Effects of nutrition and cytokins on the long-term outcomes of kidney transplantation

PhD Thesis

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INTRODUCTION

The prevalence of end stage renal disease and is growing in the western countries mainly because of the growing incidence of diabetes mellitus and hypertonia. The prevalence of ESRD is more than 10 percent and the number of incidence cases are growing. The treatment of ESRD requires a strong and stable health care system and the adherence of the patient. The best choice of treatment is kidney transplantation from many aspects, from that the patients and the health care system can benefit the most.

In Hungary and in our clinic the kidney transplantation program started 40 years ago. At the beginning of the era acute rejection was the main cause of mortality, however with the development of immunosuppressive methods the most prevalent cause of death has changed. The renal replacement therapies also went through an enormous improvement resulting in a better survival for ESRD patients. All these improvements led to a change and nowadays the main cause of death in renal transplant recipients is cardiovascular mortality that might be explained with the uremic and with the altered immunological milieu caused by the implanted graft. These changes could lead to atherosclerosis and endothelial dysfunction worsening the survival of renal transplant recipients.

The messengers of inflammation and nutritional status are the inflammatory cytokines and the adipocyte-synthetized adipocytokines. The well-known members of inflammatory cytokines are the interleukins and the TNF-Alpha, that have a lot of function modulating the immune system. Besides the effects on inflammation, they also modulate the nutritional processes and induce structural change mainly in the vascular system, and also in the kidneys. In renal transplant recipients, the examined TNF-Alpha and IL6 levels are elevated, especially in those who are going through rejection. Furthermore, they might be responsible for cardiovascular death in the general population with normal kidney function.

Adipocytokines might also play an important role in the long term of outcomes of renal transplant recipients. The discovery of adipocytokines took place in beginning of the nineties. The adipocytokines are synthetized in adipocytes and their main function is the regulation of metabolism. Their family is growing year by year by newly discovered members, the most known adipocytokines are the leptin, adiponectin, resistin and visfatin. Furthermore, they not only regulate metabolism, they also have effects on inflammatory and hormonal actions. Their role in the survival and comorbidities of renal transplant recipients slowly becomes indisputable.

Both the cytokines and the adipocytokines have important role in the regulation of metabolism and nutrition of renal transplant recipients. Their dysfunction could lead to malnutrition, cachexia or protein-energy wasting syndrome. This is why the association of nutritional status and inflammation in kidney transplanted patients gained more interest in the last decade, considering that their relation has important effects on the clinical outcomes. In this dissertation I analyzed the association of nutrition and inflammation in renal transplant recipients and examined the possible messengers of their relationship.

OBJECTIVES

In the last two decades, perceptions about the role of body fat and inflammation have changed. The connection between inflammation and nutrition is driven by adipocytokines and pro-inflammatory cytokines that modulate endocrine and immune homeostasis. Many studies have been investigating the effect of inflammation and nutrition in renal transplant recipients, which suggest they play an important role in the outcomes of renal transplant recipients. Kidney transplant recipients are a unique and relevant population, given their altered nutritional and immune status and subsequent dysregulation of adipocytokines and pro-inflammatory cytokines.

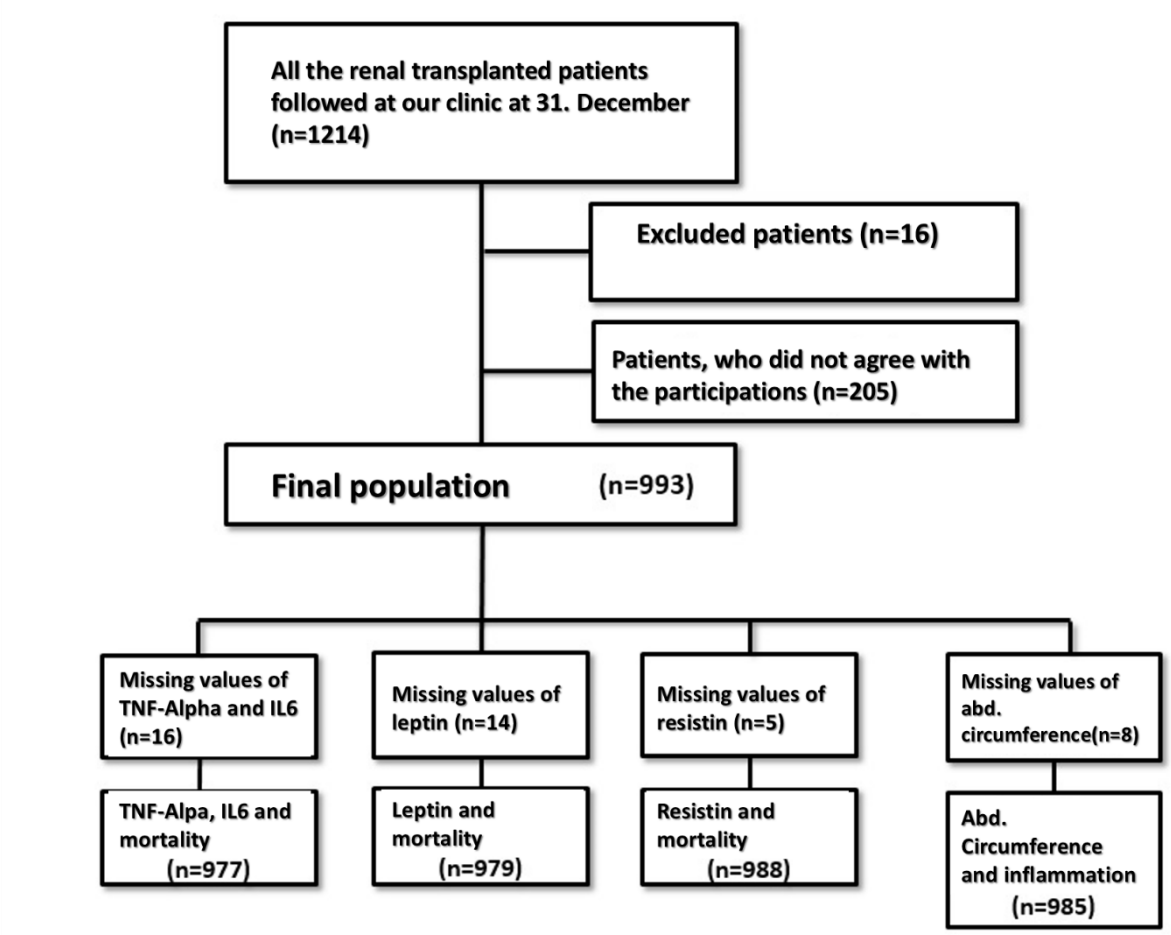
In this PhD work, I analyzed the connection of inflammation and nutritional status in renal transplant recipients. If there is an existing link, the messengers of this connection are presumably the pro-inflammatory cytokines and adipocytokines. This is why I also investigated the role of cytokines, such as leptin, resistin, TNF-Alpha and IL6, in the long-term clinical outcomes of renal transplant recipients. My objectives were:

1. Is there a connection between nutritional status and inflammation in renal transplant recipients? For the examination of the association I used abdominal circumference and BMI as the markers of nutrition and CRP, WBC, TNF-Alpha and IL6 as the parameters of inflammation.
2. Is there any association between serum resistin levels, as an adipocyte-synthetized pro-inflammatory adipocytokine, in the long term outcomes of renal transplant recipients?
3. Is there any association between serum leptin levels, as an adipocyte-synthetised metabolic adipocytokine, in the long term outcomes of renal transplant recipients?

4. Is there any association between serum TNF-Alpha levels, as a pro-inflamamtory cytokine, in the long term outcomes of renal transplant recipients?
5. Is there any association between serum IL6 levels, as a pro-inflamamtory cytokine, in the long term outcomes of renal transplant recipients?

METHODS

We recruited all prevalent kidney transplant recipients (n=1,214), who were followed at a single transplant outpatient clinic at the Department of Transplantation and Surgery at Semmelweis University Faculty of Medicine in Budapest, Hungary during the inclusion period of December 31, 2006 to December 31, 2007 (Malnutrition-Inflammation in Transplant - Hungary Study [MINIT-HU Study]). Baseline defined as the data detected at the time of study entry for the prevelant transplant recipients in the inclusion period. We excluded patients who at the time of study entry experienced acute rejection within the last 4 weeks; were hospitalized at the study entry; received kidney transplantation in the previous 3 months; or had acute infection or bleeding. The study cohort algorithms is shown in **Figure 1**:



Data collection

Medical history and socio-demographic data were collected at baseline, including information on age, sex, etiology of chronic kidney disease, co-morbidities (the modified Charlson Comorbidity Index(CCI)), and transplantation-related data including immunosuppressant medications. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. The study was approved by the Ethics Committee of Semmelweis University (49/2006). Before enrollment, patients received detailed written and verbal information regarding the aims and protocol of the study and gave written consent to participate.

All laboratory data were collected and measured at the baseline clinic visit and included resistin, leptin, adiponectin, TNF-Alpha, IL-6, blood hemoglobin (Hb), serum CRP, serum creatinine, blood urea nitrogen (BUN) and serum albumin levels. Serum cytokine concentrations were measured using immunoassay kits based on solid-phase sandwich enzyme

linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, Coefficient of Variation<10%).

Transplant-related data were obtained from the medical records and included medications (including current immunosuppressive treatment), transplant vintage (i.e., time elapsed since the date of transplantation), length of time on dialysis prior to transplantation, type of allograft, history of acute rejection(s) that were treated after transplantation, human leukocyte antigen (HLA) mismatch, panel reactive antibodies titer (PRA), cold ischemia time (CIT), donor age and sex, and history of delayed graft function. Total time with end stage renal disease (ESRD) was defined as the total time on any type of renal replacement therapy including any type of dialysis or kidney transplantation. Standard immunosuppressive therapy included prednisolone, with either cyclosporine (CsA) A microemulsion formulation (Neoral) or tacrolimus, combined with mycophenolate-mofetil (MMF) or azathioprine or sirolimus.

Patients were followed for a median (IQR) period of 76 (46-79) months. The primary outcome of interest was all-cause death with a functioning graft. We also assessed the association between baseline serum resistin level and death-censored graft loss as a secondary outcome. Deaths and re-initiations of maintenance dialysis were ascertained from hospital medical records. Deaths were validated by cross-referencing with the Hungarian Central Office of Administrative and Electronic Public Service record, which is the government agency maintaining official vital status data.

Statistical Analysis

Statistical analyses were carried out using STATA 13 (StataCorp, College Station, TX) software. Descriptive data were summarized using proportions, means (±standard deviation, SD) or medians [IQR] as appropriate. Categorical variables were compared using chi-square tests, and continuous variables were compared using Student's t-test or the Mann-Whitney U test as appropriate and p for trend test. Correlations between covariates were assessed by Pearson correlation coefficients. In all analyses, two-sided tests were used and the results were considered statistically significant if the p-value was less than 0.05.

Three regression models were examined with incremental levels of multivariable adjustment for analyzing the association of nutritional status and inflammatory markers: 1) Unadjusted model; 2) Case-mix model was age, gender, eGFR, ESRD time, Charlson Comorbidity Index, steroid use, blood hemoglobin, serum transferrin, soluble transferrin receptor, ferritin, calcium, phosphate and parathyroid hormone; 3) Final model was adjusted

for variables in case-mix model, as well as serum albumin, prealbumin, cholesterol, HDL-cholesterol and triglyceride level.

The association between baseline serum cytokin levels and deaths with a functioning graft was assessed using Cox proportional regression analysis and Kaplan-Meier plots with the log rank test. Analogous analyses were also conducted for death-censored graft loss as a secondary outcome. Proportional hazards assumptions were tested using scaled Schoenfeld residuals. The variables entered in the multivariable-adjusted models were selected based on theoretical considerations; we included predictors in the models which were known to be associated both with resistin levels and with mortality based on scientific evidence, and which were available in our database.

Association of serum resistin level and clinical outcomes:

1) Unadjusted model; 2) Model 1 was adjusted for age and sex; 3) Model 2 was adjusted for variables in model 1, as well as baseline eGFR, CCI, ESRD time, and diuretic treatment at baseline; 4) Model 3 was adjusted for variables in model 2, as well as serum albumin level and body mass index (BMI); 5) Model 4 was adjusted for variables in model 3 and cold ischemic time, PRA level, HLA mismatch, number of transplantations, TNF-Alpha, IL6 and CRP.

Association of serum leptin level and clinical outcomes:

1) Unadjusted model; 2) Case-mix model was age, gender, eGFR, ESRD time, Charlson Comorbidity Index, steroid use, blood hemoglobin, serum transferrin, soluble transferrin receptor, ferritin, calcium, phosphate and parathyroid hormone; 3) Final model was adjusted for variables in case-mix model, as well as serum albumin, prealbumin, cholesterol, HDL-cholesterol and triglyceride level.

Association of serum TNF-Alpha level and clinical outcomes:

1) Unadjusted model; Final Model: age, donor age, eGFR, total ESRD time (including total time on renal replacement therapy and time after being transplanted), Charlson Comorbidity Index, albumin, abdominal circumference, cold ischemia time, PRA level and HLA mismatch.

Association of serum IL6 level and clinical outcomes:

1) Unadjusted model; Final Model: age, donor age, eGFR, total ESRD time (including total time on renal replacement therapy and time after being transplanted), Charlson Comorbidity Index, albumin, abdominal circumference, cold ischemia time, PRA level and HLA mismatch

RESULTS

Association of Abdominal Circumference and Inflammation in Kidney Transplant Recipients

Mean±SD age was a 51±13 year, 57% were men and 21% were diabetics. Patients with abdominal circumference above the median had higher body mass index and were older (mean±SD: 23.9±3.6 vs. 30.1±3.9 kg/m², p<0.001; and 48±14 vs. 54±11 years, p<0.001). Furthermore patients with higher abdominal circumference had higher inflammatory parameters: median (Inter-Quartile Range) CRP (mg/L): 2.3 (3.9) vs. 4.1 (6.2), p<0.001; and IL6 (pg/mL): 1.9 (2.2) vs. 2.3 (2.4), p<0.001. In multivariable adjusted linear regression models higher abdominal circumference showed significant linear associations with inflammatory markers (standardized regression coefficients (β) of abdominal circumference for lnCRP: $\beta_{\text{abdominal circumference}}$=0.29, p<0.001; and for lnIL-6: $\beta_{\text{abdominal circumference}}$=0.09, p=0.018). Moreover, in multivariable adjusted linear regression models higher BMI showed significant linear associations with inflammatory markers (standardized regression coefficients (β) of BMI for lnCRP: β_{BMI}=0.24, p<0.001; and for white blood cells: β_{BMI}=0.07, p=0.041).

Association between serum resistin level and outcomes in kidney transplant recipients

The mean±SD age of the study population was 51±13 years, among whom 57% were men and 21% were diabetics. Median serum resistin concentrations were significantly higher in patients who died with a functioning graft as compared to those who did not die during the follow-up period (median [IQR]: 22[15-26] vs. 19[14-22] ng/mL, respectively; p<0.001). Higher serum resistin level was associated with higher mortality risk in both unadjusted and fully adjusted models: HRs (95%CI): 1.33(1.16-1.54) and 1.21(1.01-1.46), respectively. Compared to patients whose serum resistin levels were in the lowest tertile, those in the middle tertile had similar mortality risk (HR (95%CI): 1.01(0.68-1.49)), but patients with the highest tertile had a higher risk of mortality: HR (95%CI): 1.22(0.82-1.84) in multivariable adjusted models.

Association between serum leptin level and mortality in kidney transplant recipients

Serum leptin levels showed moderate negative correlation with eGFR(R=-0.21,p<0.001) and positive correlations with BMI(R=0.48,p<0.001) and C-reactive protein(R=0.20,p<0.001). Each 10 ng/ml higher serum leptin level was associated with 7%

lower risk of death with functioning graft(Hazard Ratio(HR)(95%Confidence Interval(CI)): 0.93(0.87-0.99)), and this association persisted after adjustment for confounders: HR(95%CI): 0.90 (0.84-0.98). Similar associations were found with all-cause death as outcome. The association between serum leptin level and risk of graft loss was non-linear, and only low serum leptin level was associated with higher risk of graft loss.

Association of serum TNF-Alpha and clinical outcomes in renal transplant recipients

The mean±SD age of the study population was 51±13 years, 57% were men, 21% were diabetics. Median serum TNF-Alpha concentration was significantly higher in patients who died with a functioning graft as compared to those who did not die during the follow-up period(TNF-Alpha: median[IQR]: 1.92[1.43-2.67] vs. 2.25[1.63-3.08, p<0.001]pg/mL. Higher serum TNF-Alpha was associated with higher mortality risk in both unadjusted and fully adjusted models: TNF-Alpha:$HRs_{(1\ pg/ml\ increments)}$ (95%CI): 1.24(1.13-1.36) and 1.19(1.08-1.32); respectively. Compared to patients whose serum TNF-Alpha was in the lowest tertile, those in the middle tertile had similar mortality risk (TNF-Alpha: HR(95%CI): 1.09(0.74-1.61); but patients in the highest tertile reported higher risk of mortality: TNF-Alpha: HR(95%CI): 1.45(1.01-2.09); in multivariable adjusted models.

Association of serum IL6 and clinical outcomes in renal transplant recipients

The mean±SD age of the study population was 51±13 years, 57% were men, 21% were diabetics. Median serum IL6 concentration was significantly higher in patients who died with a functioning graft as compared to those who did not die during the follow-up period (IL6: median[IQR]: 1.91[1.21-3.02] vs. 2.81[1.65-4.97]pg/mL, p<0.001). Higher serum IL6 level was associated with higher mortality risk in both unadjusted and fully adjusted models: IL6: $HRs_{(1\ pg/ml\ increments)}$(95%CI): 1.06(1.03-1.09) and 1.03(0.99-1.06), respectively. Compared to patients whose serum IL6 levels were in the lowest tertile, those in the middle tertile had similar mortality risk (IL6: HR(95%CI): 1.05(0.68-1.62)), but patients in the highest tertile reported higher risk of mortality: IL6: HR(95%CI): 1.55(1.04-2.32) in multivariable adjusted models.

CONCLUSIONS

1. Abdominal circumference is an independent and significant predictor of inflammatory markers in prevalent kidney transplant recipients. Using abdominal circumference and BMI as parameters of nutrition and CRP, WBC, TNF-Alpha and IL6 as inflammatory markers, there is an existing link between inflammation and nutrition in renal transplant recipients.
2. In prevalent kidney transplant recipients, serum resistin was an independent predictor of death with a functioning graft. The elevated serum resistin concentration is associated with higher mortality risk and with higher risk of graft loss.
3. In prevalent kidney transplant recipients, lower serum leptin was an independent predictor of death. The elevated serum leptin level is associated with lower risk of mortality, however the connection of leptin concentration and graft loss is non-linear.
4. In prevalent kidney transplant recipients, serum TNF-Alpha was independently associated with death with a functioning graft. The elevated serum TNF-Alpha concentration is associated with higher mortality risk and with higher risk of graft loss.
5. In prevalent kidney transplant recipients, serum IL6 was independently associated with death with a functioning graft. The elevated serum IL6 level is associated with higher mortality risk and with higher risk of graft loss, however the results are not significant in every model.

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