype A5 is required for glucose stimulated insulin secretion and the EphA-ephrin-A mediated interaction between β-cells is bidirectional[65]. The blood vessel ECs within pancreatic islets express Eph subtype A4 receptors[66] but how these ligands and receptors play a role between EC and β-cell crosstalk is subject to investigation.

Extracellular vesicles

Recent reports establish extracellular vesicles (EVs) as a novel player in cell-to-cell communication[67,68] and have been characterized both in human islets[69] and in experimental models of human islet xenotransplantation in SCID mice[70]. Studies exploring the functional contribution of β-cell EVs on islet ECs demonstrate that islet-derived EVs have the capacity to affect the surrounding ECs, which are then able to internalize the islet EVs in a dose dependent manner[69]. Furthermore, internalization of islet EVs results in transfer of multiple RNAs, including insulin mRNA and various microRNAs. Uptake of islet EVs conferred endothelial cell resistance to apoptosis and up-regulated expression of numerous proangiogenic factors[69]. In a different study, endothelial progenitor cell EVs, when internalized by islet α-, β- and ECs resulted in improved glucose-stimulated proliferation and angiogenesis[70].

THE ENDOCRINE EFFECT OF ISLET ECS ON β-CELLS

Islet ECs, apart from their pivotal role in angiogenesis, also possess endocrine function. They produce multiple factors (Figure 1) that govern proliferation, survival, and gene expression, which contribute to the physiology and function of the β-cell[71-75].

Basement membrane

ECM proteins provide biochemical cues interpreted by cell surface receptors and initiate signalling cascades controlling morphogenesis, cell survival, proliferation, differentiation, and stem cell state[76-78]. Islets are surrounded by a peri-islet basement membrane (BM) and an associated interstitial matrix containing multiple components such as collagen, laminin, fibronectin, perlecans, nidogens, and heparin sulphate[79,80]. Beta-cells depend on intra-islet ECs to synthesize their ECM components[75]. It has been reported that collagen IV, secreted by islet endothelium, can potentiate insulin secretion *via* interaction with its receptor integrin $\alpha_1\beta_1$ on β-cells[81] similar to other BM components such as laminins and fibronectin which have been reported to act as endothelial signals promoting insulin gene expression and proliferation in β-cells[75,82]. Interaction of collagen IV with its receptors also contributes to β-cell differentiation, maturation, and survival[83-85]. Other BM components such as fibronectin and heparin sulfate also play roles in β-cell migration, growth, differentiation and survival[1,86-88].

Connective tissue growth factor

The β-cell proliferative factor, connective tissue growth factor (CTGF/CCN2), is a member of the CCN family of secreted ECM-associated proteins[89,90] and is expressed in ECs during development[90,91]. It induces expression of platelet derived growth factor B (PDGF-B) in ECs, required for pericyte recruitment and retention[91]. CTGF promotes β-cell regeneration[92], proliferation[93], and modulates the response to high glucose[94]. Its inactivation results in defects in islet cell lineage allocation and β-cell proliferation during embryogenesis[95].

Hepatocyte growth factor

Islet ECs release the hepatocyte growth factor (HGF)[13] which induces β-cell proliferation and differentiation in embryonic and postnatal pancreas[47,75,95-98]. HGF plays a positive role in β-cell mitogenesis, differentiation, glucose sensing, and transplant survival[99,100]. *In vitro*, VEGF-A and insulin are islet-derived factors that induce the HGF secretion within purified islet ECs. *In vivo*, utilizing of pregnant rat pancreas, where a high physiological proliferation of β cells occur, resulted in a prominent expression of HGF, coinciding with the peak of β-cell proliferation[74].

Thrombospondins

Thrombospondins are matricellular glycoproteins that participate in a regulating cell proliferation, migration, and apoptosis, and have been implicated in angiogenesis, tumour invasion, and metastasis[101,102]. Thrombospondin-1 (TSP-1) is almost exclusively expressed by the intra-islet endothelium[71,103,104] and is not downregulated by hypoxia[105]. TSP-1 is mainly known for its antiangiogenic properties[106] but also may alter the morphology of pancreatic islets and function as a major activator of transforming growth factor TGFβ-1[107]. Animals deficient of this glycoprotein are characterized by hypervascular islets[107] and the EC-derived TSP-1 is important to maintain β-cell function postnatally[71].

Endothelins

Endothelin is a vasoconstrictive protein. Endothelin-1 (ET-1) predominantly is found to have strong effects on native islet blood vessels[108] while ET-1 and ET-3 may directly stimulate β-cell insulin secretion and release[73,109]. The gene expression of ET-1 in both ECs and islet endocrine cells is regulated by hypoxia[110,111]. Insulin can also stimulate the expression and secretion of ET-1 from bovine ECs[112] and endogenous insulin can regulate circulating ET-1 concentrations in humans[113]. ET-1 also upregulates the expression of the *FOXO1* gene

(encoding a transcription factor) on ECs contributing to its survival[114].

Endoglin

Endoglin (Eng) is a homodimeric transmembrane glycol protein within the TGF-β superfamily and is expressed by vascular ECs[115-118]. Studies have identified two distinct Eng positive cell types within human and mouse islets: The ECs and the mesenchymal stromal cells[119]. EC-specific endoglin expression in islets is sensitive to VEGF playing partial roles in driving islet vascular development[120].

IMPLICATIONS OF β-CELL AND ENDOTHELIAL CROSSTALK ON ISLET TRANSPLANTATION

Islet transplantation and revascularization

The human islet isolation technique completely severs the islet vasculature[121,122]. During the enzymatic digestion step, islets undergo a number of cellular assaults such as ischemia, mechanical stress, loss of basement proteins, and partial disruption of intra-islet ECs[123-125] resulting in a substantial loss of viability before transplantation. Other than being devoid of ECs to support rapid revascularization, cytotoxic damage and cell death account for a loss of up to 80% of transplanted islets[126,127]. Rapid and adequate revascularization is critical for survival and function of transplanted islets[121,128,129]. Transplanted islet grafts initially have a significant reduction in vascular supply and low oxygen tension in comparison to normal islets[130-132]. The return of islet function depends on re-establishment of new vessels within islet grafts to derive blood flow from the host vascular system[123,133]. Islet engraftment is a slow process, while the islet blood flow re-establishment requires about two weeks, vessel maturation is likely to take a much longer period. Using immunosuppressive drugs such as rapamycin further affect this process by exerting antiangiogenic activities on mouse and human islet endothelium[134].

Though transplanted islets are considered avascular, freshly isolated islets retain angiogenic capacity as they contain intra-islet ECs. These cells can be triggered by various inducers such as VEGF to form vessels *via* angiogenic sprouting[33,135,136]. Revascularization is an important process for adequate engraftment of islets. Prevascularizing islets prior to transplantation could potentially improve islet survivability and function by aiding islet-to-host inosculation[25]. The intra-islet vasculature can also act as a barrier against infiltrating insults of autoreactive cells in type 1 diabetes (T1D) thereby implicating ECs as an important target in type 2 diabetes (T2D)[137-139].

Studies involving cell and tissue engineering approaches have considered factors such as pancreatic islet size-dependency[140], use of stem cells[141-144], creating engineered vascular beds and hydrogels[145-147], endothelial progenitor cell derived microvesicles[70], and repurposed biological scaffolds[148] to improve islet revascularization potential. The angiogenic capacity of islet ECs has been previously determined[136]. A number of factors which may potentially improve islet transplantation involve ECs. For example, vascular ECs of the embryonic aorta induce the development of endocrine cells from pancreatic epithelium in mice[149,150] and the overexpression of VEGF-A in transplanted mouse islets improves insulin secretion and blood glucose regulation in recipient mice[33,53]. Identifying novel factors and understanding nature of mechanisms that underlie bidirectional communication between β-cells and ECs should be of immense relevance for improved human islet transplantation or preventing pancreas associated diseases such as pancreatitis and diabetes.

ECs and β-cell crosstalk: Islet pathophysiology, current perspectives and future directions

Evaluation of factors contributing to mechanisms responsible for regulating the interaction between β-cells and intra-islet ECs would broaden our understanding of pancreatic tissue function, growth, and disease. In this context, VEGF-A has been the most well studied molecule[51,53]; however, reports have suggested the detrimental effects of VEGF on islets. Continued β-cell overexpression of VEGF-A impairs islet morphology and function by eliciting an inflammatory response[57,151]. Elevated levels of serum VEGF, Ang-2, and soluble Tie-2 have also been associated with T2D and vascular dysfunction[152-154]. Achieving an optimal VEGF-A dose to potentiate islet vascularization is subject to further investigation. The HGF production is increased during pregnancy in adult rats[74] and helps balance high glucose levels in diabetes induced mice[155]. *HGF* gene therapy has been suggested as a potential approach for improving islet transplantation rates and treatment of diabetes[156,157].

The dense pancreatic vasculature along with its associated ECM plays a key role in the physiology and disease associated with pancreatic islets. The islet is an ideal "tissue" model because of its heterogeneous cell population embedded within the ECM. Understanding the nature of how these cells communicate with each other and with their underlying BM is crucial for normal islet physiology and pathology. The β-cells rely on intra-islet ECs to synthesise their ECM components[75]. This dependency may potentially be compromised in chronic inflammatory pancreatic diseases such as chronic pancreatitis which is characterized by a number of alterations within ECM formation and composition resulting in destruction of acinar and islet cells, and subsequent replacement by connective tissue[158,159]. This connective tissue appears to result from an increased deposition and disorganization of the ECM proteins including collagens, fibronectins, and laminins[160-163]. Moreover, reports also suggest that one of the most

enriched groups of over-expressed proteins in pancreatitis (mild and severe) and pancreatic ductal adenocarcinoma include those involved in the ECM structure and organization[164,165]. In addition, glycoproteins, especially those with N-linked glycosylation sites, are significantly enriched among the over-expressed proteins in mild and chronic pancreatitis[164]. Collagen, proteoglycans, and other ECM specialized glycoproteins such as fibrillin, fibronectin, and laminin, all part of the peri-islet BM, contain various degrees of glycosylation[166].

The connection between ECs and β-cells has been previously evaluated[28,51,57,167,168], particularly where different approaches have been utilized to increase β-cell mass and thereby insulin production. New factors have also been identified which may potentially contribute in further understanding islet cell communication and function. For example, R-spondins-1, an intestinal growth factor containing a thrombospondin domain, has been identified as a novel β-cell growth factor and insulin secretagogue[169]. It has potential to enhance β-cell growth and function in patients with T2D, and enhance of β-cell mass[170]. Connexins, ephrins, and cadherins, members of the transmembrane family of proteins are expressed in pancreatic islets. The major β-cell connexin is Cx36[171], Cx43, and Cx45 are specifically expressed on intra-islet ECs[172] whereas Cx30.2, recently identified, is expressed at cell-cell junctions in both cell types[173].

A number of studies have demonstrated that ECs play a very critical role within the islet microenvironment. A dysfunctional intra-islet vascular endothelium may contribute to the severity or progression of pancreatic disease etiologies. A deeper knowledge of islet endothelial phenotype and function will help identify specific targets and strategies for T1D prevention and successful outcomes for islet transplantation. Identifying and validating the potential therapeutic benefits of novel factors which either maintain the integrity of EC and β-cell communication or reinstate and balance the disrupted crosstalk is likely to benefit patients with diabetes and other pancreatic disorders.

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MINIREVIEWS

Smoking in Renal Transplantation; Facts Beyond Myth

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Abstract

Smoking is one of the preventable leading causes of death worldwide. Most of the studies focused on the association between smoking and cardiovascular disease, pulmonary diseases, malignancy and death. However, the direct effect of smoking on the renal system was undermind. There are emerging evidence correlating tobacco use with pathological changes in the normal kidneys. The effect is more obvious on the renal allograft most probably due to the chronic immune suppression status and the metabolic effect of the drugs. Several studies have documented a deleterious effect of smoking on the renal transplant recipients. Smoking was associated with lowering patient and graft survival. Smoking cessation proved to improve graft survival and to a lesser extent recipient survival. Even receiving a renal transplant from a smoker donor increases the risk of death for the recipient and carries a poorer graft survival compared to non-smoking donors. Most of the studies investigating the effect of smoking were based on self-reporting questioners, which may be misleading due to poor recall or the desire to give socially acceptable answers. This made the need of a reliable biomarker of ultimate importance. Cotinine was proposed as a promising biomarker that may help to provide objective evidence regarding the status of smoking and the dose of nicotine exposure, yet there are still some limitations of its use. The aim of this work is to review the current evidence to improve our understanding of this critical topic. Indeed, this will help to guide better-designed studies in the future.

Key words: Smoking; Kidney donor; Kidney recipient; Renal transplantation

Core tip: There are several studies addressing the effect

of smoking on different body systems, yet, there are only few exploring the effect of smoking on the outcome of renal transplantation. Our present article summarizes all the available data published over the past 2 decades for better understanding of this topic and may also guide future studies.

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INTRODUCTION

Smoking is a challenging health care problem; it has a well-established correlation with many serious medical conditions like cardiovascular diseases, pulmonary diseases, malignancy and death[1]. Cigarette smoking assumes to have a role in atherosclerosis, endothelial dysfunction, progression of vascular disease progression of proteinuria, as it contains large amounts of free radicals[2]. This makes smoking a significant renal risk factor, with considerable consequences on health care budget[3].

The effect of smoking is aggravated in renal transplant recipients due to the effect of immune suppression medications on carcinogenesis, in addition to the effect of chronic kidney disease itself on cardiovascular risk and mortality[1].

Despite the extensive smoking-related research, yet studies that investigated this phenomenon in the transplant populations are relatively few, and most of them are retrospective, poorly randomised or small sample size[2].

EFFECTS OF SMOKING ON THE KIDNEY

The hazards of smoking were investigated thoroughly in association with cardiovascular disease, lung disease and oncogenesis. However, the effect of smoking on healthy kidney and progression of primary kidney diseases did not attract great attention[3]. Indeed, many studies confirmed the role played by smoking in the progression of many intrinsic renal diseases (*e.g.*, diabetic nephropathy, IgA nephropathy and autosomal dominant polycystic kidney disease)[3].

Ritz *et al*[4] studied the effect of smoking on healthy normotensive volunteers. They reported a significant increase in arginine vasopressin levels (from 1.27 ± 0.72 to 19.9 ± 27.2 pg/mL) and serum epinephrine (from 37 ± 13 to 140 ± 129 pg/mL). There was an increase in renal vascular resistance by 11% and a decrease in the glomerular filtration rate (GFR) by 15%. They assumed these effects are secondary to nicotine itself as these findings were reproduced by using nicotine containing gum[4].

Pinto-Sietsma *et al*[5] performed a leading cross-sectional study on 7476 participants to evaluate the effect of smoking on the development of albuminuria and abnormal kidney functions in non-diabetic population. They documented the presence of a dose-dependent association between smoking and development of both microalbuminuria and renal impairment in this screening. These findings were less obvious or absent in former smokers[5].

RECIPIENT SMOKING AND TRANSPLANTATION OUTCOME

Smoking is strongly correlated to some of the potentially fatal outcomes, and there is some evidence that these complications are aggravated in solid organ transplant recipients[6].

Smoking is a well-known risk factor for cardiovascular disease. Ponticelli *et al*[7] have addressed the role of cardiovascular disease as the leading cause of death in renal transplant recipient. The development of de novo cardiovascular insult in the first year post-transplant was associated with pre-existing cardiovascular disease, older age, pre-transplant hypertension, smoking and duration of dialysis[7].

The second leading cause of death post-transplantation was malignancy[2,7] with a clear association between smoking and increased risk for certain types of malignancy[1].

The effect of smoking on renal transplant recipients was investigated in relatively few studies, and most of them are retrospective. Table 1 summarises the results of most of these studies[1,8-20].

It worth to mentioning that Zitt *et al*[16] had a unique approach by studying the relation between smoking and renal biopsy findings of 76 kidney transplant recipients. Current smokers had an increase in the severity of vascular intimal fibrous thickening (P = 0.004). While the degree of chronic sclerosing nephropathy (P = 0.05) and arteriolar hyalinosis (P < 0.001) were associated with the duration of time post-transplantation[16].

Most of these studies have revealed a clear benefit of smoking cessation on graft survival, but the effect on patient survival is less clear possibly reflecting the permanent atherosclerotic effect on the vascular system[20].

EFFECT OF SMOKING HABIT OF KIDNEY DONOR ON THE OUTCOME OF TRANSPLANTATION

It may be logic that the recipient smoking will affect his own survival, but surprisingly, even the donor smoking will affect the recipient survival years after transplantation[21,22].

Lin *et al*[21] have analysed data from the United Network for Organ Sharing from 1994 to 1999, and

Table 1 The impact of smoking on kidney transplant recipient

Ref.	Year	Study design	No. of cases		Results	Conclusion
			Total	smokers		
Arend *et al*[8]	1997	Retrospective analysis	916	394	RR 2.2 of mortality after the first year of transplantation (95%CI)	The risk of mortality after the first year was higher in older patients, men, diabetics, hypertensive and smokers
Cosio *et al*[9]	1999	Retrospective analysis	523	147	Patient survival shorter in smokers by Cox regression (P = 0.0005), univariate and multivariate analysis (P = 0.0004)	History of smoking correlates with decreased patient survival, the effect of smoking on transplant recipient is quantitatively similar to the effect of diabetes
Kasiske *et al*[10]	2000	Retrospective analysis	1334	330	RR 1.3 of graft loss with smoking more than 25 pack/yr at transplantation (95%CI) and increase the risk of death (RR = 1.42, 95%CI)	The effect of smoking dissipates after five years from quitting
Doyle *et al*[11]	2000	Retrospective analysis	206	155	RR 8.1 for graft loss (P < 0.001) and RR 7.9 for mortality (P < 0.001)	Tobacco use was associated with worse patient and graft survival compared to those who never smoked or those who quit smoking at least two months before transplantation
Matas *et al*[12]	2001	Retrospective analysis	2540	Not mentioned	Pre-transplant smoking has RR 2.1 for graft loss	Pre-transplant smoking, peripheral vascular disease or dialysis more than one year were all associated with worse long-term outcome
Sung *et al*[13]	2001	Retrospective analysis	645	156	RR 2.3 for graft loss, graft survival in smokers *vs* non-smokers were (84% *vs* 88%) at 1 yr, (65% *vs* 78%) at 5 yr and (48% *vs* 62%) at 10 yr follow up (P = 0.007)	Smoking significantly affects graft survival, an effect that is not explained by increases in rejection or patient death. Smoking cessation has beneficial effect on graft survival
Yavuz *et al*[14]	2004	Retrospective analysis	226	97	There was no significant relation between pre-transplant smoking and graft loss (P = 0.129), or mortality (P = 0.138)	They suspected that the non-significant effect of smoking might be attributed to the limited number of cases included
Kheradmand *et al*[15]	2005	Retrospective analysis	199	41	Pre-transplant smoking was associated with reduced overall graft survival (P = 0.01)	Smoking contributes to graft loss but has no significant relation with rejection episodes
Zitt *et al*[16]	2007	Retrospective analysis	279	62	Smokers had higher serum creatinine levels. Transplant biopsy was indicated more often in smokers compared to non-smokers (39% *vs* 24%, P = 0.02)	Smoking was associated with vascular fibrous intimal thickening in transplanted kidneys so that it may have a role in the development of chronic allograft nephropathy and graft loss
Gombos *et al*[17]	2010	cross-sectional study	402	102	In spite that kidney functions in smokers were not affected after one month of transplantation, yet, there was significant lower kidney function in smokers after three years (P < 0.05). This correlates with the intensity of smoking (P < 0.05)	Smoking is common following kidney transplantation in Hungary, and this may be a risk of a poor long-term outcome
Nogueira *et al*[18]	2010	Retrospective analysis	997	329	Patient and graft survival were worse in smokers (AHR for patient survival was 1.6, 95%CI, P = 0.02, and graft survival AHR 1.47, 95%CI, P = 0.01). Glomerular filtration rate after one year was lower in smokers	History of smoking will negatively affect patient and graft survival. Also, it increases the risk of early rejection
Hurst *et al*[19]	2011	Retrospective analysis	41705	5832	New onset smokers have increased risk of graft failure (AHR = 1.46, P < 0.001) and death (AHR = 2.32, P < 00.1) compared with never smokers	New onset smoking post-transplant associated with lower patient and graft survival
Agarwal *et al*[20]	2011	Prospective observational study	604	133	Current smokers have increased risk of graft failure compared to recipients who never smoke (HR = 3.3, P = 0.002). While past smokers had an almost similar risk of graft failure compared to non-smokers (HR = 1.1, P = 0.7) On the other hand, current and past smokers were at higher risk of mortality compared to non-smoker recipients (HR = 2.1, 95%CI: 1.1-3.8, P = 0.016, and HR = 2.4, 95%CI: 1.4-4.0, P = 0.001, respectively)	Current smoking is a risk factor for graft failure and mortality Despite the finding that smoking cessation may not alter the risk of mortality, but at least it will improve the graft survival

Opelz *et al*[1]	2016	Retrospective analysis	46548	15086	Patients who quit smoking before transplantation had clear benefits regarding patient and graft survival when compared to those who continues to smoke {all-cause graft failure (HR 1.1 *vs* 1.5, $P < 0.001$), all-cause mortality (HR 1.1 *vs* 1.6, $P < 0.001$) and death with functioning graft due to malignancy (HR 1.4 *vs* 2.6, $P = 0.001$)} However, they still have a higher risk for graft loss, malignancy and death compared to those who never smoke before	Smoking cessation before transplantation improve patient and graft survival. There is also a substantial reduction in certain types of malignancy compared to those who continued to smoke (lower incidence of respiratory, urinary tract, female genital organs, lips and oral cavity tumours)

AHR: Adjusted hazard ratio.

they declared that smoking habit of the donor has mild, yet statistically significant effect on recipient survival (HR = 1.06, $P < 0.05$), and graft survival (HR = 1.05, $P < 0.05$).

Underwood *et al*[22] studied a retrospective analysis of 602 kidney transplant recipients and their living donors. The effect of donor smoking on graft survival was statistically insignificant (HR = 1.19, $P = 0.515$), unlike the recipient smoking which proved to be significant (HR = 1.74, $P = 0.05$). However, the recipient survival was negatively correlated to donor smoking (HR = 1.93, 95%CI: 1.27-2.94, $P = 0.002$) and recipient smoking (HR = 1.74, 95%CI: 1.01-3.00, $P = 0.048$)[22].

Heldt *et al*[23] evaluated GFR of 100 living donors and their recipients, the recipients of smoking donors had lower calculated GFR (37.0 mL/min per 1.73 m^2 *vs* 53.0 mL/min per 1.73 m^2; $P < 0.001$) at a mean follow-up of 38 mo.

SMOKING BIOMARKER AND RENAL TRANSPLANTATION

Smoking exposure and analysis of dose of smoking depends on self-reporting in most of the studies[24], which we strongly believe it lacks accuracy. A proper estimation of the risks associated with tobacco use depends on accurate measurement of exposure, which may be difficult in certain population such as pregnant women and parents of young children, where smoking considered socially unaccepted[24]. Some patients may not recall the number of cigarettes accurately (digit bias)[25], and finally the tobacco dose differs between individuals due to the difference between cigarettes as well as the difference in inhaling habits (passive smoking)[25]. All these factors made the development of a valid and accurate biomarker for tobacco smoking of ultimate importance.

Cotinine is the major metabolite of nicotine. It has a relatively constant level due its long half-life (16 h *vs* 2-3 h for nicotine), which can be measured in plasma or urine. For these reasons, cotinine is considered a promising biomarker of smoking exposure[25].

Hellemons *et al*[25] studied 603 renal transplant recipients for a mean follow-up of 6.9 years. The aim was to investigate the relation of self-reporting and cotinine exposure in transplant population and to evaluate the use of cotinine as an alternative for self-report[25]. They concluded that active smoking had a negative impact on patient and graft survival, while former smokers had increased the risk of mortality but not graft failure. They documented that cotinine measurement (especially plasma cotinine) provides a valid alternative to self-reported smoking exposure, and it may even be preferred over self-reporting in epidemiological studies[25].

The use of cotinine also has its limitations. Cotinine level is a reflection of smoking over the past few days, and this may be misleading if the patient is smoking occasionally (like in weekends) or if the patient was smoking less due to a period of illness. The second limitation lies in its inability to differentiate between never-smoking and former-smoking[25]. Differentiating never-smoking from former-smoking is clinically relevant as former-smoking was proved to be associated with increasing risk of recipient mortality[20,25].

We believe that the combination of cotinine measurement and self-reporting of smoking exposure will be the most reliable approach in evaluating renal transplant population.

CONCLUSION

Smoking remains a major modifiable health care challenge; it is the leading cause of variable morbidities and mortality. The use of smoking biomarkers proved to be reliable in evaluation and quantification of smoking exposure in the transplant population. Donor smoking and recipient former smoking proved to have a negative impact on survival. Transplant community should pay more attention to donor and recipient smoking cessation programs.

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MINIREVIEWS

Past, present and future of kidney paired donation transplantation in India

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Abstract

One third of healthy willing living kidney donors are rejected due to ABO blood group incompatibility and donor specific antibody. This increases pre-transplant dialysis duration leading to increased morbidity and mortality on the kidney transplantation waiting list. Over the last decade kidney paired donation is most rapidly increased source of living kidney donors. In a kidney transplantation program dominated by living donor kidney transplantation, kidney paired donation is a legal and valid alternative strategy to increase living donor kidney transplantation. This is more useful in countries with limited resources where ABO incompatible kidney transplantation or desensitization protocol is not feasible because of costs/infectious complications and deceased donor kidney transplantation is in initial stages. The matching allocation, ABO blood type imbalance, reciprocity, simultaneity, geography were the limitation for the expansion of kidney paired donation. Here we describe different successful ways to increase living donor kidney transplantation through kidney paired donation. Compatible pairs, domino chain, combination of kidney paired donation with desensitization or ABO incompatible transplantation, international kidney paired donation, non-simultaneous, extended, altruistic donor chain and list exchange are different ways to expand the donor pool.

In absence of national kidney paired donation program, a dedicated kidney paired donation team will increase access to living donor kidney transplantation in individual centres with team work. Use of social networking sites to expand donor pool, HLA based national kidney paired donation program will increase quality and quantity of kidney paired donation transplantation. Transplant centres should remove the barriers to a broader implementation of multicentre, national kidney paired donation program to further optimize potential of kidney paired donation to increase transplantation of O group and sensitized patients. This review assists in the development of similar programs in other developing countries.

Key words: Living donor kidney transplantation; Kidney paired donation; Renal replacement therapy; Developing country

Core tip: Over the last decade kidney paired donation is most rapidly increased source of living kidney donors. Here we describe different successful ways to increase living donor kidney transplantation through kidney paired donation. Compatible pairs, domino chain, combination of kidney paired donation with desensitization or ABO incompatible transplantation, international kidney paired donation, non-simultaneous, extended, altruistic donor chain and list exchange are different ways to expand the donor pool. Transplant centres should remove the barriers to a broader implementation of multicentre, national kidney paired donation program to further optimize potential of kidney paired donation to increase transplantation of O group and sensitized patients.

Kute VB, Patel HV, Shah PR, Modi PR, Shah VR, Rizvi SJ, Pal BC, Modi MP, Shah PS, Varyani UT, Wakhare PS, Shinde SG, Ghodela VA, Patel MH, Trivedi VB, Trivedi HL. Past, present and future of kidney paired donation transplantation in India. *World J Transplant* 2017; 7(2): 134-143 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i2/134.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i2.134

INTRODUCTION

Low insurance coverage, poor public health system leading to out of pocket health expenditure and unavailability of adequate trained doctors and staff are problems of renal replacement therapies in developing country. Living donor kidney transplants have a greater long-term graft survival rate than deceased donor kidney transplantation (primarily from brain-dead donors). Kidney paired donation has all advantages of living donor kidney transplantation (similar patient survival, graft survival and outcome). Successful kidney paired donation program requires healthy mixture of enthusiasm, mathematical modeling, patience and team work. Learning curves, need of infrastructural support, additional cost are not required in kidney paired donation. It can be done at center of their choice under their primary nephrologist. Worldwide kidney paired donation has increased access to living donor kidney transplantation in national and single centre programs in the last decade[1-10]. Here we describe different successful ways to increase living donor kidney transplantation through kidney paired donation[11-13]. This review assists in the development of similar programs in other developing countries.

CONVENTIONAL BALANCED KIDNEY PAIRED DONATION

The pair 1 (A patient and B donor) exchanges kidney with pair 2 (B patient and A donor) and both the pairs are benefitted resulting in two ABO compatible kidney transplantation. Kidney paired donation initially started in Dutch program as closed loop of 2-way kidney exchange. It can be arranged as 3-way, 4-way and n-way exchanges. Two way single centre kidney paired donation program increases waiting time to find suitable donor in kidney exchange program. It has less match rate and has limited scope to increase transplant rate. The 3-way exchange increases match rate from 54% to 66% in one simulation study[11]. Dutch program reported that 3 way exchange is the most optimum length of kidney paired donation to achieve good match rate and to carry out simultaneous kidney transplantation especially for newly starting single centre kidney paired donation programs[7-10]. The longer chains do not lead to significantly more kidney transplantation. Multiple simultaneous kidney transplantation surgeries increase logistic burden on the transplant team, and requires stringent and careful transplant coordination.

UNCONVENTIONAL KIDNEY PAIRED DONATION WITH USE OF COMPATIBLE PAIRS

The ABO incompatible pair 1 (O patient and non-O donor) exchange kidney with ABO compatible but ABO non-identical pair 2 (non-O patient and O donor). This is also known as altruistically unbalanced paired donation. The compatible pairs can be offered benefit by better HLA matched donor or younger donor. Transplant surgery should not be delayed for the compatible pair to find better matched donor especially in developing countries where the morbidity and mortality on long term maintenance dialysis is high. Bingaman *et al*[12,13] reported increase in match and transplant rate with use of compatible pairs. The compatible pairs increase the match rate for incompatible pairs (28.2% to 64.5% for single-centre program, 37.4% to 75.4% for national program). Legal, logistical, and governmental controversies, lack of awareness and counselling have limited the growth kidney paired donation with compatible pairs. KPD transplantation can be offered to non-HLA identical compatible pairs with

donors over 45 years to get better (HLA or younger donor) matched donor[12,13].

Over the last three decades the short term graft survival is improved but long term graft survival and outcome is similar with use of modern potent immunosuppression. The age of ESRD patient in developing countries like India is younger than developed countries. The leading cause of morbidity and mortality after kidney transplantation in India is Infection. Better HLA matched kidney transplantation for the compatible pairs will result in better long term outcome and need of re-transplantation which is common cause of sensitization. Commercial interest should be carefully ruled out in such kind of exchange with careful selection. Basu *et al*[14] reported the need of large donor pool (multicentre or national kidney paired donation program) to find better HLA matched donor. The willingness of ABO compatible pairs to participate in kidney paired donation should be evaluated in more studies to increase the long term graft survival[15,16].

Multiple studies have demonstrated HLA-matched transplant had higher rates of survival, a lower incidence of rejection, and a lower risk of graft loss due to immune injury[17]. The Collaborative Transplant Study, the United Kingdom Transplant and Euro-transplant data showed that DR matching having a much greater effect than that of B or A. In India majority of living donors are females and most of them are spousal donors. If all spousal donors above 45 years of age even though ABO compatible (especially blood group O donors) are included in national kidney paired donation program, it will increase the number of transplants of O group and sensitized recipients[12,13].

NON-DIRECTED ANONYMOUS DONORS

Non-directed anonymous donors (Good Samaritan or altruistic donors) are donors who want to donate a kidney, but do not have an intended recipient. Non-directed anonymous donors from the general population can initiate the kidney paired donation chain to increase transplant rate for O group and sensitized patients in kidney paired donation[18-21]. One of the key to the success of Canadian kidney paired donation program is non-directed anonymous donors chains, where non-directed anonymous donors facilitated transplants in 61% of all incompatible kidney paired donation pairs[4]. There should be legal permission for non-directed anonymous donors as per organ act of the country. Transplantation of Human Organs Act (THOA), India did not permit non-directed anonymous donors transplants.

USE OF KIDNEY PAIRED DONATION TO INCREASE ACCESS TO LIVING DONOR KIDNEY TRANSPLANTATION FOR SENSITIZED PATIENTS

Kidney paired donation in the presence of low-level donor specific antibody can be performed in carefully selected highly sensitized patients with minimal to no desensitization. The patients should be aware of possible poor long term outcomes with low level donor specific antibody and negative flow cross-match due to the impact of memory responses[22]. The use of ABO incompatible pairs also increases match rate for highly sensitized patients. Kidney paired donation combined with desensitization protocol can be performed with donor of low immunological risk in absence of other better option for the carefully selected highly sensitized patients. This strategy is used in Johns Hopkins Hospital[23,24]. The Global kidney exchange will increase the living donor kidney transplantation opportunity for sensitized and O group patients by direct benefit of increase in donor pool and benefit from differences in heterogeneity of blood types distribution in the population, antigens and antibodies profile. It will also improve the quality and quantity of transplant.

DOMINO PAIRED DONATION

Kidney exchange transplants can be increased by 20% with domino paired donation[25]. In one South Korean centre, 179 living donor kidney transplantations were performed, with 70 domino chains initiated by an altruistic living non-directed donor. The patient and graft survival rates at 1-year and 5-year were 97.2% and 90.8%, and 98.3% and 87.7%, respectively. Multi-centre domino kidney paired donation increases access to living donor kidney transplantation, with similar outcome to conventional kidney paired donation[26].

KIDNEY PAIRED DONATION COMBINED WITH ABO-INCOMPATIBLE TRANSPLANTATION

Patient donor pair with high ABO titres [for examples pair 1: patient 1 (O group) and donor 1 (A group) with anti-A isoagglutinin titer $\geqslant$ 512; pair 2: patient 2 (O group) and donor 2 (B group) with anti-B isoagglutinin titre $\geqslant$ 512] exchange kidney to get donor with low ABO titres [pair 1: patient 1 (O group) and donor 2 (B group) with anti-B *isoagglutinin* titer $\leqslant$ 64; pair 2: patient 2 (O group) and donor1 (A group) with anti-A isoagglutinin titre $\leqslant$ 64]. This will minimizes cost, deceases need of immunosuppression and improve long term outcome of ABO incompatible kidney transplantation and increases match rate for the sensitized patients. ABO-incompatible transplantation in the absence of donor specific antibody with low baseline ABO titre $\leqslant$ 1:64 has good outcome[27,28]. The cut-off value of high ABO antibody titre may vary as per experience of the transplant unit. This strategy is used effectively in the various national kidney paired donation program (Australian > United Kingdom > Canada)[28].

INTERNATIONAL KIDNEY PAIRED DONATION

The single centre kidney paired donation program which

is commonly practiced in India has inherent limitations to expand the donor pool. Garonzik-Wang *et al*[29] reported international kidney exchange between the United States and Canada in a 10-way domino chain kidney transplantation between September 2009 to July 2010. The success was attributed to close geography reducing kidney transport time, close collaboration, similar language and philosophical understandings between the Canada and the United States transplant team. Three international living donation kidney transplantation from kidney exchange program between May 2013, and March 2014 were reported in Turkey where national kidney paired donation program increased living donation kidney transplantation by 5%[30]. The international organ exchange from deceased donors substantially contributed (7.2% of deceased donor kidney transplantation) to the Swiss transplant activity during the period 2009-2013[31]. Each state, region and all the developing countries needs a more robust, organised kidney sharing scheme and efforts should be made to establish a national/regional pool of kidney sharing registry as is the case with the European, North American and other developed countries. Local/regional/national kidney sharing options should be fully explored prior to embarking on international kidney sharing. Global registry of incompatible pairs from diverse population of patient-donor pairs is expected to yield transplant to these pairs.

LIST EXCHANGE AND INDIA

In a living donor list exchange program, the living donor in ABO or HLA incompatible pair donate kidney to the deceased donor kidney transplantation waitlist patient and in return the incompatible patient get top priority on the deceased donor kidney transplantation waitlist. Melcher *et al*[32] reported utilization of deceased donor kidneys to initiate living donor kidney transplantation chains. Ross *et al*[33] reported to restrict list paired exchanges to A, B, AB blood group and sensitized patient donor pair excluding O group patients. The deceased donor kidney transplantation waiting time is prolonged for O group patients with use of list exchange. Single centre kidney paired donation program in Ahmedabad India, demonstrated that deceased donor - living donor list exchange is not required for A and B blood group patient donor pair as they can be readily transplanted in living donor kidney paired donation within reasonable waiting time[34]. The graft half-life of deceased donor and living donor kidney is 13.8 and 21.6 years respectively[35]. This shows that including non-O blood group (A and B group) patient donor pair in list exchange will be unfair as the intended patient will receive a deceased donor kidney rather than a living donor kidney. Patient donor pairs were more willing to participate in living donor kidney paired donation as compared to deceased donor -living donor exchange program. The major reason for this was their intended recipient received kidney from a living donor as compared to deceased donor and intended recipients would get transplants at the same time. Similar findings were also reported by Waterman *et al*[36]. This could be the reason for the significant increase in living donor kidney paired donation program compared to living donor –deceased donor list exchange in the last decade all over the world. The older, diabetic and highly sensitized patients could get benefit from accepting deceased donor kidney of lower quality as compared to living donor kidney early after end stage renal disease, whereas younger, A and B group patients benefit from receiving higher quality living donor kidney even with longer dialysis exposure[37].

In India, allocation of deceased donor kidney is done according to waiting time and not by HLA matching. There is no provision of list exchange in Transplantation of Human Organs October 2013, India. For deceased donor-living donor list exchange program, deceased donor wait list should be transparent with uniform enrolment rules for patients. Deceased donor should be standard criteria donor with uniform donor acceptance policy and definitely should not be the expanded criteria donor. Cold ischemia time should be minimized to improve long term outcome. Donor associated infections should be carefully ruled out. The quality of the kidney should be confirmed by frozen section biopsy whenever required. Every attempt should be made to improve the quality of organ to improve the long term survival. More studies are required to address this issue to balance principal of utility and justice of kidney transplantation.

ALLOCATION ALGORITHMS IN KIDNEY PAIRED DONATION

The virtual cross-matching is used effectively for donor allocation by the various national kidney paired donation program. The manual allocation can be performed by transplant team member with bonus points to sensitised patient, difficult to match patient (O group patient and non - O donor), retransplantation, donor age similarity, dialysis time, HLA match and waiting time[38,39].

KEY ELEMENTS OF FOUR NATIONAL KIDNEY PAIRED DONATION REGISTRIES

Dedicated central support staff, multi-way and domino exchanges, frequency of match cycles every 3-4 mo, donor allocation algorithm with the virtual cross-match, accepts ABO incompatible donor matching (Australia and United Kingdom program), Donor travel (The Netherlands and Canada) or organ transport (Australia and United Kingdom program), and good HLA laboratories support are the key components of four national kidney paired donation registries. The match and transplant rates from two-way and three-way exchanges are not dependent on donor pool size at the time of allocation. Dutch kidney paired donation program reported that the success of a living donor kidney exchange program depends on good co-ordination between the participating transplant centres, common protocol for the selection of donor and patient,

Table 1 Outcome of single center kidney paired donation program India[40-44]

	Pahwa *et al*[41]	Waigankar *et al*[40]	Jha *et al*[42]	Kute *et al*[43]	Kute *et al*[44]
Duration	2006-2011	2008-2011	2010-2013	2000-2012	2013
Patients (*n*)	44	14	26	70	56
2-way exchange	22	7	13	35	25
Follow up	3 yr	12-18 mo	20 mo (median)	2.72 yr (mean)	1 yr
Graft survival	100%	100%	92.30%	81%	97.50%
Patient survival	97.70%	100%	96.16%	90%	94.60%
Acute rejection	-	14.20%	11.50%	14.20%	16%
Reason for joining kidney paired donation (*n*)					
ABO incompatible	40	8	26	56	52
Sensitized	4	0	0	14	4

Table 2 Advantages and disadvantages of single *vs* multicentre kidney paired donation transplant

	Singe center	Multicenter
Donor pool	Less	More
Donor transport	Not required	Required
Shipping of kidneys	Not required	Required
Surgical team skills	Same	Different
Surgical team requirement	More	Less
Cold ischemia time	Less	More
Hospital atmosphere	Familiar	Unfamiliar
Follow up	Same center	Difficult follow up
Administrative cost	Less	More

supervision by an independent allocation organization and a central HLA and tissue typing laboratory responsible for the cross-matches. The protocol consisted of four different steps the registration procedure for participants, allocation - and matching criteria, cross-match procedure in the central national reference laboratory and surgical and follow-up procedures.

OUTCOME OF SINGLE CENTER KIDNEY PAIRED DONATION PROGRAM IN INDIA

Between January 2000 and July 2016, 3616 living donor kidney transplantation and 561 deceased donor kidney transplantation were performed at Institute of Kidney Diseases and Research Centre, Dr HL Trivedi Institute of Transplantation Sciences, Ahmedabad, India with 300 of them (8.3%) using kidney paired donation. Kidney paired donation contributed to 56 kidney paired donation transplantations in 2013 and 2014 leading to increase living donor kidney transplantation by 15.8% and 18.1% respectively[40-59]. Seventy seven kidney paired donation increased the living donor kidney transplantation rate by 25% in one year in 2015. Our centre in Ahmedabad India has used different forms of kidney exchanges including 2-way, 3-way, 4-way, 6-way kidney exchange, use of compatible patient donor pairs, kidney exchange with desensitization, non-simultaneous kidney exchange and international kidney exchange[51-57] (Table 1).

Advantages and disadvantages of single *vs* multicentre kidney paired donation transplant are given in Table 2. In absence of computer allocation system and national kidney paired donation program, the single center can start manual allocation of 2-way or 3-way exchange of ABO incompatible pairs. Matching at the single-centre kidney paired donation program would eliminate the need for co-ordination between different transplant centres, common standard protocols between centres for medical selection of donor-recipient pair; privacy and legal concerns. The virtual cross matching can be used in case of cross match positive pairs. Multicenter or national kidney paired donation program can increase match rate for difficult to match patients like O group and cross match positive donor-recipient pair.

The single centre study showed that outcome (patient survival, graft survival, and rejection rate) of living related donor kidney transplantation (n = 190) is similar to kidney paired donation (n = 34) at 2 years follow up[47]. The use of carefully selected older living donor and patient-donor age difference has no significant impact on long term graft survival in living donor kidney transplantation (n = 49). This is useful in single centre kidney paired donation program with limited donor pool.

POTENTIAL AND SUSTAINABILITY OF A SINGLE-CENTRE KIDNEY PAIRED DONATION PROGRAM

Methodist San Antonio kidney paired donation program reported outcome of 134 kidney paired donation transplants (117 incompatible pairs and 17 compatible pairs) performed over a 3-year period (November 2007 to February 2011)[12,13]. There was significant increase

in access to living donor kidney transplantation with kidney paired donation over the 3 years in Methodist San Antonio kidney paired donation program (11%, 27%, 35%). These data also validate impact of single centre kidney paired donation program. Key elements of the Methodist San Antonio kidney paired donation program were computer allocation, storage of blood specimens for future cross-match testing with consent of patient-donor pairs, A1 and A2 subtype of all blood type A donors and use of more compatible pairs. All patients had negative cross match at the time of transplant, prospective counselling of all patient-donor pairs regarding kidney paired donation, comprehensive immunological assessment with donor specific antibody and HLA testing of all patient-donor pairs, combination of kidney paired donation with desensitization for highly sensitized patients were the strategies implemented by single centre program like San Antonio. It has increased access to kidney paired donation transplantation for traditionally disadvantaged cohorts of patients (female recipients (61%) and previous transplant (32%).

Key to success of the single centre kidney paired donation program in India[40,41] are formation of registry to maintain database of incompatible pairs, awareness and mandatory counselling about advantages of living donor kidney paired donation program, expert transplant coordinator, dedicated HLA laboratory, patient-mentorship program to increase awareness about kidney paired donation, dedicated transplant team for evaluating donors and recipients and supporting the patients to overcome a variety of logistical barriers, dedicated transplant team to run the living donation kidney transplantation program, use of compatible pairs and active participation of patients. Medical profession, government and politicians willingness and support is required for the expansion of kidney exchange in India. In a high volume living donor kidney transplantation program all A and B blood group donor recipient pairs without sensitization can be transplanted with kidney paired donation within reasonable waiting time even with manual allocation without using the computer allocation[40,41].

MATCH RATES BY PATIENT-DONOR PAIR CHARACTERISTICS TO DECIDE ABOUT KIDNEY PAIRED DONATION *VS* DESENSITIZATION

Panel reactive antibodies indicate the ability to match in kidney paired donation. Donor specific antibody indicates ability to desensitize. Panel reactive antibodies and donor specific antibody in combination help to predict which modality (kidney paired donation, desensitization or a combination of both) increases early access to cost effective living donation kidney transplantation with best long term outcome. Donor-recipient pair who are easy in kidney paired donation and desensitization [low panel reactive antibodies, low-strength donor specific antibody (narrow sensitization), O donor] should be tried in kidney paired donation first for the few months and if no match is found in kidney paired donation should undergo desensitization therapy with written informed consent of the pairs. Donor-recipient pair who are easy to match in kidney paired donation and hard to desensitize [low panel reactive antibodies, high-strength donor specific antibody (highly sensitized), O donor] should wait in kidney paired donation. Donor-recipient pair who are hard to match in kidney paired donation and easy to match in desensitization [high panel reactive antibodies, low-strength donor specific antibody (narrow sensitization), non-O donor (specially AB), O recipient] should first look in kidney paired donation pool but probably not worth waiting for the long time and if no match found in kidney paired donation within few months should undergo desensitization therapy with written informed consent of the pairs. Donor-recipient pair who are hard to match in kidney paired donation and hard to desensitize [high panel reactive antibodies, high-strength donor specific antibody (highly sensitized), non-O donor (specially AB), O recipient] may not benefit by single modality of kidney paired donation or desensitization therapy. They should be considered for the combination of the kidney paired donation and desensitization therapy to find a "better" donor. Risk associated with HLA incompatible higher than that associated with ABO incompatible. Kidney paired donation should be preferred over the desensitization therapy. Patients who are hard-to-desensitize (high-strength donor specific antibody) should wait for a match in kidney paired donation, unless they are also hard-to-match (high panel reactive antibodies).

Kidney paired donation limitations and expansions

The expansion of kidney paired donation can be achieved if all the limiting factors are properly solved.

Coercion

The potential kidney donor can deny for donation due to medical reasons like ABO incompatible or cross match positive. Kidney paired donation can increases pressure on the donors for donation. The care should therefore be taken that kidney donor is motivated for the donation and there is no pressure on the donor for the indirect donation.

Anonymity: Kidney paired donation initially started as an anonymous transplantation. The advantage of anonymity is that transplantation team will save the time of organising meetings between the different donor-recipient pair. There will be no extra psychological pressure or conflicts between the two pairs when the results of the two transplantations are not equal especially in the simultaneous single centre kidney transplantation. Donor-recipient pair will not withdraw from the kidney paired donation due to non-medical reasons like cast, *etc*., after meeting with the intended donor. A disadvantage of anonymity is that the donor will

not be informed about the functioning of the donated kidney. In fact formal meeting between the two donor-recipient pair increases the trust between donor-recipient pair and transplant team. They should be counselled that although kidneys are exchange of similar good quality, post-transplant outcome can be different in the two patients depending on the patient related factors like immunology. In the Indian scenario authorization committee take the meeting of the 2 donor-recipient pair together and evaluate about the consent to participate in kidney paired donation. Anonymity is very difficult to maintain in case of simultaneous transplant surgery in single centre kidney paired donation program.

DISTRIBUTION OF BLOOD GROUP TYPES IN INCOMPATIBLE DONORS AND PATIENTS

One of the limitations of kidney paired donation is imbalance between O donor and non-O recipients in the ABO blood group type distribution in general population and incompatible donor recipient pairs. In typical kidney paired donation pools, participation of donor recipients pairs with type O blood group recipients, and non-O blood group donors is more. The compatible pairs would greatly alleviate this imbalance and increases transplant rate for O group and sensitized patients.

Reciprocal match requirement

The kidney paired donation matches require reciprocal compatibility.

Simultaneous donor nephrectomy requirements

It is standard practice to consider simultaneous donor nephrectomy and transplant surgery in kidney paired donation. Majority of Indian transplant centres perform simultaneous two way kidney exchanges and long chains are not preferred due to limited transplant team (operating rooms and surgical staff) and infrastructure. More than 2-way exchanges and long chains can be performed with single centre non-simultaneous kidney paired donation or multi-centre simultaneous kidney paired donation. Multi-centre simultaneous kidney paired donation requires donor travel or transport of kidney. The long term graft survival is not significantly affected when cold ischemia time is short (< 8 h). Despite prolonged cold ischemia time for interstate exchanges, the Australian kidney exchange program preferred to transport donor kidneys rather than kidney donors[60]. However, there is no multi-centre kidney paired donation transplant practice in India. This requires uniform pre-transplant evaluation and acceptance criteria for living donors and fitness of patients among the participating transplant centres. Hospital atmosphere would be unfamiliar for the donor and donor-recipient pair may not trust on the transplant team in other hospital in case of multicentre simultaneous kidney paired donation. In India, only one report of multi-centre simultaneous kidney paired donation of 5 donor-recipient pairs has been reported[58]. Careful selection, written informed consent of pairs and permission from authorization committee is required in single centre non-simultaneous kidney transplantation. In non-simultaneous kidney transplantation, the long chain can break if donor reneges or recipient become medically unfit. Proper counselling of the pairs can avoid donor reneging and standard criteria deceased donor kidney can be allocated on priority in case of donor reneging. All the patients should remain medically fit for transplantation in non-simultaneous kidney transplantation.

Kidney paired donation for O group patients with non-O donor

Living donor kidney transplantation options for O group patients with non-O kidney donor and low ABO titer (< 1:64) are participation in kidney paired donation with compatible pair, international kidney paired donation, global kidney exchange, ABO incompatible kidney transplantation.

Kidney transplantation options for O group patients with non-O kidney donor and high ABO titer are participation in kidney paired donation with compatible pair, international kidney paired donation, global kidney exchange, kidney paired donation combined with ABO-incompatible transplantation, living-deceased donor kidney exchange and deceased donor kidney transplantation.

There is a need for Indian guidelines for incompatible pairs but there is ever more need to develop practice algorithms at least for this part of the world. This should focus on cost, long term patient/graft survival, availability of therapy and local resource limitations.

Legal barriers and new hope

Kidney paired donation is underutilized in India despite tremendous potential for the growth. It could be attributed to lack of national database about incompatible pairs, lack of awareness/counselling about kidney paired donation and administrative challenges (legal permission, *etc.*). This is new hope to overcome administrative challenges from different state authorization committee. In India, Transplantation of Human Organs Act 2011 gives legal permission for kidney paired donation[59]. When the donor-recipient pairs are from different geographic area and state of residence, it was mandatory to take legal permission from authorization committee from all the states rather than only from authorization committee from the state in which transplantation is proposed to be done. This increases waiting time in administrative legal permission. According to Transplantation of Human Organs Act 2013, cases of kidney paired donation from near relative from different states Governments can be approved by authorization committee of hospital in which kidney transplantation is proposed to be done. It will promote multicentre and national kidney paired donation program. The altruistic donors are not allowed for organ donation in kidney paired donation in India.

Global kidney exchange[61,62]

There is financial barrier to kidney transplantation in developing world due to poverty and lack of national health insurance. Poor patient (A blood group patient and O blood group donor) could not undergo kidney transplantation despite having healthy, willing, compatible living kidney donor. The barrier to kidney transplantation in developed world is immunological (O blood group patient and A blood group donor) rather than financial. In global kidney exchange, these two patient donor pairs in developing and developed world exchange kidney with each other to overcome the barriers for kidney transplantation. Global kidney exchange is cost effective even if the cost of both kidney transplantations including the immunosuppression is paid by the health insurance payer of the developed country. Legal and logistical problems should be carefully solved for successful implementation of this strategy. More studied are required to address willingness of patients, health care professionals to participate in global kidney exchange.

Regulated compensation for living kidney donation

Most United states voters view living kidney donation positively, and reported that they would be motivated toward organ donation if offered compensation for living kidney donation of $50000[63]. Certain compensation amounts or health insurance to donor/family members could motivate the public to donate without being perceived as an undue inducement. The direct payment of money and paid leaves are the most preferred forms of compensation. A program of government compensation of kidney donors would provide the following benefits[64,65]: (1) Cost effective as dialysis is more expensive than transplant; (2) Increase living donor kidney transplantation will be available for the poor and productivity of society will increase and a good deal for taxpayers also; and (3) This will decrease morbidity and mortality of long term dialysis and increase quality of life for transplanted patients. The recent study from India reported that live donors should be given incentives for donating their kidney[66]. More studied are required to address regulated compensation for living kidney donation.

CONCLUSION

An effective kidney paired donation program should be implemented in each transplantation centre. Kidney paired donation has all advantages of living donor kidney transplantation (similar patient, graft survival, cost and outcome) without long waiting time for deceased donor kidney transplantation. Successful kidney paired donation program requires healthy mixture of enthusiasm, mathematical modeling, patience and team work. Transplant centres should remove the barriers to a broader implementation of multicentre, national kidney paired donation program to further optimize potential of kidney paired donation to increase transplantation of O group and sensitized patients.

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META-ANALYSIS

Systemic meta-analysis assessing the short term applicability of early conversion to mammalian target of rapamycin inhibitors in kidney transplant

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Abstract

AIM

To consolidate the present evidence of effectiveness in renal functioning and graft survival following early introduction of mammalian target of rapamycin (mTOR) inhibitors with or without calcineurin inhibitors (CNIs) in renal transplant recipients.

METHODS

We analysed the current literature following PROSPERO approval describing the role of immunosuppressive agent, mTOR inhibitors as an alternative to CNI within six months of renal transplant by searching the PubMed, EMBASE, Cochrane, Crossref, and Scopus using MeSH terms.

RESULTS

Six articles of early withdrawal of CNI and introduction of mTOR-inhibitors within six months of renal transplantation were sought. Glomerular filtration rate (GFR) and serum creatinine were significantly better in mTOR inhibitor group with equivalent survival at 12 mo, even though Biopsy Proven Acute rejection was significantly higher in mTOR-inhibitor group.

CONCLUSION

The evidence reviewed in this meta-analysis suggests

that early introduction mTOR-inhibitors substantial CNI minimization. The mTOR inhibitors such as everolimus and sirolimus, due to their complementary mechanism of action and favourable nephrotoxicity profile; better glomerular filtration, lower serum creatinine with equivalent survival. Having said that, due to the higher rejection rate, may influence the use of these regimens to patients with moderate to high immunological risk patients.

Key words: Adverse events; Calcineurin inhibitors; Graft failure; Kidney transplantation; Mammalian target of rapamycin inhibitors

Core tip: Early calcineurin inhibitor withdrawal seems to be more pragmatic approach as it bestows better renal functioning in the low immunological risk renal transplant recipients.

Kumar J, Reccia I, Kusano T, Julie BM, Sharma A, Halawa A. Systemic meta-analysis assessing the short term applicability of early conversion to mammalian target of rapamycin inhibitors in kidney transplant. *World J Transplant* 2017; 7(2): 144-151 Available from: URL: http://www.wjgnet.com/2220-3230/full/v7/i2/144.htm DOI: http://dx.doi.org/10.5500/wjt.v7.i2.144

INTRODUCTION

Inventions in medical science enhance life which has been realized in the concept of kidney transplantation and add significant amount of productive years to the patients of chronic kidney disease[1]. The calcineurin inhibitors (CNIs), cyclosporine A (CsA) and tacrolimus (Tac) were instituted in clinical practice in 1980's. and established themselves as an effective immunosuppressive agent with more than 90% one-year graft survival whilst maintaining a rejection rate of less than 20%[2]. Anyhow, the superlative results of short-term allograft survival have not been maintained for long that could be because of slow, steady decline in renal functioning as, eGFR reduced to below 50% in a span of ten years[3]. Studies have reported chronic allograft nephropathy as the most common cause of late graft loss in 40% kidney transplant patients, whilst the mortality incidence with delayed functioning graft (DFG) was reported in 43% cases. The cardiovascular diseases and malignancies are considered as the most important causes of DFG in transplant patients[4].

The CNI induced nephrotoxicity is considered as an important cause of long-term graft failure in 96.8% of allograft biopsies by virtue of increased production of vasoconstrictors, such as thromboxane and endothelin, together with decreasing the turn-out of vasodilators, such as nitric oxide, prostaglandin E2, and prostacyclin[5,6]. Nankivell *et al*[7] (2004) outlined that more than 50% of kidney allograft biopsies unveiled attestation of chronic CNI toxicity following ten years transplant as 79.2%-100% exhibit histological alterations as tubular atrophy, nodular arteriolar hyalinosis, tubular vacuolization, luminal narrowing, interstitial fibrosis, focal or global segmental sclerosis and micro-calcifications. Surprisingly, the reward of minimal early acute rejection has not been translated into any long term benefits. In addition, CNIs have been associated with development of various cardiovascular risk factors such as hyperlipidemia, hypertension, and new onset diabetes mellitus after transplantation[8,9].

However, the biggest challenge with immunosuppression therapy is to maintain the balance of immunosuppression need in order to avert any rejection episode whilst keeping the check on the toxicities. The recent introduction of better and more efficient non-nephrotoxic immunosuppressive agents such as the mammalian target of rapamycin (mTOR) inhibitors, sirolimus (SRL) and everolimus (EVR), with mechanism of action similar to that of CNIs, forms the basis of use of these drugs[10,11].

CNIs as Tacrolimus (Tac) and Cyclosporin A (CsA) attach with the intracellular proteins called FKBP and immunophilins to form complex which blocks the effect of calcineurin which normally potentiates the intracellular processes associated with the activation of T-lymphocytes. This causes decreased production of interleukin-2 and inhibit the proliferation of T-cells[12,13].

In the similar manner mTOR inhibitors as SRL and EVR form a complex with FKBP to reduces T-cell activation by blocking growth-factor-mediated cell proliferation in the response to an alloantigen[14-17]. The distinct immunological properties with and limited nephrotoxic potential of mTOR-inhibitors have prevailed clinicians to use them as a surrogate to CNIs in renal transplantation[18-21].

The main aim of this review is to focus on the short term benefit early conversion to mTOR-inhibitors with or without CNI in renal transplant recipients in terms of graft functioning and graft survival.

MATERIALS AND METHODS

This meta-analysis was performed following registration in PROSPERO an international database of prospectively registered systematic reviews (CRD42017054458). An extensive search of all the published literature on the role of early conversion to mTOR inhibitors as an alternative to CNI has been made on National Library of Medicine Database (PubMed), EMBASE, Cochrane, Crossref, and Scopus databases on 30th August 2016. The search covered the period 2001 (the year of the first reported early CsA withdrawal with sirolimus in the literature) to September 30th, 2016[22]. The following medical subject headings (MeSH) terms: "Adverse events", "calcineurin inhibitors", "cyclosporin",

Table 1 Criteria for the inclusion of early mammalian target of rapamycin inhibitor conversion studies

Study design	Prospective cohort design with a well-defined study population
Study group	Post renal transplant
Conversion time	Period of 2 wk to 6 mo post-transplant
Study size	> 30 patients
Length of follow-up	Any
Source	Peer-reviewed journals
Language	English
Outcome measure	Patient safety, exposure-response relationships, adverse events, and graft functioning and long-term survival

"everolimus", "graft rejection", "graft survival", "kidney transplantation", "mTOR inhibitors", "sirolimus", "tacrolimus" were searched.

Study selection methodology

The original English literature articles published between 2001-September 2016 were included. Only studies which systematically and quantitatively assessed the graft functioning and graft survival of more than or equal to 12 mo following early conversion to mTORI with or without CNI in different randomised clinical studies were analysed. All kind of comparative studies, retrospective and prospective were included. We have excluded publications as editorials, reviews and letters (Table 1).

Data extraction

Two separate physician reviewers Kumar J, Reccia I reviewed all the articles. Disagreements were resolved through discussion, whilst in scenarios were consensus could not be achieved were resolved by a third author (Ahmed Halawa). We have analysed all papers with empirical studies using a standardised quality assessment tool and pre-specified inclusion and exclusion criteria. The present meta-analysis was performed using the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines and registered in PROSPERO an international database of prospectively registered systematic reviews (Figure 1).

Statistical analysis

The QUADAS-II (quality assessment of diagnostic accuracy studies-II) based analysis was done to assess the internal validity of pre-specified inclusion and exclusion criteria of the various studies. QUADAS-2 is an evidence-based bias assessment tool to evaluate the quality of diagnostic accuracy studies in a systematic review.

A total of six peer-reviewed multi-institutional studies were included in the present meta-analysis. We reviewed each study comprehensively, and data were extracted for the outcomes such as patient safety, exposure-response relationships, adverse events, and various shortcomings or weaknesses to improve the graft functioning and long-term survival (Table 2).

Review Manager (RevMan) Version 5.3 was used to analyse continuous and dichotomous trial data when at least two trials reported. Odds ratios (OR) for dichotomous outcomes, mean difference (MD) for continuous outcomes including a 95%CI, heterogeneity between the trials was measured using the statistic with > 30% considered as significant. The random effects model was used in cases of significant heterogeneity by visualizing the forest plot of involved trials.

RESULTS

The initial search yielded a total of 112 manuscripts. After careful evaluation, 98 articles were excluded on basis period of introduction was not within six months of transplantation. Eventually, a total of six articles matched the previously described inclusion criteria, *i.e.*, ZEUS trial (2011), CENTRAL trial (2012), CONCEPT trial (2009), SMART trial (2010), Spare the Nephron trial (2010)[23-27] and Heilman *et al*[28] (2011) (Table 2). The comprehensive data of all these studies summarizing the renal functioning, Biopsy Proven Acute rejection (BPAR), survival and adverse events were included in Table 3, below we have further analyzed these studies in the time frame of 12 mo following transplantation.

Renal function

The 12 mo estimated renal function (eGFR) was significantly better in the mTOR inhibitor group compared to CNI group (six trials, 1257 patients, mean difference 5.24 mL/min per 1.73 m^2, 95%CI: 2.18 to 8.29, $P = 0.00$, $I^2 = 70\%$) (Figure 2). Similarly, the measured serum creatinine was significantly lower in the mTOR inhibitors groups at 12 mo (six trials, 1256 patients, mean difference = -11.59 μmol/L, 95%CI: -20.08 to -3.09, $P < 0.00$, $I^2 = 73\%$) (Figure 3).

BPAR

The incidence of BPAR was significantly higher in mTORs groups compared to CNIs groups (six trials, 1265 patients, OR = 2.11, 95%CI: 1.43 to 3.11, $P = 0.00$, $I^2 = 3\%$) at 12 mo (Figure 4).

Graft survival and adverse events

At 12 mo, the rates of graft survival were comparable for mTOR inhibitor group and the CNI groups (Table 3). There was no significant difference in the incidence of serious adverse events/infection between the mTOR inhibitors and CNI groups in majority of studies.

DISCUSSION

The initiation of mTOR-inhibitors in early post-transplant period is one of the arduous decision taken by clinicians as it should be done following the period of the heightened immunological risk is over, but no evidence of CNI related toxicity evolved[29,30]. Various

Table 2 Summary of Different Early Conversion Clinical Trials

Ref.	Study design	Time of conversion	Group 1	Group 2
Everolimus				
Budde *et al*[23], 2011 (ZEUS Study)	Multicentre, Prospective, Randomized Study (n = 300), 12 mo	4.5th month	EVR (C0, 6-10 ng/mL) Induction: Basiliximab (n = 155)	CsA (C0, 120-180 ng/mL till 4.5-6 mo then decreased to 100-150 ng/mL) Induction: Basiliximab (n = 145)
Mjörnstedt *et al*[24], 2012 (CENTRAL trial)	Multicentre, Prospective, Randomized Study, (n = 269), 12 mo	7th week	EVR (C0, 6-10 ng/mL) + MMF (1.4 g/d till 2 wk then decreased to 1.08 g/d) + S (n = 92)	Low CsA (C0, 75-200 ng/mL till 2 wk then decreased to 50-150 ng/mL) + MMF (1.4 g/d) + S (n = 90)
Sirolimus				
Lebranchu *et al*[25], 2009 (CONCEPT Study)	Multicentre Prospective, Randomized Study, (n = 193), 12 mo	3rd month	SRL (C0, 8-15 ng/mL till 39 wk then decreased to 5-10 ng/mL) + MMF + S (Induction: Daclizumab) (n = 95)	CsA (C0, 500-800 ng/mL) + MMF + S (Induction: Daclizumab) (n = 97)
Guba *et al*[26], 2010 (SMART Trial)	Multicentre Prospective, Randomized Study, (n = 140), 12 mo	10-24th day	SRL (C0, 8-12 ng/mL then decreased to 5-10 ng/mL) + MMF (1.5 g/d) + S (Induction: ATG) (n = 69)	CsA (C0, 150-200 ng/mL then decreased to 100-150 ng/mL) + MMF (2 g/d) + S (Induction: ATG) (n = 71)
Weir *et al*[27], 2010 (Spare the Nephron Trial)	Multicentre, Prospective, Randomized Study, (n = 299), 12 mo	Within 115 d	MMF + SRL (n = 148)	MMF + CNI (n = 151)
Heilman *et al*[28], 2011	Multicentre Prospective, Randomized Study, (n = 122), 12 mo	1 mo	SRL (C0, 9.8 ± 3.6 ng/mL) + MMF + S (Induction: Basiliximab) (n = 62)	TAC (C0, 6.9 ± 4.6 ng/mL) + MMF + S (Induction: Basiliximab) (n = 60)

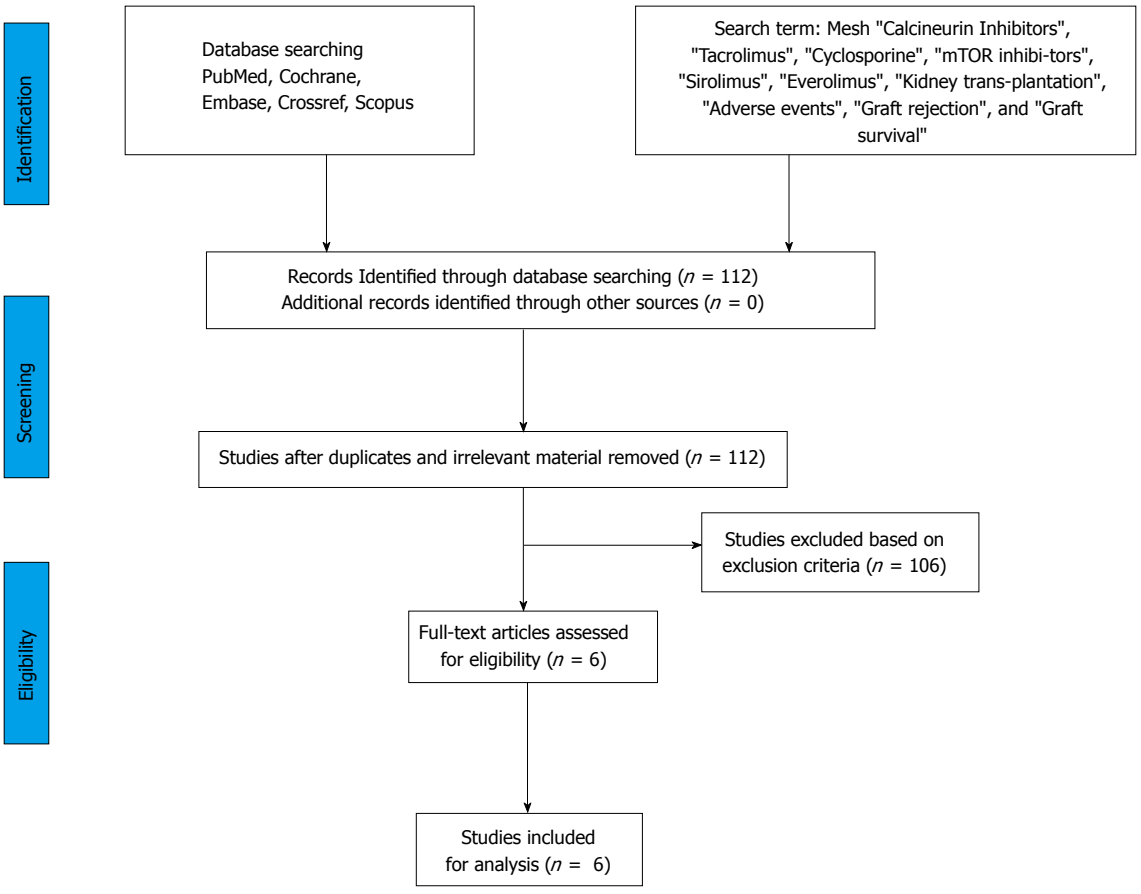

Figure 1 Search strategy and study selection used in this systematic review as per PRISMA protocol.

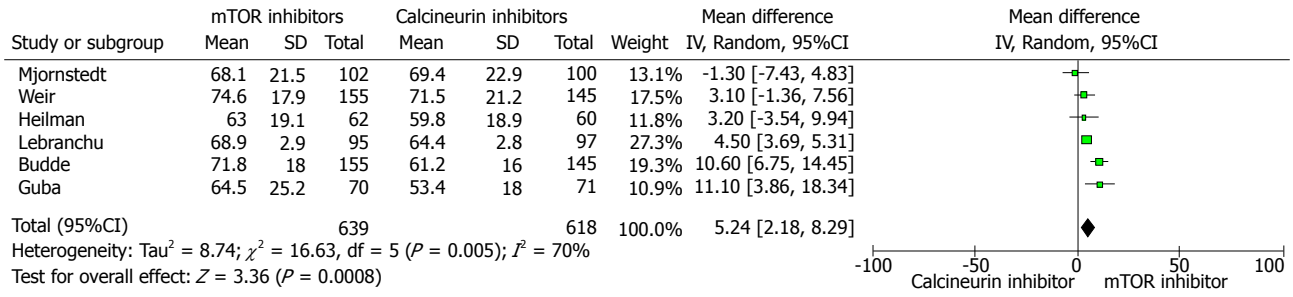

Figure 2 Forest plot represents the glomerular filtration rate at 12 mo in kidney transplant recipients when treated with mammalian target of rapamycin inhibitor or calcineurin inhibitor therapy. Squares represent size effects of studies, comparing the weight of the study in the meta-analysis. The diamond summary effect shows significant favour towards mTOR inhibitors. 95%CIs represented in horizontal bars. mTOR: Mammalian target of rapamycin.

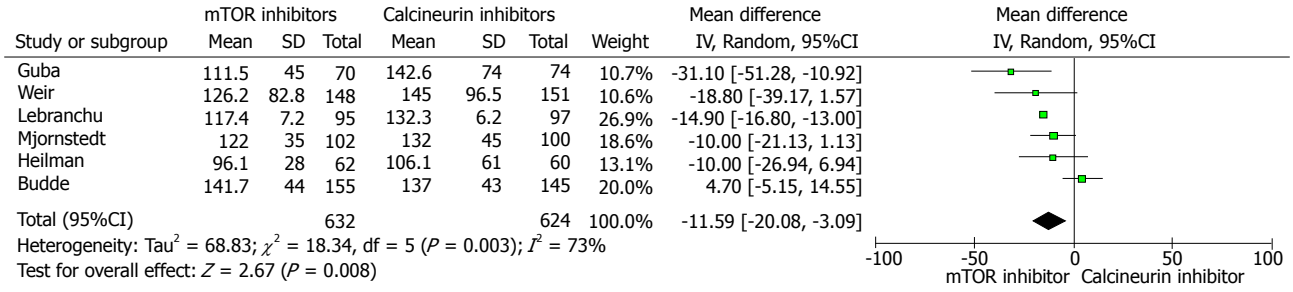

Figure 3 Forest plot represents the serum creatinine at 12 mo in kidney transplant recipients when treated with mammalian target of rapamycin inhibitor or calcineurin inhibitor therapy. Squares represent size effects of studies, comparing the weight of the study in the meta-analysis. The diamond shows summary effect towards mTOR inhibitors with 95%CIs represented in horizontal bars. mTOR: Mammalian target of rapamycin.

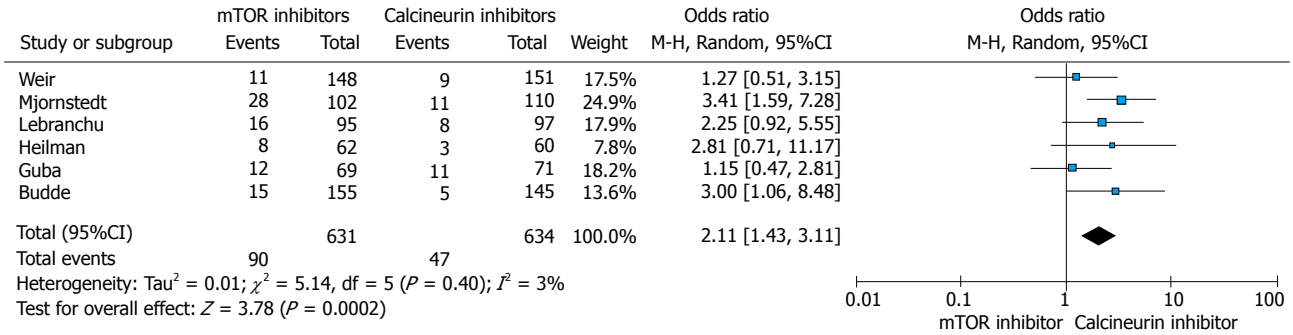

Figure 4 Forest plot represents the biopsy proven acute rejection at 12 mo in kidney transplant recipients when treated with mammalian target of rapamycin inhibitor or calcineurin inhibitor therapy. Squares represent size effects of studies, comparing the weight of the study in the meta-analysis. The meta-analysis significantly favours CNI, with 95%CIs represented in horizontal bars. CNI: Calcineurin inhibitor.

CNI free or reduced dosing regimens have been tried to minimize nephrotoxic adverse effect. The peril of increased risk of rejection with the denovo use of CNI free protocols, has been pared down with the early introduction mTOR inhibitors. Howbeit, data regarding optimal transmutation time to mTOR inhibitor based immunosuppression is not clear. Though, the present literatures support the notion of early conversion to mTOR inhibitors within the six months of transplant whereas the reward of conversion after month 6 is not that encouraging. The major hindrance in the expected outcome following late conversion might be because the CNI related nephrotoxicity has already settled in[23,25].

In the present rationale, mTOR inhibitors should be introduced within a period of 2 wk to 6 mo, *i.e.*, following the period of increased risk for rejection and wound infection has been over.

In a ZEUS study, which was multicenter randomised trial done by Budde *et al*[23] (2011) considered early conversion from CsA to everolimus at 4.5 mo after renal transplantation. Two hundred and sixty-nine patients were randomised into two groups the first group received everolimus with MMF, while another group was maintained on gradually tapered lower dose of CsA with MMF. The group has reported a statistically significant improvement in renal functioning, *i.e.*, eGFR for the everolimus group (71.8 ± 18 mL/min *vs* 61.2 ± 16 mL/min; $P = 0.000$), at 12 mo while, BPAR was a higher in the everolimus group (13.9% *vs* 7.5%, $P = 0.09$). Nevertheless, they heralded no difference in terms of graft and patient survival[23].

In a CENTRAL trial by Mjörnstedt *et al*[24] (2012)

Table 3 Summary of outcomes in Different Early Conversion Clinical Trials

Ref.	Renal function (Gp1 *vs* Gp 2)	BPAR (Gp1 *vs* Gp 2)	Adverse event (Gp1 *vs* Gp 2)	Remarks
Everolimus				
Budde *et al*[23], 2011, (ZEUS Study)	12 mo Sr. Cr: 141.7 ± 44 μmol/L *vs* 137.0 ± 43 μmol/L (*P* = NS) eGFR: 71.8 ± 18 mL/min *vs* 61.2 ± 16 mL/min (*P* = 0.000)	9.7% *vs* 3.4% (*P* = 0.03)	SAE/Infection: 61% *vs* 59% (*P* = NS) UTI: 57.0% *vs* 53% (*P* = NS) Diarrhoea: 36% *vs* 27% (*P* = NS) HPL: 14% *vs* 10% (*P* = NS)	Graft survival: 100% *vs* 100% (*P* = NS) Patient survival 100% *vs* 99% (*P* = NS)
Mjornstedt *et al*[24], 2012 (CENTRAL trial)	12 mo Sr. Cr: 122.0 ± 35 μmol/L *vs* 132.0 ± 45 μmol/L (*P* = NS) eGFR: 68.1 ± 21.5 mL/min *vs* 69.4 ± 22.9 mL/min (*P* = NS)	27.5% *vs* 11.0% (*P* = 0.004)	SAE/Infection: 53.9% *vs* 38.0% (*P* = 0.025) CMV infection: 8.8% *vs* 13.0% (*P* = NS) Edema: 29.4% *vs* 21.0% (*P* = NS) Anaemia: 16.7% *vs* 6.0% (*P* = 0.02) HPL: 12.7% *vs* 9.0% (*P* = NS) Proteinuria: 4.9% *vs* 0% (*P* = 0.06) Acne: 12.7% *vs* 2.0% (*P* = 0.006) Mouth Ulceration: 12.7% *vs* 2.0 % (*P* = 0.001)	Graft survival: 100% *vs* 100% (*P* = NS) Patient survival 98% *vs* 98% (*P* = NS)
Sirolimus				
Lebranchu *et al*[25], 2009 (CONCEPT Study)	12 mo: Sr. Cr: 117.4 μmol/L *vs* 132.3 μmol/L (*P* < 0.001) eGFR: 68.9 mL/min *vs* 64.4 mL/min (*P* = 0.017)	16.8% *vs* 8.2% (*P* = NS)	Peripheral Edema: 28.1% *vs* 22.6% (*P* = NS) SAE/infection: 60% *vs* 44% (*P* = 0.025) Diarrhoea: 30.2% *vs* 9.2% (*P* < 0.001) Dyslipidemia: 5.20% *vs* 4.12% (*P* = NS) Proteinuria: 9.3% *vs* 3.09 % (*P* = NS) NODAT: 3.1% *vs* 2.06% (*P* = NS) Apthous Stomatitis: 45.8% *vs* 5.15% (*P* < 0.001)	Graft Survival: 99% (*P* = NS) Patient Survival 97% (*P* = NS)
Guba *et al*[26], 2010, (SMART Trial)	12 mo: Sr Cr: 111.5 ± 45 mg/dL *vs* 142.6 ± 74 mg/dL (*P* = 0.004) eGFR: 64.5 ± 25.2 mL/min *vs* 53.4 ± 18.0 mL/min (*P* = 0.001)	17.4% *vs* 15.5% (*P* = NS)	Wound Healing Disorder: 10.1% *vs* 11.3%, (*P* = NS) Infection: 52.2% *vs* 60.6% (*P* = NS) CMV: 7.3% *vs* 28.2% (*P* < 0.001) HPL: 20.3% *vs* 7.0% (*P* = 0.02) Diarrhoea: 13.0% *vs* 9.9% (*P* = NS) Lymphocele: 27.5% *vs* 23.9% (*P* = NS)	Graft Survival: 99% *vs* 97% (*P* = NS) Patient Survival 99% *vs* 99% (*P* = NS)
Weir *et al*[27], 2010 (Spare the Nephron Trial)	12 mo Sr. Cr: 126.2 ± 82.8 μmol/L *vs* 145.0 ± 96.5 μmol/L (*P* = NS) eGFR: 74.6 ± 17.9 mL/min *vs* 71.5 ± 21.2 mL/min (*P* = 0.06)	7.4% *vs* 6.0% (*P* = NS)	Infection: 16.2% *vs* 18.3% (*P* = NS) HPL: 24.3% *vs* 10.5% (*P* = 0.000) CMV: 4.7% *vs* 9.2% (*P* = NS) Polyoma virus: 2% *vs* 4% (*P* = NS) Diarrhoea: 29.7% *vs* 9.8% (*P* = 0.001) Malignancy: 4.7% *vs* 6.5% (*P* = NS)	Graft Survival: 98% *vs* 97.4% (*P* = NS) Patient Survival 100% *vs* 98% (*P* = NS)
Heilman *et al*[28], 2011	12 mo Sr. Cr: 96.1 ± 28 μmol/L *vs* 106.1 ± 61 μmol/L (*P* = NS) eGFR: 63.0 ± 19.1 mL/min *vs* 59.8 ± 18.9 mL/min (*P* = NS)	13% *vs* 5% (*P* = NS)	CMV: 13% *vs* 13% (*P* = NS) Polyoma virus: 2% *vs* 4% (*P* = NS)	NA

eGFR: Estimated renal function; NA: Not Available; Not Significant; NODAT: New-onset diabetes after transplantation; CMV: Acute cytomegalovirus.

they studied the effect of early conversion from CsA to everolimus in the seventh week of the post-transplant. About two hundred and two patients who were randomised to receive intervention group everolimus (C0, 3-8 ng/mL) and were compared with CsA (C0, 75-200 ng/mL for two weeks then reduced, further maintained at 50-150 ng/mL) with oral steroids and MMF. They didn't report significant improvement in GFR in everolimus group (68.1 ± 21.5 mL/min *vs* 69.4 ± 22.9 mL/min, *P* = NS) at 12 mo, although serum creatinine was lower in mTOR inhibitor group (122.0 ± 35 μmol/L *vs* 132.0 ± 45 μmol/L, *P* = NS).

Though the reported incidence of BPAR was significantly higher in EVR group than in CsA group (27.5% *vs* 11.0%, *P* = 0.004), the survival outcomes were similar at 12 mo. The reported side effects as proteinuria, anaemia, hyperlipidemia, acne and mouth ulceration were significantly more frequent in the everolimus group[24].

In the CONCEPT study 2009 by Lebranchu *et al*[25], instituted Sirolimus by replacing CsA in the third month of the post-transplantation. Their literature listed significantly better eGFR (68.9 mL/min *vs* 64.4 mL/min) and significantly lower serum creatinine (117.4 μmol/L *vs* 132.3 μmol/L, *P* < 0.001) in the sirolimus group at 12 mo. The detailed BPAR was similar for entire period of observation. The side effects such as diarrhoea, SAE, aphthous stomatitis, proteinuria and new onset diabetes mellitus were either significantly higher or higher in the sirolimus group[25].

Guba *et al*[26] (2010) carried out a multicenter randomised SMART trial, to explore the effects of very early conversion to sirolimus from CsA only 10 to 24 d after the renal transplantation. They randomised one hundred and forty-one patients were into two groups to confer sirolimus with MMF and steroid, on the other hand the second group was maintained on gradually tapered lower dose of CsA with MMF and steroid. They

reported statistically significant improvement in renal functioning, eGFR (64.5 ± 25.2 mL/min *vs* 53.4 ± 18 mL/min; *P* = 0.001) with significantly reduced serum creatinine (111.5 ± 45 μmol/L *vs* 142.6 ± 74 μmol/L, *P* = 0.004) for the sirolimus group at 12 mo. The detailed incidence of BPAR (17.4% *vs* 15.5%, *P* = NS) was similar in both groups, likewise, the graft and patient survival were quite similar. In addition, the recipients in the sirolimus group reported a significantly higher number of adverse effects such as acne, hyperlipidemia and lower number CMV viremia withal the incidence of BPAR was similar in both groups (20.2% *vs* 19.7%, *P* = NS)[26].

In Spare the Nephron Trial, Weir *et al*[27] (2010) randomized 299 kidney transplant recipients into two groups following 115 d of the transplant. The first group received sirolimus with MMF while the second group was maintained on CNI and MMF. They reported significant improvement in renal function in terms of higher eGFR (74.6 ± 17.9 mL/min *vs* 71.5 ± 21.2 mL/min; *P* = 0.06) and lower serum creatinine (126.2 ± 82.8 μmol/L *vs* 145.0 ± 96.5 μmol/L, *P* = NS) in the sirolimus group. They delineated the likewise patient and graft survival in both groups. However, patients in the sirolimus group reported a significantly higher number of adverse effects as hyperlipidemia and diarrhoea[27].

In the 2011 study by Heilman *et al*[28], sirolimus introduced in the first month of the renal transplant. They have given the account of significant improvement in eGFR (63.0 ± 19.1 mL/min *vs* 59.8 ± 18.9 mL/min; *P* = NS) and set out lower serum creatinine in the sirolimus group at 12 mo while the reported BPAR was likewise in both groups[28].

Publication bias is an important point to consider in a meta-analysis because all the researches which take place are not published. Studies with a significant result are more likely to be published. Studies with a significant result are more likely to be placed in a higher impact journal compared to the studies with null results. Moreover, well controlled and properly carried out studies are less likely to achieve significance.

In general, early CNI withdrawal in the wake of mTOR inhibitor based regimen institution seems a more empirical and constructive approach towards immunosuppressive management of renal transplant recipients. Natheless, taking into account of the high rejection rate contemplated in these studies, it will be a judicious decision of not to proffer this therapy to patients with moderate to high immunological risk though additional studies with long duration of follow-up are demanded to confirm present conjecture[29-33].

Despite the fact that the data on the Tac minimization strategies are limited, the present evidence suggest that treatment with mTOR-inhibitors allows early and substantial CNI minimization and provides better renal functioning at the end of first year of transplantation. Thus, it is not judicious to extend these regimens to patients with moderate to high immunological risk. However, further trials directed towards different ethnicity and geography are needed to determine further evidence.

COMMENTS

Background

The aim of this review is to assess the one-year effectiveness of the early introduction of mammalian target of rapamycin (mTOR) inhibitors with or without calcineurin inhibitors (CNIs) within six months of renal transplantation.

Research frontiers

The current literature was reviewed to assess the role of immunosuppressive agent, mTOR inhibitors as an alternative to CNI within six months of renal transplant in terms of better renal functioning and survival by assessing glomerular filtration rate (GFR), serum creatinine, Biopsy Proven Acute Rejection (BPAR) and survival.

Innovations and breakthroughs

The major advantages were observed regarding better renal functioning, GFR and serum creatinine were better in mTOR inhibitor group at 12 mo. BPAR was significantly higher in the mTOR-inhibitor group though survival was comparable.

Application

In general, early CNI withdrawal seems to be a more empirical and constructive approach as it provides better renal functioning in the low immunological risk transplant recipients.

Peer-review

This study is a systemic review and meta-analysis of the effect on renal function and graft survival following early conversion of CNI to mTOR inhibitors with or without CNI after kidney transplantation. The authors initially selected 112 manuscripts, and of them, only 6 papers were useful for meta-analysis. They conclude that introduction of mTOR-inhibitors allows early and substantial CNI minimization.

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META-ANALYSIS

Living related and living unrelated kidney transplantations: A systematic review and meta-analysis

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Abstract

AIM

To compare the outcomes between related and unrelated kidney transplantations.

METHODS

Literature searches were performed following the Cochrane guidelines. We conducted a systematic review and a meta-analysis, which included 12 trials that investigated outcomes including the long-term (ten years), mid-term (one to five years), and short-term (one year) graft survival rate as well as the acute rejection rate. Meta-analyses were performed using fixed and random-effects models, which included tests for publication bias and heterogeneity.

RESULTS

No difference in graft survival rate was detected in patients who underwent living related kidney transplantations compared to unrelated (P = 0.44) transplantations after ten years. There were no significant differences between the graft survival rate in living related and unrelated kidney transplantations after a short- and mid-term follow-up (P = 0.35, P = 0.46). There were no significant differences between the acute rejection rate in living related and unrelated kidney transplantations (P = 0.06).

CONCLUSION

The long, mid and short term follow-up of living related and unrelated kidney transplantation showed no significant difference in graft survival rate. Also, acute rejection rate was not significantly different between groups.

Key words: Transplantation; Living related; Living unrelated; Graft survival rate

Core tip: The long, mid and short term follow-up of living related and unrelated kidney transplantation showed no significant difference in graft survival rate. Also, acute rejection rate was not significantly different between groups.

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INTRODUCTION

Renal failure is a disease with a high rate of morbidity and mortality. By the end of 2001, with the help of dialysis and renal transplantations, approximately 1479000 people were kept alive. This number increased to 1783000 by the end of 2004[1]. Nowadays, renal transplantation has become the optimal treatment for patients with end-stage renal disease[2]. The recipients of renal transplant had a higher quality of life and a greater survival rate in comparison to patients who underwent dialysis. Due to these results, the demand of renal transplantations has increased over time, but the gap between supply and demand has widened. Consequently, the number of patients who are on the renal transplant waiting list for deceased-donor transplantation has increased and thousands of patients have died while waiting for their renal transplantation. This has made it necessary to search for alternatives.

During the past two decades, several approaches have been adopted to increase living related organ donations, but living unrelated donors remain an underutilized source. The result of living unrelated transplantations was widely disputed. While the Brazilian[3], Iranian[4,5], and Egyptian[6] experiences resulted in excellent outcomes that were superior to those in cadavers and were comparable to living related-donor transplantations, there were contradictory reports in several studies[7,8]. To our knowledge, there was no systematic review and meta-analysis that evaluated outcomes in patients who underwent living related *vs* unrelated kidney transplantations. This systematic review and meta-analysis was designed to compare the outcomes including the long-, mid- and short-term graft survival rate, and the acute rejection rate between related and unrelated kidney transplantations.

MATERIALS AND METHODS

Literature search

The review was conducted in accordance with the guidelines described in the Cochrane handbook for the systematic review and meta-analysis of interventions.

Eligibility criteria and study characteristics

The criteria for studies included the following: (1) the patients considered had undergone living related or unrelated kidney transplantations; (2) the study involved the comparison of the outcomes in patients whom underwent kidney transplantation from related *vs* unrelated kidney donations; and (3) the primary outcome was long-term (ten years) graft survival rate, while the secondary outcomes were short-term (one year) and mid-term (one to five years) graft survival rate and acute rejection rate.

Both English language studies and non-English language studies were included in the meta-analysis.

Study identification and data abstraction

Two independent reviewers completed a systematic computerized search of online databases, including PubMed, Ovid, MEDLINE, EMBASE, the Cochrane Controlled Trials Register, HealthSTAR, CINAHL, Google, and Google Scholar to locate studies exploring the evaluation outcomes of patients who underwent kidney transplantation from living related *vs* unrelated kidney donations published in any language throughout March 2016. The keywords used for the search included kidney transplant, related, unrelated, and living. Thereafter, a search on MEDLINE was refined to clinical trials. We also searched the Cochrane Database of Systematic Reviews, the Cochrane Central Register of Controlled Trials, Clinical Trials (www.clinicaltrials.gov), Centre watch (www.centerwatch.com), Trials Central (www.trialscentral.org/ClinicalTrials.aspx), and the United Kingdom National Research Register (www.nrr.nhs.uk).

After reviewing the titles of these studies, we retrieved the abstracts that were appropriate for use in our study. We independently reviewed these abstracts and chose those studies that were potentially relevant to our work. We reviewed the bibliographies of all of the studies that were included to identify any additional studies which required inclusion. A data-extraction form was designed and agreed upon by the authors. Initially, two authors independently extracted the data, which were later reviewed jointly to reach an agreement on its accuracy. The data that were collected from all the manuscripts included the following fields: Number of patients, mean follow-up, recipient mean age, recipient sex, Immunosuppression regimen, the short-term, mid-term and long-term survival rate and the acute rejection rate, mean serum creatinine at 1 year and final follow-up, and post-transplant infectious complications. Disagreements were resolved by consensus or consultation with senior authors (Table 1). The authors of individual trials were contacted directly to provide additional information when necessary. We analysed the quality of studies with a questionnaire and only the studies that had a score greater than eight were included in our study (Table 2). In cases where the full text or data were not accessible, we tried to contact the authors in order to have them provided.

Table 1 Study design

Patients	Patients underwent kidney transplantation
Literature search	Keyword search in PubMed, Google scholar and Scopus
Databases	Pubmed, Ovid, MEDLINE, EMBASE, the Cochrane Controlled Trials Register, HealthSTAR, CINAHL, Google, and Google Scholar
Limits	Only comprehensive articles without time limit Humans In English
Keywords	Kidney transplantation Renal transplant Related Unrelated
Eligibility criteria	Article in Full-text (no abstracts) Unique publication (no duplicate articles) Reported each of the interested outcomes (graft survival rate, and acute rejection rate) Original report as determined from reading the abstract or if necessary the full text Outcome reported in a usable form (each surgical approach was reported as a separate cohort, no additional confounding treatments, no missing or unreliable data; could not have > 10% difference in values between text and tables Reported on surgical approaches of interest
Exclusion criteria	Duplicate patient population, where some or all of the same patients were included in a different study reporting on the same parameters (prevents double counting) Early case experience (prevents bias toward approaches with more experienced surgeons)
Data abstraction	Articles needed to report which contain each of outcome of interest to be included in the analysis Data were abstracted by two individuals into a custom database table including list of variables. 50% of articles were abstracted by one reviewer and other 50% with other one. The data for 50% of the articles was double-entered by a second individual, and any discrepancies were resolved through repeated review and discussion prior to data analysis All primary outcomes were then double-checked and any discrepancies resolved Variables in four types were abstracted from each study: Those necessary to determine inclusion and exclusion criteria, surgical approach, baseline patient characteristics, and clinical outcomes All studies were reviewed by two independent reviewers using the total QASs (Table 3) to assess the methodological quality of the studies that were included. Although the QASs were reported for each study, they were not used to weight the studies in the meta-analysis
Primary outcomes	Graft survival rate
Secondary outcomes	Acute rejection rate
Controls for Publication bias	Performed a funnel plot analysis

QASs: Quality assessments.

Table 2 Quality assessment items and possible scores

Was the assigned treatment adequately concealed prior to allocation?
2 = method did not allow disclosure of assignment
1 = small but possible chance of disclosure of assignment or unclear
0 = quasi-randomized or open list/tables
Were the outcomes of participants who withdrew described and included in the analysis (intention-to-treat)?
2 = withdrawals well described and accounted for in analysis
1 = withdrawals described and analysis not possible
0 = no mention, inadequate mention, or obvious differences and no adjustment
Were the outcome assessors blinded to treatment status?
2 = effective action taken to blind assessors
1 = small or moderate chance of unblinding of assessors
0 = not mentioned or not possible
Were the treatment and control groups comparable at entry? (likely confounders may be age, partial or total rupture, activity level, acute or chronic injury)
2 = good comparability of groups, or confounding adjusted for in analysis
1 = confounding small; mentioned but not adjusted for
0 = large potential for confounding, or not discussed
Were the participants blind to assignment status after allocation?
2 = effective action taken to blind participants
1 = small or moderate chance of unblinding of participants
0 = not possible, or not mentioned (unless double-blind), or possible but not done
Were the treatment providers blind to assignment status?
2 = effective action taken to blind treatment providers
1 = small or moderate chance of unblinding of treatment providers
0 = not possible, or not mentioned (unless double-blind), or possible but not done
Were care programes, other than the trial options, identical?
2 = care programes clearly identical
1 = clear but trivial differences
0 = not mentioned or clear and important differences in care programes
Were the inclusion and exclusion criteria clearly defined?
2 = clearly defined
1 = inadequately defined
0 = not defined
Were the interventions clearly defined?
2 = clearly defined interventions are applied with a standardized protocol
1 = clearly defined interventions are applied but the application protocol is not standardized
0 = intervention and/or application protocol are poorly or not defined
Were the outcome measures used clearly defined? (by outcome)
2 = clearly defined
1 = inadequately defined
0 = not defined
Were diagnostic tests used in outcome assessment clinically useful? (by outcome)
2 = optimal
1 = adequate
0 = not defined, not adequate
Was the surveillance active, and of clinically appropriate duration?
2 = active surveillance and appropriate duration
1 = active surveillance, but inadequate duration
0 = surveillance not active or not defined

Statistical analysis

The Review Manager Database (RevManversion 5.0, The Cochrane Collaboration 2008) was used to analyse the selected studies. Continuous data for each arm of a particular study were expressed as mean and standard deviation. Dichotomous data were expressed as proportions or risks, with the treatment effect reported as a relative risk with 95%CI.

The data were analysed for the outcomes that were of interest to us. The risk ratio (RR) was defined as the number of patients with a successful graft survival rate. The RR referred to the multiplication of the rate of graft

surveillance that occurred with the use of related and unrelated kidney transplantations. The heterogeneity between the studies was assessed using the χ^2 test and the I^2 statistic. The latter is a measure of the percentage of variation in data that results from heterogeneity as opposed to chance. A P value of < 0.1 and an I^2 value > 50% were considered suggestive of statistical heterogeneity, prompting a random effects modelling estimate. Conversely, a non-significant chi-squared test result (a P value $\geqslant$ 0.1 and an I^2 value $\leqslant$ 50%) only suggested that there was no evidence of heterogeneity; it did not necessarily imply that homogeneity existed because there may have been insufficient power to detect heterogeneity. The Mantel-Haenszel (M-H) method was used to combine the studies. If their significant heterogeneity were indicated (P < 0.1 and I^2 > 50%), a random-effect model was used; if not, a fixed-effect model was used. In addition, funnel plots were constructed for the outcomes to assess publication bias, *i.e.*, the tendency not to publish studies with negative results; the more asymmetric the funnel plot is, the more potential bias there is. The statistical significance was set at P < 0.05.

RESULTS

Study selection

Using our search terms, 376 references were identified. The first search of studies exploring the evaluation of the outcomes of patients yielded the following results: PubMed (n = 11590), Ovid (n = 24), EMBASE (n = 3300), the Cochrane Controlled Trials Register (n = 9719), and Google Scholar (n = 1430). Out of these, we included 12 studies after applying our eligibility criteria to their titles and/or abstracts, excluding duplicates (Figure 1).

The eligible trials included 12 relevant comparisons (Table 3) involving 9954 participants. We could not assess the differences in the outcomes between post-operative infections, post-operative hypertension, diabetes, and post-operative creatinine due to the lack of data.

Study presentation

Cortesini *et al*[9] evaluated 527 kidney allografts from living donors. Of these, 302 living donors were first-degree relatives of the recipient and shared one haplotype (living related donor) and 172 were unrelated. They showed actuarial graft survival rates in the living related and living unrelated groups, which were 91% and 87% in 1 year, 77% and 79% in 5 years, and 66% and 69% in 9 years. In conclusion, they reported that kidney transplantation between unrelated donors and recipients might be a valid alternative in view of the cadaver organ shortage, its success as a procedure and its potential to provide the "gift of life" to both the patient and the family.

Voiculescu *et al*[10] evaluated 62 out of 112 potential living donors for types of rejections, complications, and kidney functions. Of them, 38 cases were living related and 24 cases were living unrelated. They showed that acute rejection rate was similar in both groups (52.2% *vs* 54.2%); however, there were more complications of infection in the living related group (66.7% *vs* 36.4%) and a trend showing more surgical complications in living related transplantations (28.9% *vs* 8.3%). They concluded that the results for the living unrelated group are equivalent to the living related transplantation group. They determined that careful selection of donors and recipients is a prerequisite for success.

Kizilisik *et al*[11] evaluated 109 living donor kidney transplants. Seventy-eight percent of living donors were from living related donors and 22% were from living unrelated donors. The resultant one- and three-year patient survival rates were 97.6% and 93.2%, with 1- and 3-year graft survival rates of 93.2% and 88.3%, respectively. Among the patients of Kizilisik *et al*[11], there were 6 delayed graft functions (5.5%), 16 acute cellular rejections (10%), and 10 chronic rejections (9%). They suggested that living donors represent a valuable source because of the limited number of cadaveric kidneys available for transplant and stated that the use of living-unrelated donors has produced an additional supply of organs.

Park *et al*[12] evaluated 77 living-donor renal transplants (41 were living unrelated and 36 were living related transplants). They reported that 11 recipients lost their grafts (6 from living unrelated and 5 from living related); most of these losses were due to chronic rejection (n = 7). Overall 3-, 5- and 10-year graft survival rates in live donors were 92.8%, 86.6% and 76.9%, respectively; for the living unrelated, the graft survival at 3-, 5- and 10-years was 91.9%, 88.5% and 74.7% *vs* 94%, 84% and 78.8% for the living related transplants. They concluded that acute rejection episodes markedly decreased long-term graft survival in live donor renal transplants, the use of living related transplants provides graft survival comparable with living related transplants, and proper management of acute rejection is essential for long-term graft survival.

Wolters *et al*[13] evaluated 95 living donor transplantations (69% related, 31% unrelated). They showed that at a mean follow-up of 35 mo, 94.7% of grafts were functioning. Three grafts were lost due to acute (in related transplants) or chronic (in unrelated transplants) rejection or due to multi-organ failures. They concluded that although HLA mismatching was significantly different between related and unrelated donors, no difference in the outcome was observed.

Simforoosh *et al*[14], between 1984 and 2004, evaluated 2155 kidney transplantations; out of this, 374 were from living related donors and 1760 were from unrelated donors. The resultant 1-, 3-, 5-, 10- and 15-year graft survival rates among the related group were 91.6%, 81.7%, 76.4%, 64.4% and 48.4%; and for unrelated group, these rates were 91.5%, 86.7%, 81.4%, 68.2%

Table 3 The characteristics of included study which reported related *vs* unrelated living kidney transplantation outcomes

Ref.	Number	Mean follow up (mo)	Recipient mean age (yr)	Recipient sex M/F	Immunosuppression regimen	One year graft survival rate	five years graft survival rate	10 yr graft survival rate	Acute rejection rate	Mean serum Cr at 1 yr	Mean serum Cr at final follow up	Post-transplant infectious complications
Cortesini *et al*[9], 2002	302 *vs* 172	42	32.8 ± 7.3 *vs* 44 ± 9.9	215/87 *vs* 133/39	Cyclosporine	275 (91) *vs* 150 (87)	232 (77) *vs* 136 (79)	199 (66) *vs* 118 (69)	N/D	1.9 ± 0.8 *vs* 2.0 ± 0.8	2.0 ± 0.8 *vs* 1.9 ± 0.8	N/D
Simforoosh *et al*[5], 2016	411 *vs* 3305	N/D	27.6 ± 10.1 *vs* 35.6 ± 15.6	270/138 *vs* 2164/1136	Cyclosporine	89% *vs* 90%	288 (70.2) *vs* 2697 (81.6)	225 (54.9) *vs* 2350 (71.1)	N/D	N/D	N/D	N/D
Voiculescu *et al*[10], 2003	38 *vs* 24	19.6 ± 15.4	37.7 ± 12.1 *vs* 53.6 ± 7.8	26/12 *vs* 14/10	Steroids, cyclosporine, mycophenolate mofetil	36 (94.8) *vs* 24 (100)	N/D	N/D	20 (52.5) *vs* 13 (54.2)	N/D	1.76 ± 0.6 *vs* 1.62 ± 0.5	25 (66.7) *vs* 9 (36.4)
Ahmad *et al*[15], 2008	261 *vs* 61	45	28 ± 16 *vs* 48 ± 12	N/D	Cyclosporine	247 (94.8) *vs* 60 (98.4)	N/D	N/D	107 (41) *vs* 21 (35)	N/D	N/D	N/D
Kizilisik *et al*[11], 2004	85 *vs* 24	36	N/D	N/D	Cyclosporine, azathioprine, steroid, tacrolimus, mycofenolatemofetil	81 (95) *vs* 23 (95.8)	75(88.3) *vs* 21 (87.5)	N/D	11(13) *vs* 5 (20)	N/D	N/D	7 (8.3) *vs* 8 (3.5)
Park *et al*[12], 2004	36 *vs* 41	N/D	33.6 *vs* 38.3	21/15 *vs* 28/13	Cyclosporine, steroid and mycophenolatemofetil	N/D	30 (84) *vs* 36 (88.5)	28 (78.8) *vs* 41 (74.7)	11 (30) *vs* 13 (31)	N/D	N/D	N/D
Wolters *et al*[13], 2005	66 *vs* 29	35	31 ± 12.5 *vs* 51 ± 8.5	41/25 *vs* 23/6	Cyclosporine/MMF/ prednisone *vs* MMF/prednisone	N/D	62 (94.7) *vs* 23 (94.7)	N/D	6 (9) *vs* 5 (17.2)	N/D	N/D	N/D
Simforoosh *et al*[14], 2006	374 *vs* 1760	45.68 ± 46.80	28.97 ± 9.58 *vs* 33.46 ± 14.61	N/D	Cyclosporine, azathioprine, and prednisone	342 (91.6) *vs* 1610 (91.5)	286 (76.4) *vs* 1432 (81.4)	241 (64.4) *vs* 1200 (68.2)	N/D	N/D	N/D	N/D
Ishikawa *et al*[16], 2012	66 *vs* 44	12	36.1 ± 12.4 *vs* 55.0 ± 8.8	29/15 *vs* 38/28	Plasmaphresis, tacro, celecpt, Basiliximab, rituximab, methyl prednisolone, cyclosporine, deoxypergualin	65 (98.5) *vs* 43 (97.7)	N/D	N/D	16 (24.2) *vs* 14 (31.8)	N/D	N/D	N/D
Santori *et al*[17], 2012	111 *vs* 24	128.17 ± 86.64 *vs* 103.53 ± 86.85	26.94 ± 13.51 *vs* 50.04 ± 8.86	78/33 *vs* 18/6	Cyclosporine, tacro, steroids, celecept	N/D	N/D	71 (63.8) *vs* 21 (87.8)	N/D	N/D	N/D	N/D
Matter *et al*[18], 2016	2075 *vs* 410	7.72 ± 6.15	28.8 ± 9.8 *vs* 34.8 ± 11.1	1554/521 *vs* 297/113	Steroid-Azathioprine or MMF	2012 (97) *vs* 389 (95)	1784 (86) *vs* 340 (83)	1660 (67) *vs* 270 (66)	71 (3.4) *vs* 26 (6.3)	1.38 ± 0.69 *vs* 1.35 ± 0.61	1.71 ± 1.04 *vs* 1.59 ± 0.89	N/D
Ali *et al*[19]	92 *vs* 143	5	N/D	N/D	Methyl prednisolone, Cyclosporine or tacrolimus MMF	90 (97) *vs* 141 (98.6)	80 (86) *vs* 125 (87.4)	N/D	N/D	N/D	N/D	N/D

Data is presented as *n* (%) and Mean ± SD. N/D: Not determined; MMF: Mycophenolatemofetil.

and 53.2%, respectively. Patient survivals for 1-, 3-, 5-, 10- and 15-years in the living related group were 94.6%, 91.9%, 83%, 79.5% and 73.9%; and in the unrelated group, these were 93.6%, 91.7%, 89.3%, 84% and 76.4%, respectively. They concluded that the results of living unrelated kidney transplantation upon long-term follow-up in a large number of cases was as effective as living related kidney transplantation.

Ahmad *et al*[15] retrospectively analysed the outcome of 322 living-donor renal transplants (related donors: 261; unrelated donors = 61). They reported that 33 grafts failed: 30 in the living related (11%) and 3 in the unrelated donor group (5%). Acute rejections occurred in 41% of recipients in the living related group

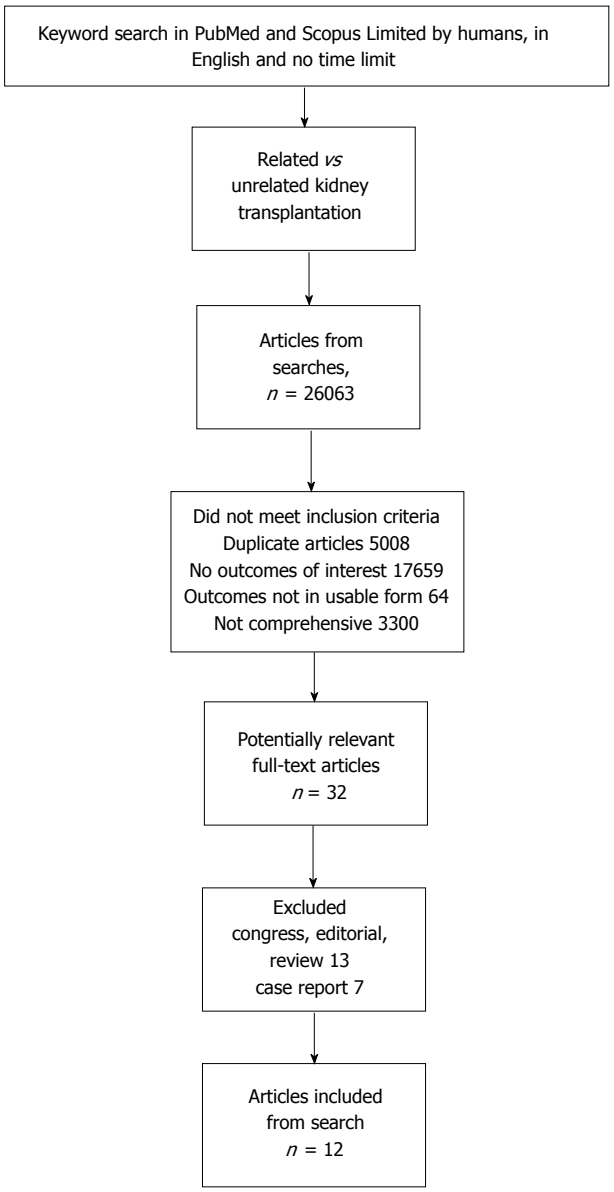

Figure 1 Study selection.

and 35% of recipients in the unrelated group. One- and 3-year patient survival for the living related and unrelated group was 98.7% and 96.3% and 97.7% and 95%, respectively. One- and 3-year graft survival was equivalent at 94.8% and 92.3% for the living related, and at 98.4% and 93.7% for the living unrelated group, respectively. They concluded that the outcome of living related donors and living unrelated donors is comparable in terms of patient and graft survival, acute rejection rate, and the estimated GFR despite the differences in demographics, HLA matching, and re-transplants of recipients.

Ishikawa *et al*[16] evaluated 112 cases of living kidney transplantations including 46 (41%) unrelated donors and 66 cases of received kidneys from living related donors. They showed that the incidences of an acute rejection episode were 31.8% and 24.2% in the living unrelated and the related groups, respectively. They demonstrated that living transplantation from an unrelated group was equivalent to related ones.

Santori *et al*[17] evaluated 135 procedures using living donors (living related: 111; living unrelated: 24). They reported no significant difference in patient survival after stratifying for donor type (living related: 93.9%; unrelated donors: 95.8%) or in graft survival after stratifying for donor type (related: 63.8%; unrelated: 87.8%). After entering donor type as an independent variable in a univariate Cox regression, they showed no significance for either recipient or graft survival. They suggested that living unrelated donor utilization should be encouraged in kidney transplantation programmes.

Simforoosh *et al*[5] evaluated 3,716 kidney transplantations (411 related donors and 3305 unrelated donors). They showed that donor age was the only statistically significant predictor of graft survival rate (hazard ratio = 1.021; 95%CI: 1.012-1.031). Patient survival and graft survival was similar in transplantations from living unrelated and related donors. They concluded that transplants from LURDs might be proposed as an acceptable management for patients with end stage renal disease.

Matter *et al*[18] from March 1976 to December 2013, divided the patients into two groups: (1) 2075 kidney transplant recipients (1554 or 74.9% male and 521 or 25.1% female) for whom the donors were living related; (2) 410 kidney transplant recipients (297 or 72.4% male and 113 or 27.6% female) for whom the donors were living unrelated. They showed the percentages of patients with acute vascular rejection were significantly higher in the unrelated group, while percentages of patients with no rejection were significantly higher in the related group, but there were no significant differences regarding patient and graft survivals between both groups.

Ali *et al*[19] evaluated 250 kidney transplantations (92 related donors, 143 unrelated donors and 15 spouse). They showed the one-year graft survival for related and unrelated donor transplants was 98.9% and 91.8%, respectively. Graft survival was lower (82.9%) in recipients with acute rejection episodes. The patient survival at one-year was 94%. The three year graft and patient survival was 91% and 90%, respectively, and five-year survival for grafts and patients was 87.1% and 88%, respectively.

Meta-analysis

Long term (ten year) graft survival rate: We conducted random effect meta-analyses (Figure 2) because the results from the studies which reported ten years graft survival rate after living related and unrelated renal transplantation showed significant heterogeneity (P = 0.001). No significant difference in graft survival rate was detected after ten years in patients who underwent living related kidney transplantations in comparison to

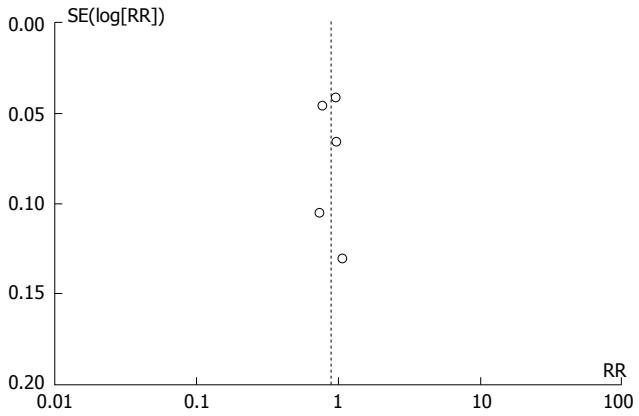

Figure 2 Significant heterogeneity in long term follow up between living related and unrelated kidney transplantation in funnel plot. RR: Risk ratio.

those who underwent unrelated kidney transplantations (P = 0.44) (Figure 3).

Mid-term (one to five year) graft survival rate: We conducted random effect meta-analyses because the results from studies reporting 1-5 years graft survival rate after living related and unrelated renal transplantation showed significant heterogeneity (P = 0.002). There were no significant differences between graft survival rate in living related and unrelated kidney transplantations after mid-term follow-ups (P = 0.46) (Figure 3).

Short-term (one year) graft survival rate: We conducted fixed effect meta-analyses because the results from the studies reporting one year graft survival rate after living related and unrelated renal transplantations showed no significant heterogeneity (P = 0.11). There were no significant differences between the graft survival rate in living related and unrelated kidney transplantations after a one year follow-up (P = 0.35) (Figure 3).

Acute rejection rate: We conducted fixed effect meta-analyses because the results from the studies reporting acute rejection rate after living related and unrelated renal transplantations showed no significant heterogeneity (P = 0.17). There were no significant differences between the acute rejection rate in living related and unrelated kidney transplantations (P = 0.06) (Figure 3).

DISCUSSION

This systematic meta-analysis showed that no significant difference existed in graft survival rate between living related and unrelated kidney transplantations in short, mid and long-term follow-ups.

In comparison to dialysis, transplantation has lengthened the patient's survival and improved their quality of life; in the medical field, it has broadened knowledge; to sponsors, it has provided a cost-effective solution for a never-ending problem. On the other hand, the shortcoming of transplantation is the unavailability of enough donors. This led to scientists using living unrelated kidney transplantations as an available source, but there were strong controversies in this respect. A detailed analysis suggests that the difference was related to a "centre effect". The inferior outcomes of living unrelated-donor transplantations were caused by the low standards of medical care in commercial transplantation programmes, the infections transmitted between the donor organs or patient non-compliance. After correcting these factors[20,21], the reports have shown no significant difference in graft outcomes when compared with living related transplantations. Our results support the finding that showed no significant difference between living related and unrelated kidney graft survival rates after mid-term and short-term follow-ups.

This systematic review and meta-analysis showed that the long-term graft survival rate has not a significant difference between the living related and the living unrelated groups. In our previous report[5], we evaluated the recipients of kidney transplants for 25 years and a comparable survival rate was found between the two groups. Park *et al*[12] reported the graft survival rates at 3, 5 and 10 years as 91.9%, 88.5% and 74.7% for the LURD *vs* 94%, 84% and 78.8% for the LRD transplants, with no significant difference. In contrast to our findings, previous studies showed no significant difference in long-term graft surveillance between the two groups[5,9,14]. This might be because of significant heterogeneity between the studies. As the funnel plot described, there is significant heterogeneity between the studies; therefore, in the future, more studies with a high quality of methodology are warranted.

While unrelated kidney transplantations are not widely accepted, the concern for transplantations continues to revolve around the issue of inadequate material benefits for potential donors[22]. The only model that resolved this issue was the model used in Iran. This model is organized by a non-profit organization known as the "Dialysis and Transplant Patients' Association (DATPA)"[23]. The DATPA's task is to assign appropriate donors for certain recipients and to offer medicolegal coverage. Donors receive a form of compensation from the government and the DATPA, and in addition, they are granted free life-long health insurance, and often, a "rewarding gift from the recipient"[23]. This model has been very successful over the past two decades in Iran, nearly eradicating the names on the transplant waiting list and gracefully providing a second chance at life for patients with ESRD; this model comprises over 75% of the total kidney transplant activity in Iran.

As a limitation, because of the lack of data, we could not evaluate the difference in HLA mismatches between the studies. Nevertheless, previous studies have reported equivalent short-, medium- and long-term outcomes of transplantation in LURD series in comparison to LRDs.

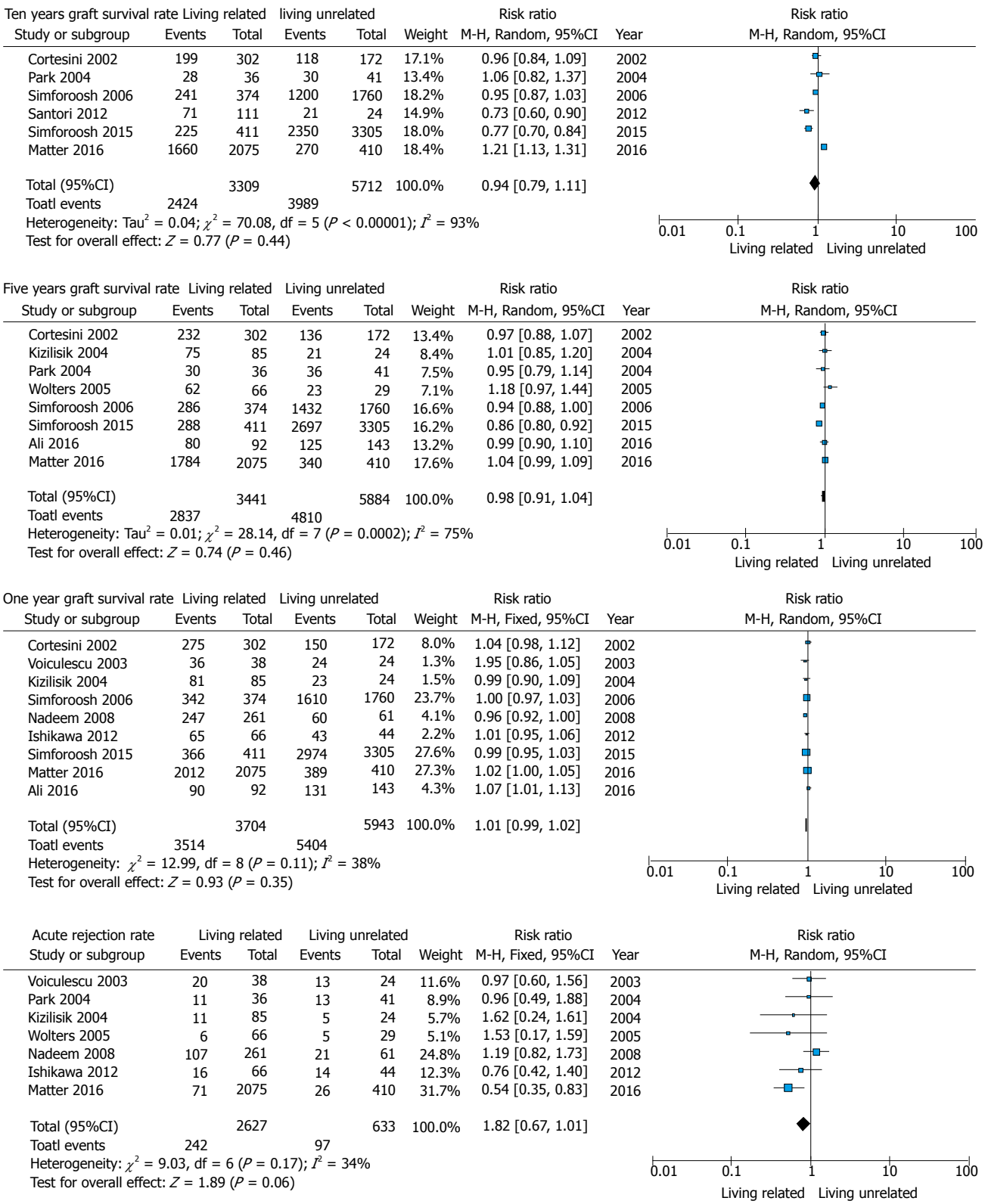

Figure 3 Comparing long, mid and short term graft survival rate and acute rejection rate between living related and unrelated kidney transplantations.

In conclusion, the long, mid and short-term follow-up of living related and unrelated kidney transplantation showed no significant difference in graft survival rate. Also, acute rejection rate was not significantly different between groups. We suggest that the Iranian model is a fair compromise because it avoids the rampant transplant commercialism.

COMMENTS

Background

The number of patients who are on the renal transplant waiting list for deceased-

donor transplantation has increased and thousands of patients have died while waiting for renal transplantation. Despite this, no systematic review and meta-analysis has been performed yet.

Research frontiers

Nowadays the outcomes of living related *vs* unrelated kidney transplantation are debatable. Worldwide research is directed towards the use of living unrelated kidney transplantation as a potential source.

Innovations and breakthroughs

In the present study, the authors investigated the outcomes of two kinds of sources in kidney transplantation by pooling results from different centres. This is the first report of a meta-analysis comparing these sources in receipts.

Applications

The present report provides an understanding of living unrelated kidney transplantation as an excellent source.

Peer-review

In this manuscript authors performed a meta-analysis to compare related and unrelated living donor kidney transplant outcome. Results indicate comparable outcome of kidney transplant from living unrelated *vs* related donors in the short, mid and long term follow up.

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