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Creatinine and cytokines plasma levels related to HLA compatibility in kidney transplant patients

Níveis plasmáticos de creatinina e citocinas relacionados com compatibilidade HLA em pacientes transplantados renais

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ABSTRACT

Introduction: The success of kidney transplantation depends on prevention of organ rejection by the recipient's immune system, which recognizes alloantigens present in transplanted tissue. Human leukocyte antigen (HLA) typing is one of the tests used in pre-renal transplantation and represents one of the most important factors for a successful procedure. **Objective:** The present study evaluated creatinine and cytokines plasma levels in kidney transplant patients according to pre-transplant HLA typing. **Methods:** We assessed 40 renal transplanted patients selected in two transplant centers in Belo Horizonte (MG). **Results:** Patients were distributed into three groups according to HLA compatibility and, through statistical analysis, the group with more than three matches (H3) was found to have significantly lower post-transplant creatinine levels, compared to groups with three or fewer matches (H2 and H1, respectively). The median plasma levels of cytokines interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and interleukin 10 (IL-10) were evaluated according to the number of matches. Pro-inflammatory cytokines (IL-6 and TNF-α) were significantly higher in groups with lower HLA compatibility. On the other hand, the regulatory cytokine IL-10 had significantly higher plasma levels in the group with greater compatibility between donor and recipient. **Conclusion:** These findings allow us to infer that pre-transplant HLA typing of donors and recipients can influence post-transplant renal graft function and may contribute to the development and choice of new treatment strategies.

Key words: renal transplantation; creatinine; cytokines; HLA.

INTRODUCTION

Kidney transplantation is the treatment of choice for patients with end-stage renal disease (ESRD). The success of a renal transplantation depends on the prevention of graft rejection by the receptor's immune system, which recognizes alloantigens present in the transplanted organ. This allorecognition is commonly initiated by T cells[1]. Rejection of the transplanted kidney comprises repeated inflammatory reactions and represents the main cause of graft dysfunction in early and late post-transplant periods[2, 3].

In the attempt to monitor this process, creatinine has been used as a biomarker of chronic kidney disease (CKD), kidney transplantation and acute kidney injury (AKI). Its measurement is quite simple, easily reproducible and performed in most clinical laboratories[4].

Additionally, the increased production of some cytokines, such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α), has also been associated with the outcome of renal transplantation. These cytokines seem to play an important role in the development of chronic inflammatory lesions in kidney transplant patients, what would allow the clinical usage of these biomarkers[3]. On the other hand, modulating cytokines, such as interleukin 10 (IL-10), have a decisive role in the development of immune tolerance, contrasting with the role of pro-inflammatory cytokines[5].

The discovery of human leukocyte antigens (HLAs) has allowed medicine to take a quantum leap forward in the chances of a successful transplant when adopting the

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compatibility criteria[6]: the greater the similarity between HLA alleles of donor and recipient the more likely the transplant will succeed, be it of a solid organ, tissue or bone marrow[6]. The number of HLA matches is one of the issues to be analyzed in the pre-transplant period[7].

Immune reaction to the transplanted kidney begins when T lymphocytes recognize foreign antigens in the graft, by means of their membrane receptor [T-cell receptor (TCR)]. This allorecognition causes an event cascade in which cytokines play an important role, culminating in clonal expansion of effector cells, production of more cytokines, and anti-HLA antibodies with cytotoxic activity[8]. When the inflammatory reaction is not modified by immunosuppressive agents, this kind of response results in lesion and destruction of the kidney graft[8].

Thus, the present study investigated the association of plasma levels of creatinine and cytokines (IL-6, TNF-α and IL-10) with HLA histocompatibility between donors and recipients of kidney transplant, in order to assess post-transplant renal function in these patients.

METHODS

This study was approved by the ethics committee of Universidade Federal de Minas Gerais (UFMG) (ETIC Report 387/09), and the informed consent was obtained from all participants. The research protocol did not interfere with any medical recommendation or prescription.

Forty kidney transplant patients were selected from two transplant units in Belo Horizonte, Minas Gerais, between 2010 and 2012, two to 147 months after transplantation. They had received kidneys from living donors. All the patients included in the study were clinically stable at the moment of blood collection, and were on immunosuppressive therapy, mainly composed of a calcineurin inhibitor (tacrolimus or cyclosporine) + mycophenolate mofetil + prednisone. Exclusion criteria were: acute rejection or suspected rejection at the moment of blood collection, patients who had undergone recent surgeries or had recent fractures, patients who returned to hemodialysis or who had indication for return at collection time, and patients with active infection or suspected infection at the moment of collection. Later on, in 2013 and 2014, these patients were again followed by means of new appointments and new reports in their medical records for updated plasma creatinine levels.

The selected patients were divided into groups according to the number of HLA matches (H1: fewer than three matches, H2: three matches and H3: more than three matches). Data on creatinine and HLA compatibility were collected from medical records. **Table 1** shows the main clinical and demographic features of the studied population.

For determination of plasma cytokines (IL-6, TNF-α and IL-10), 5 ml blood was collected in 0.109 mol/l sodium citrate (Vacuette®). Plasma aliquots were kept frozen at -80ºC until the moment of use. Cytokines were measured by flow cytometry (BD™ CBA), rigorously following the manufacturer's instructions (BD® Biosciences Pharmingen, USA). The detection limits established for the assessed cytokines were IL-6: 2.5 pg/ml, TNF-α: 2.8 pg/ml, and IL-10: 2.8 pg/ml. Samples were read in a flow cytometer BD™ FACScalibur of Centro de Pesquisas René Rachou/Fundação Oswaldo Cruz (CPqRR/Fiocruz). Cytokine levels were expressed as mean fluorescence intensity (MFI). This unit was chosen because it allows distinguishing values in the limits of the linearity range[9].

Statistical analyses were conducted using the program GraphPad Prism (version 6.0); and the comparison between groups, the one-way analysis of variance (one-way Anova) and Kruskal-Wallis test. Values of $p < 0.05$ were considered significant differences.

RESULTS

The laboratory parameters – plasma levels of creatinine and cytokines (IL-6, TNF-α and IL-10) – were assessed in 40 kidney transplant patients according to the number of HLA matches. Initially, patients in the study were clinically characterized as seen in Table 1.

Approximately 57.4% of kidney transplant patients included in the study were males. Median age for males and females were 48 and 44 years, respectively. Medians obtained for body mass index (BMI) of men (23.5) and women (25.7) revealed that most patients were within the healthy weight range (BMI between 18.6 and 24.9 kg/m^2). In 48.8% of the cases the primary diseases that led to CKD were either not known or not informed. Chronic glomerulonephritis was the most common cause of CKD in men (25.9%). In women, the most common CKD cause was diabetes *mellitus* (30.7%), along with other chronic diseases (30.7%). Segmentar focal glomerurosclerosis was more frequent in men (14.8%) than in women (7.7%); systemic arterial hypertension presented similar proportions for men (7.4%) and women (7.7%); mean time on hemodialysis was longer in female (149 months) than in male patients (47 months); the most commonly used

TABLE 1 – Clinical and laboratory information of kidney transplant patients in the study

Parameters	Males (n = 27)	Females (n = 13)	Total (n = 40)
Age (years)	48 (24-66)	44 (25-63)	45 (24-66)
BMI (kg/m^2)	23.5 (16.9-28)	25.7 (18.18-34.8)	24 (16.9-34.8)
Primary cause of CKD n (%)			
Diabetes *mellitus*	3 (11.12)	4 (30.77)	7 (17.5)
Systemic arterial hypertension	2 (7.41)	1 (7.69)	3 (7.5)
Chronic glomerulonephritis	7 (25.92)	0	7 (17.5)
Segmentar focal glomerulosclerosis	4 (14.81)	1 (7.69)	5 (12.5)
Others	5 (18.52)	4 (30.77)	9 (22.5)
Indeterminate	6 (22.22)	2 (15.38)	8 (20)
Time on HD (months)	47 (12-157)	149 (133-180)	73 (12-180)
Immunosupressive therapy n (%)			
Prednisone	21 (77.78)	10 (76.92)	31 (77.5)
Tacrolimus	15 (55.5)	4 (30.77)	19 (47.5)
Mycophenolate sodium	14 (51.85)	7 (53.85)	21 (52.5)
Rapamycin	4 (14.82)	3 (23.1)	7 (17.5)
Others	4 (14.82)	4 (30.77)	8 (20)
Indeterminate	5 (18.52)	2 (15.38)	7 (17.5)
Rejection n (%)			
Yes	12 (44.44)	7 (53.85)	19 (47.5)
No	4 (14.82)	2 (15.38)	6 (15)
Indeterminate	11 (40.74)	4 (30.77)	15 (37.5)
Creatinine (mg/dl)	2.66 (0.92-12.35)	3.48 (0.67-10.72)	2.93 (0.67-12.35)

BMC: body mass index; CKD: chronic kidney disease; HD: hemodialysis.

immunosuppressive agent was prednisone, for both men (77.8%) and women (76.9%); rapamycin was the least used in men (14.8%) and women (23.1%).

It is important to highlight that at the moment of blood collection (2010 and 2011), patients were clinically stable, with no signs or clinical suspicion of rejection. Later, during the analyses, 47.5% of the patients presented some kind of rejection, 53.8% of them were females. These data on rejections were collected in 2013 and 2014.

As to the distribution of HLA alleles, **Table 2** shows the most frequent types among donors and recipients of kidney transplants in this study. The HLA types encountered were used to classify the groups according to the number of matches between the transplant recipient and its donor, with group H1: fewer than three matches, group H2: three matches, and group H3: more than three matches. HLA A2 was the most common subtype for both recipients and donors, with 42.5% and 55%, respectively. The least frequent alleles were A3, in donors (15%), A3 and DR13, in recipients (20%).

Later, after clinical characterization of the study participants, patients were assessed as to plasma levels of creatinine and cytokines.

TABLE 2 – Distribution of the most frequent alleles in donors and recipients of kidney transplant

HLA	Recipients (%)	Donors (%)
A2	17 (42.5)	22 (55)
A3	8 (20)	6 (15)
A30	9 (22.5)	9 (22.5)
B44	11 (27.5)	11 (27.5)
B35	10 (25)	7 (17.5)
DR4	13 (32.5)	12 (30)
DR7	10 (25)	13 (32.5)
DR13	8 (20)	12 (30)

HLA: human leukocyte antigen.

The comparison of median plasma creatinine levels observed in the different groups with different numbers of matches is presented in **Figure 1**. Our findings demonstrated significantly lower levels of creatinine in group H3 (which had more than three matches between donor and recipient) than in group H2 (p = 0.03). The median creatinine levels in group H3 were also lower than those in group H1, which had less than three matches between donor and recipient, although with non-significant p value (Figure 1).

The median plasma levels of the assessed cytokines (IL-6, TNF-α and IL-10) were also statistically compared according to the

number of HLA matches, what is shown in **Figure 2**. The groups with the smallest numbers of matches between donor and recipient (H1 and H2) had IL-6 and TNF-α (pro-inflammatory cytokines) significantly higher than group H3 (more than three matches), with $p < 0.0001$ and $p = 0.0067$, respectively. The group with the largest number of matches (H3) presented plasma levels of the regulating cytokine IL-10 significantly higher than the others (H1 and H2), with smaller numbers of matches ($p = 0.0004$).

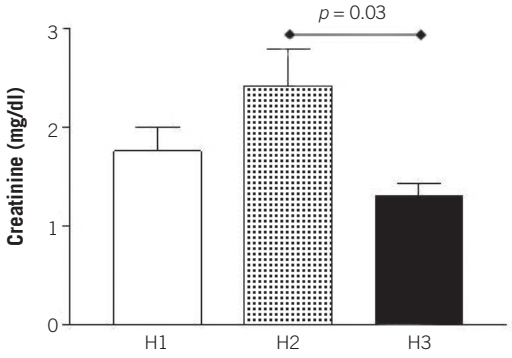

FIGURE 1 – *Creatinine plasma levels (mg/dl) in the groups of kidney transplant patients (H1, H2, and H3) according to the number of HLA matches*

H1: fewer than three matches; H2: three matches; H3: more than three matches.

DISCUSSION

Recipient's immune response against the transplanted tissue is the chief obstacle to a successful transplant, in most cases[10]. Long ago, HLA histocompatibility was considered one of the main factors responsible for tolerance to the transplanted organ. Thus, it was expected that the greater the similarity between HLA molecules of donor and recipient the longer the graft survival[10, 11]. Conceivably, patients who receive organs with low HLA compatibility generally present a significantly higher chance of producing antibodies against the donor[9].

In the present study, recipients of the renal graft that had a greater number of HLA matches (H3) also presented lower levels of post-transplant plasma creatinine. In agreement with our findings, Mao *et al.*[12] demonstrated significant correlation between patients with anti-HLA antibodies and creatinine plasma levels higher than 4 mg/dl. Actually, at another prospective study performed with 2,231 kidney transplant patients, the influence of anti-HLA antibodies in graft function was investigated during two years[13]. Those researchers observed a decline in graft survival related to the progressive increase of creatinine serum levels in patients with anti-HLA antibodies.

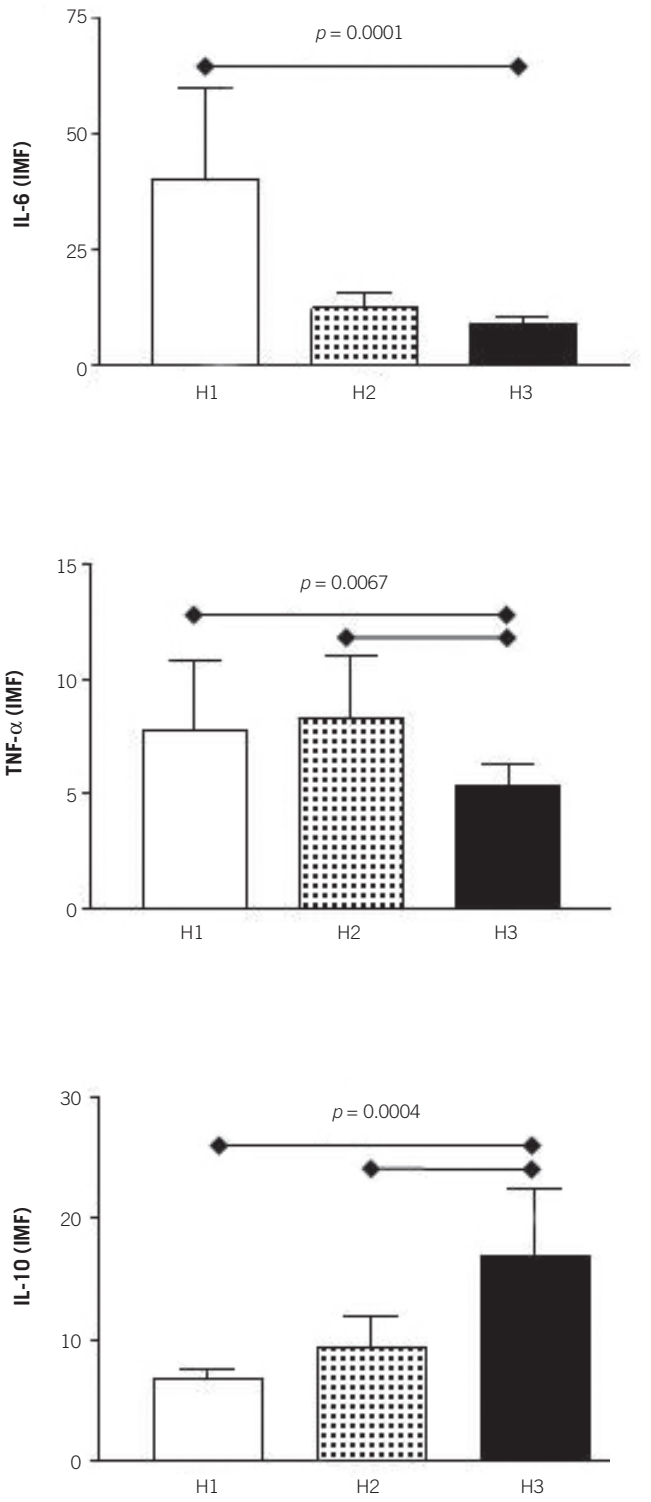

FIGURE 2 – *Plasma levels of inflammatory (IL-6 and TNF-α) and regulating (IL-10) cytokines in the groups of kidney transplant patients (H1, H2, and H3) according to the number of HLA matches*

H1: fewer than three matches; H2: three matches; H3: more than three matches; MFI: mean fluorescence intensity. Data presented as median.

Creatinine is a classic marker of renal function, freely filtered by the glomerulus and neither absorbed nor metabolized by the kidney. Approximately 10%-40% urine creatinine comes from tubular secretion of organic cations in the proximal tubule, being more significant as glomerular filtration rate (GFR) gets lower. The secreted amount is not constant and depends on the individual and the creatinine serum concentration, making it difficult to determine a secretion constant[14]. In general, renal dysfunction is not detected until creatinine plasma levels are considerably increased, what would make it hard to prevent early graft loss[15]. Notwithstanding, the research conducted by Spanaus *et al*.[16] showed that creatinine remains relevant in the assessment of renal function. In that study serum creatinine was compared with cystatin C and β-trace protein (BTP), and the three biomarkers proved to be equivalent, both in diagnostic performance and for risk prediction of kidney disease progression[16].

Plasma levels of anti-HLA antibodies, as well as those of creatinine, play an important role in kidney graft survival and are associated with both post-transplant early rejection and late loss of graft function[17].

In this regard, two aspects are highly relevant in the transplant area: HLA compatibility, which has high degree of polymorphism and is important in organ transplant immunology[18], and the existence of pre-formed antibodies (which can be detected in the recipient's serum before the graft fails) against donor-specific HLA antigens. The presence of anti-HLA antibodies is a known and independent risk factor for rejection and graft failure[19]. In the present study, plasma levels of anti-HLA antibodies were not assessed, however it is possible to affirm that cytokine levels may be, under a certain aspect, associated with the presence of these markers. For these reasons, it is believed that patients with higher levels of pro-inflammatory cytokines (IL-6, TNF-α and IL-10, for example) could also present a type of anti-HLA antibody[20]. The assessment of these antibodies and their relationship with plasma cytokines is one of the perspectives of this study. To this end, patients included in the present work will be continuously monitored by the assessment of their clinical records.

Our findings showed higher plasma levels of pro-inflammatory cytokines in patients with small numbers of HLA matches, what reinforces the importance of immunological similarity between donor and recipient. In fact, Canossi *et al*.[20] demonstrated that high levels of TNF-α and IL-6 are associated with the production of anti-HLA antibodies and rejection episodes. The same authors showed that some genetic polymorphisms influence interindividual differences in the production of these cytokines, and these variations have the potential to influence inflammatory response to the graft[20].

IL-6 is a high-sensitivity marker to early detect loss of renal function of the graft, especially over the years. When creatinine levels increase twofold, IL-6 levels tend to go high in the same proportion[21]. It is also known that the decreased rhythm of renal filtration can affect levels of inflammatory molecules, because both IL-6 and C-reactive protein (CRP) are inversely associated with creatinine clearance. Thus, renal function decline would contribute to decrease the clearance of pro-inflammatory substances, which are directly (due to high production) or indirectly (due to renal retention) involved in inflammation and dysfunction of endothelial cells found in transplant patients[22].

The role of pro-inflammatory cytokine TNF-α, which stimulates macrophage function and enhances major histocompatibility complex (MHC) class II antigen expression, has also been demonstrated in acute and chronic graft rejections[23]. TNF-α is a potent inducer of inflammatory response and a central regulator of innate immunity. TNF-α inflammatory responses are mediated both directly and by means of stimuli of other pro-inflammatory cytokines[23].

The consequences of pro-inflammatory cytokines TNF-α and IL-6 activity are: increased expression of MHC antigens and higher expression of co-stimulating molecules and adhesion molecules, which stimulate the recognition of major and minor histocompatibility antigens of the recipient by donor-derived mature T cells after transplantation[24]. When properly activated, donor T cells produce a group of Th1-type cytokines, such as TNF-α and IL-6, which initiate an inflammatory event cascade[24].

In the present study, the plasma levels of IL-6 and TNF-α were associated with the number of HLA matches. Groups with lower compatibility between donor and recipient (H1 and H2) have plasma levels of such cytokines significantly higher than group H3. This fact may be related to the development of new cytotoxic antibodies, especially in those patients that present some HLA incompatibility with the donor[25]. Therefore, graft rejection could be accentuated by the induced proliferation of pro-inflammatory cytokines (for instance, IL-6 and TNF-α). These molecules are counterbalanced by Th2-type cytokines, mainly IL-10, and by several types of T-suppressor cells[24].

In our study, the patients who presented larger numbers of HLA matches also presented the highest levels of modulating cytokine IL-10, one of the responsible for immune tolerance[26].

According to the exposed, Newell *et al*.[27] showed that immunologically tolerant patients exhibited higher levels of IL-10 with larger numbers of transient circulating B cells when compared with immunosuppressed non-tolerant patients[27]. This study also observed a significant increase in transient T1 and T2 B cells (which produce regulating cytokine IL-10) in the immunologically tolerant group in relation to the stable group (which used immunosuppressive drugs), and to the control group (non-transplanted subjects)[28].

Conversely, Sagoo *et al*.[29] did not find significant differences for the studied groups as to IL-10 production concerning kidney transplant immune tolerance. Regulating cells search to suppress immune response, by means of cell-cell interactions and production of soluble molecules such as IL-10, although this mechanism is still not completely clear[30].

With this in mind, the balance between production of pro-inflammatory and regulating cytokines seems to be one of the most important immune events in kidney graft acceptance or rejection[26]. In our study, we verified that the group with the largest number of HLA matches (H3) presented significantly higher plasma levels of the modulating cytokine IL-10, when compared with the other groups with fewer matches. In agreement with our findings, Uboldi *et al*.[31] demonstrated a positive correlation between HLA compatibility and modulating cytokine IL-10. In this study, patients with higher production of modulating cytokine IL-10, with HLA incompatibility class I and HLA compatibility class II were discovered to be protected from chronic rejection. This finding may relate to the fact that higher HLA compatibility is linked to the recipient's lower immune response to the graft, with IL-10 having an important regulatory participation in the process[5, 32].

Partially disagreeing with our findings, Conassi *et al*.[20] confirmed that cytokine IL-10 seems to have a contradictory effect. If on the one hand it may be considered an anti-inflammatory cytokine, capable of suppressing the synthesis of other cytokines (such as interferon gamma [IFN gamma]), on the other hand it may also promote differentiation of B cells and intense humoral response with the production of antibodies[20]. Therefore, further studies would be necessary to elucidate the role of IL-10 in kidney graft survival and in mechanisms of immune tolerance.

CONCLUSION

The obtained data allow concluding that the greatest HLA compatibility between kidney donors and recipients results in better plasma levels of creatinine and pro-inflammatory cytokines, especially IL-6 and TNF-α, in kidney transplant patients. The results of the present study showed that a higher HLA compatibility index is fundamental for a successful transplant, confirmed by the levels of creatinine and cytokines. These cytokines could be employed as potential biomarkers of renal function in post-transplant monitoring. However, we believe that the role of IL-6, TNF-α and IL-10 in kidney transplant needs to be more exhaustively examined in future studies.

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RESUMO

Introdução: *O sucesso de um transplante renal depende da prevenção da rejeição ao órgão por parte do sistema imune do receptor ao reconhecer aloantígenos presentes no tecido transplantado. A tipagem de antígenos leucocitários humanos (HLA) é um dos testes empregados no pré-transplante renal e constitui um dos fatores mais importantes para o transplante bem-sucedido.* ***Objetivo:*** *O estudo em questão avaliou os níveis plasmáticos de creatinina e citocinas em pacientes transplantados renais em função da tipagem HLA realizada no período pré-transplante.* ***Métodos:*** *Foram avaliados 40 pacientes transplantados renais de dois centros de transplantes em Belo Horizonte (MG).* ***Resultados:*** *Os pacientes foram distribuídos em grupos de acordo com o número de compatibilidades HLA e constatou-se, por meio de análises estatísticas, que o grupo com mais de três compatibilidades (H3) apresentou níveis significativamente menores de creatinina pós-transplante em relação aos grupos com três ou menos compatibilidades (H2 e H1, respectivamente). As medianas dos níveis plasmáticos das citocinas interleucina 6 (IL-6), fator de necrose tumoral alfa (TNF-α) e interleucina 10 (IL-10) também foram avaliadas em função do número de compatibilidades. Observou-se que as citocinas pró-inflamatórias (IL-6 e TNF-α) estavam significativamente maiores nos grupos com menor compatibilidade HLA. Por outro lado, a citocina reguladora IL-10 apresentou níveis plasmáticos significativamente maiores no grupo com mais compatibilidades entre doador e receptor.* ***Conclusão:*** *Esses achados permitem inferir que a tipagem HLA de doadores e receptores pré-transplante pode influenciar na função renal do enxerto pós-transplante, bem como contribuir para o desenvolvimento e a escolha de novas estratégias de tratamento.*

Unitermos: *transplante renal; creatinina; citocinas; HLA.*

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