Correlating Levels Of Cyclosporine Drug And Clinical Presentations In Renal Transplant Patients: An Approach To Understand Drug Induced Adverse Effects

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Abstract

Purpose: Cyclosporine is a calcineurin inhibitor that acts as an immunosuppressant by blocking T-cell activation and is used for immune-suppression in renal transplantation. Its adverse effects include tremors, hyperplasia, hirsutism, hypertension, hyperuricemia, hyperlipidemia, etc. We aim to correlate clinical presentations and cyclosporine drug level in renal transplant patients and explore cyclosporine toxicity in clinical practice.

Methods: We enrolled 50 renal transplant patients (44 males and 6 females; age range: 26 to 58 years) at 3 months post-transplant in this observational study, which was conducted at Nephrology Department, Al Basrah General Hospital (January 2016-June 2017). Clinical examination was conducted for tremors, gum hypertrophy and hirsutism. Regular monitoring of cyclosporine trough (C0) drug level was carried out along with other routine investigations.

Results: Mean cyclosporine trough drug level was calculated to be 270 ng/mL ±123.2. Our study showed significantly increased incidence of cyclosporine toxicity in male patients (22%) as compared to female patients (10%) and in the age group of 30-50 years (24%) (p=0.017). Higher cyclosporine trough levels significantly increased incidences of tremors in 13 (26%), gum hypertrophy in 12 (24%), bone pain in 6 (12%), hirsutism in 13 (26%), nephrotoxicity in 5 (10%), hyperuricemia in 7 (14%) and hyperkalemia in 7 (14%) renal transplant patients as compared to patients with normal cyclosporine therapeutic trough level (p=0.0001 for all).

Conclusion: Overall data emphasizes that concurrent evaluation of clinical presentations and monthly cyclosporine drug level is essential to achieve optimal medical care for renal transplant patients and avoid drug-induced adverse effects.

Trial Registration: Not available

Key words: cyclosporine trough level, renal transplant, nephrotoxicity, adverse effects, clinical presentations, immunosuppressant

INTRODUCTION

Transplantation of kidney is established as a safe treatment strategy for patients with end-stage renal disease for the past few decades. Operated patients are at a risk of disturbed balance of immunosuppression; therefore, successful renal transplantation requires a potentially efficient immunosuppressive therapy without toxicity [1]. The major backbone of these immunosuppressive agents includes calcineurin inhibitors such as cyclosporine (CA), which is a lipophylic cyclic polypeptide that interacts with cyclophilin to form a complex [2]. This complex further inhibits activation of calcineurin, which prevents nuclear translocation of NF-AT, a transcription factor, thereby, inhibiting the activation of another downstream signaling molecule NF-κB. This causes inhibition of IL-2 gene expression, which has an autocrine function for induction of proliferation and clonal expression of T-cells and also helps in the production of other cytokines [3].

With respect to drug pharmacokinetics, after its initial absorption of CA, the peak level of blood concentration is achieved in the duration of first 2-3 h; therefore, it is time for maximum inhibition of calcineurin. Further metabolism of drug is mainly performed by the cytochrome P450 3A enzyme system in the liver along with intestinal cytochrome P450 3A4 and by P-glycoprotein counter transport, leading to the fall in the level of CA. It is also termed as elimination phase and reaches to the lowest value, which is recorded before administration of the next dose, termed as trough concentration or C0[4].

CA has a very narrow therapeutic window; therefore, therapeutic drug monitoring is important for its optimization for immunosuppressive treatment and to avoid drug-related toxicities [5].

In spite of improvement in rates of acute rejection and increased survival rates, long-term survival data is not promising, with five-year survival of 81.4% in living related donors and 71.6% in those who have been transplanted with deceased donors as reported by a European study. One contributing factor for this data not showing promising long-time survival could be nephrotoxic effects of CA [6,7]. The nephrotoxic effect of CA could be "acute nephrotoxicity," which is reversible, and "chronic nephrotoxicity," which is irreversible [8]. It has been explored by various studies that oxidative stress induced by CA is mainly responsible for its nephrotoxic effect, as CA induces endoplasmic reticulum stress along with increased production of reactive oxygen species in mitochondria, which modifies the redox balance, causing nephrotoxicity. Various transcription factors and signaling molecules like p38, ERK, JNK and MAPK have a role in CA-induced nephrotoxicity. Renal fibrosis caused by CA is found to be contributed by fibrogenic cytokine TGF-β1 [9]. It has been explored recently that metabolic enzymes and metabolism of CA also play a role in CA-induced nephrotoxicity. Metabolites of CA are less toxic, but the metabolic rate of CA in kidney is much slow than in liver; this is one of the reasons for nephrotoxicity along with metabolites like AM19, which are directly associated with nephrotoxicity [10].

Other major side effect induced by CA is tremors, which have been shown in various studies [11,12]. Use of calcineurin inhibitors such as CA has been proved to increase the prevalence of hypertension post-transplantation in nearly 60-85% of transplant patients on these drugs [13]. CA has been found to induce acute toxicity on cardiac parameters by causing an imbalance between vasoconstrictors and vasodilators, leading to decreased renal blood flow. It also causes arterial stiffness, which could be related to increased blood pressure [14]. Other side effects include gingival hyperplasia, hirsutism, hyperuricemia and hyperlipidemia [15-19].

Above said side effects indicate critical importance of monitoring levels of CA given to renal transplant patients and keeping in check most of the clinical presentations that could occur due to increased CA level so that dose could be optimized for better use with less adverse effects. Regulation of blood levels of CA needs to be done intensively in renal transplant patients, and one of the oldest methods to assess the level of drug is measurement of pre-dosage blood trough (C0) level or two hours post dose level (C2), which is used till date [20-22]. These assays to regularly measure CA levels are significant for the management of renal transplant patients because there are both inter and intra-patient variations in CA absorption and metabolism [23]. A multicentric prospective study recruiting 334 patients showed that measuring the levels of CA 2 days post-transplant could be the strongest predictor for acute graft rejection. It was found that trough levels of CA between 300 to 400 ng/ml were associated with lower risk and lower than 300 ng/ml resulted in doubling the risk of rejection. Moreover, levels above 400 ng/ml failed to provide protection from graft rejection [22]. Einecke et al. monitored C2 and C0 levels in 127 long-term allograft recipients over a time period of nearly 13 months along with repeated monitoring in 46 stable patients. Their observational study concluded that C2 concentration between 500 and 600 ng/ml was well tolerated by the transplant patients [24]. These studies further indicate that monitoring levels of CA could help in successful renal transplantation.

Keeping all the above studies and importance of monitoring CA for successful renal transplant in consideration, we attempted to correlate interdependency of various clinical presentations and CA drug level in renal transplant patients and look for CA toxicity in clinical practice.

METHODOLOGY

Patient recruitment and clinical examinations

We enrolled 50 renal transplant patients (age range: 26-58 years), three months post transplantation, in this study. Of the included patients, 44 were males and 6 females.

The study was conducted at the Nephrology Department of Al Basrah General Hospital from January 2016 to June 2017. Ethical clearance was taken from institutional review board for conducting the study. Proper data sheets were prepared mentioning ages, gender and case history of the patients. Clinical examination was conducted in all the patients for tremors, gum hypertrophy and hirsutism. Routine investigations such as blood urea, serum creatinine, electrolytes as serum potassium, uric acid, and low-density lipoprotein cholesterol were performed in addition to cyclosporine trough (C0) drug level.

Drug used

Oral solution of CA (Sandimmune® Novartis Drug Company) available at a concentration of 100 mg/ml stored at 68 to 77 °F (20 to 25 °C) was used in this study.

CA concentration assay

Apparatus and reagents used

Level of CA drug was measured with Elecsys® reagent kit on the Cobas e411 Analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The kit contains 3 reagents: a CA specific antibody, a ruthenium (Ru)-labeled CA and microparticle reagents contain streptavidin microparticles.

Pre-treatment of samples

Assays were performed as per manufacturer's protocol. In short, whole blood was pre-treated for precipitating proteins and extracting CA. Samples including calibrator, control and patient samples were combined with Elecsys Immuno Suppressive Drug (ISD) sample in 1:1 ratio along with a methanol-based solution containing zinc sulfate, in a micro centrifuge tube. Post mixing for 180 sec, this tube was centrifuged for 4 min at 15,700 *g*. Supernatant obtained after centrifugation was separated in a sample cup, and further analysis was done using Cobas e411 Analyzer.

Assay procedure

20 µL of pretreated sample was incubated for 9 min with CA specific biotinylated antibody as a first step of the assay. Further to this sample, streptavidin coated magnetic microparticles were added and incubation was done again for 9 min. Through the interaction between biotin and streptavidin during the second incubation step, entire complex is bound to the solid phase. This reaction mixture was aspirated to the measuring cell and magnetic force was used to attract microparticles towards the electrode. Washing was done to remove the unbound substances. Charging of the electrodes caused oxidation and further reduction was caused by tripropylamine. This induced chemiluminescent emission of the bound complexes which was dependent on the concentration of CA in the sample. Emission intensity of calibrator operated in the same manner was used for measuring the sample concentration.

Doses of CA

The doses of CA were adjusted to maintain 12-hour trough (C0) levels of 100 to 200 ng/mL for subsequent three months post-transplantation. It was considered as "normal therapeutic range." Cyclosporin toxicity was considered as "high trough level" if CA drug trough (C0) levels were more than 200 ng/mL for subsequent three months of post-transplant period. It was correlated with clinical features of toxicity (Roche Diagnostics Elecsys® Cyclosporine package insert, 2013). CA nephrotoxicity was considered in the case of increased blood urea and/or serum Creatinine.

Statistical Analysis

We coded the data and stored it in the computer. Analysis was done on SPSS version 20, for determination of statistical significance among different variables. $p<0.05$ was considered as significant and calculated by method of Pearson Chi square equation. The percentage in result tables were considered for total numbers.

RESULTS

Study design and CA levels

We had enrolled 50 renal transplant patients (males: 44; females: 6, age range: 26-58 years), 3-months post transplantation for this study (Supplementary Table 1). Mean age was calculated to be 39.7 years ± 10.9 SD. Mean CA trough drug level was calculated to be 270 ng/ml ± 123.2 SD (Figure 1, table 2).

Gender and age distribution with CA trough level

Our data suggested significantly increased incidence of CA toxicity in male patients (n=11; 22%) as compared with female patients (n=5; 10%) in the total population of the recruited patients (p=0.004) (Table 1).

We found significantly increased incidences of CA toxicity in renal transplant patients in the age group of 30-50 years (n=12; 24%) as compared to other age groups (p=0.017) (Table 2) in the recruited patients.

Adverse effects associated with CA trough level

We found in Table 3 increased incidences of CA toxicity in renal transplant patients with higher CA trough levels in the form of clinical presentations such as tremors (n=14; 28%), gum hypertrophy (n=12; 24%), bone pain (n=8; 16%), hirsutism (n=13; 26%), nephrotoxicity (n=10; 20%), hyperuricemia (n=14; 28%) and hyperkalemia (n=8; 16%) as

compared to patients with normal CA therapeutic trough level. This difference was statistically significant in all the clinical presentations (p=0.0001 for all).

DISCUSSION

In our study, tremors emerged as the major clinical presentation due to toxicity of high CA levels, with 28% patients with tremors having high CA levels. This was significantly higher compared to those having normal therapeutic levels of CA. On the contrary, a similar study conducted in Iranian population found that none of the patients with CA at levels higher than therapeutic level had tremors while 12-14% patients with adequate CA levels and low CA levels had tremors (25). However, our findings corroborated with a recent finding that showed nearly 60% of patients on CA post-transplantation had tremors.[11] Various studies have showed that a large percentage of patients taking CA develop various neurological side effects including tremors. However, studies have also showed that these tremors do not affect daily life activities of the individual if the dose of CA is adequate.

Usually, higher blood levels of CA are associated with tremors of large amplitude. It has been found that CA affects dopaminergic neurotransmission, physiology of receptors, phosphorylation pathways and transcription [12,25,26]. A study on Göttingen minipigs showed that none of the pigs had any behavioral signs or lesions in MRI, characteristic of CA encephalopathy. Gross pathological examination showed the brain of all pigs was normal. Histological examination showed that only one out of 5 pigs showed signs of neuronal, perivascular, and meningeal granulocytic and mononuclear infiltrates [27]. Thus, the low dose of CA did not affect brain, emphasizing that monitoring of therapeutic dose of CA can reduce the chances of tremor-like adverse effects.

Gum hypertrophy was observed as another important clinical manifestation, which was significantly higher in transplant patients having high CA levels when compared with patients having therapeutic levels of CA. Similar results were obtained by Ghafari et al. who enrolled 200 renal transplant patients undergoing CA treatment for at least 12 months. About 35% of these patients developed gingival enlargement, and significant correlation was obtained between the concentration of CA and intensity of enlargement of gingival area.[16] This finding was supported by another case study of a 9-year-old recipient who underwent renal transplant, was on CA, and developed gingival overgrowth [28]. This CA-induced gingival hypertrophy has been attributed to increased production of IL-6 and TGF-β1 levels and also involves signal transduction molecules p38, PI3K and MAPK essential for gingival fibroblast proliferation. Chae et al. showed that exposure to CA up regulates DNA synthesis, cell-cycle and proliferation rate of human gingival fibroblasts [29]. CA was also shown to increase the expression of keratinocyte growth factor receptor, which is in line with increased proliferation of epithelial cells in CA-induced gum hypertrophy [30].

Another side effect of CA is nephrotoxicity, which was significantly higher in the group with higher levels of CA as compared to patients having normal therapeutic levels of CA. CA-induced nephrotoxicity has been reported in various studies [31]. As discussed in the introduction, CA could induce both acute and chronic nephrotoxicity. Acute nephrotoxicity induced by CA is explained by the activation of rennin-angiotensin system, afferent arteriolar vasoconstriction, increased vascular resistance of afferent and efferent arterioles along with the decrease in Glomerular Filtration Rate. CA has also been shown to increase vasoconstrictor factors, like endothelin and thromboxane, and decrease prostacyclin, prostaglandin E2, and nitric oxide (NO), which are vasodilatory factors. Alteration in transcription of genes such as NO synthase, Collagen I and IV, Bcl-2 along with transforming growth factor beta (TGF β), ET 1 by CA are responsible for chronic nephrotoxicity induced by it [7,32].

Hirsutism was also observed to be significantly higher in patients with high levels of CA, which was also reported in other studies [33]. Hair growth promoting activity of CA could be ascribed to its growth promoting effect on epithelial cells of hair, along with down-regulation of protein kinase C isozymes in hair epithelial cells [34].

We further observed significantly high levels of uric acid in renal transplant patients having higher levels of CA, indicating a strong correlation between high levels of CA and hyperuricemia. Similar results have been found in a study which analyzed 17,686 blood samples and showed that high trough level of CA is a major risk factor for increased uric acid, along with few other studies. [19,35].

Hyperkalemia is a significant clinical manifestation observed more in patients having high level of CA as compared to transplant patients with normal therapeutic level of CA. This data was supported by similar findings in various other studies [36-38]. It has been postulated that CA alters the function of various transporters, decrease the activity of renin-angiotensin-aldosterone system and causes impairment in tubular response to aldosterone, thereby, reducing excretion of potassium. Some in vitro data suggested that CA causes impairment of potassium secreting cells in cortical collecting tubule by reducing activity of Na+, K+-ATPase pump, inhibiting luminal K channels, and increasing the absorption of chloride both paracellular or transcellular, which prevents negative potential generation in lumen, which stimulates K secretion [39,40].

Overall data corroborates most of the clinical presentations with high blood levels of CA, indicating the importance of monitoring CA levels in renal transplant patients not only to overcome graft rejection but also to avoid various serious

adverse effects for a healthy daily-life of a renal transplant patient. One of the major limitations of this study is small cohort of patients. A larger cohort would further support the observations of this study.

CONCLUSIONS

This study supports the monitoring of CA trough level in patients who have undergone renal transplant and follows the clinical presentations to achieve optimal medical care of renal transplant patients. The predictors for CA toxicity in our study were male patients in the age group of 30-50 years with high CA trough level. The important clinical presentations of CA toxicity are tremors, gum hypertrophy, bone pain, hirsutism, nephrotoxicity, hyperuricemia and hyperkalemia.

RECOMMENDATIONS

Nephrologist who care for renal transplant patients taking CA should be aware of its trough level and signs of CA toxicity and consider meticulous follow-up by full history and clinical examinations in addition of monthly CA drug level.

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Supplementary Table 1: Doses of cyclosporine used in the study

Oral dose of Cyclosporine per mg as twice daily		Age			Total
		< 30 years	30-50 yr	>50yr	
Dose	75.00	1	0	0	1
	100.00	5	10	4	19
	150.00	9	6	0	15
	170.00	0	1	0	1
	175.00	2	4	1	7
	200.00	0	2	1	3
	250.00	0	3	1	4
	Total	17	26	7	50

Table 2 Distribution of age with cyclosporine trough level

Age	Cyclosporine trough level				Total (%)	
	Normal therapeutic range		High trough level			
	NO.	%	NO.	%	NO.	%
Less than 30 years	16	(32%)	1	(2%)	17	(34%)
30 – 50 years	14	(28%)	12	(24%)	26	(52%)
More than 50 years	4	(8%)	3	(6%)	7	(14%)
Total	34	(68%)	16	(32%)	50	(100%)

*P = 0.017, Pearson Chi square=8.1

Table 3 Distribution of adverse effects with cyclosporine trough level

Adverse effects	**Cyclosporine trough level**				***P* value**
	Normal therapeutic range		**High trough level**		
	NO.	**%**	**NO.**	**%**	
Tremors	**3**	6%	**14**	28%	**0.0001**
Gum hypertrophy	**3**	6%	**12**	24%	**0.0001**
Bone pain	**2**	4%	**8**	16%	**0.0001**
Hirsutism	**2**	4%	**13**	26%	**0.0001**
Nephrotoxicity	**2**	4%	**10**	20%	**0.0001**
Hyperuricemia	**3**	6%	**14**	28%	**0.0001**
Hyperkalemia	**2**	4%	**8**	16%	**0.0001**

*Table 3 shows statistically significant increase in incidences of cyclosporine toxicity in renal transplant patients with clinical presentations such as tremors 14 (28%) , gum hypertrophy 12 (24%) , bone pain 8 (16%) , hirsutism 13 (26%) ,

nephrotoxicity 10 (20%) ,hyperuricemia 14(28%) and hyperkalemia 8 (16%)as compared to normal cyclosporine therapeutic trough level of studied patients (p=0.0001 for all)