

Original Research

Impact of Cyclosporine A on White Blood Cell Counts and Some Physiological Parameters in Renal Transplanted Patients: A Case Control Study

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Abstract:

Background:

Cyclosporine A (CyA) is a lipophilic cyclic undecapeptide originally derived from the filamentous fungus *Tolypocladium inflatum*. CyA has been used as a potent immunosuppressive agent for the treatment of autoimmune diseases and a number of organ transplantations, such as kidney, liver, heart, and bone marrow, resulting in a significant improvement in clinical outcome. This study involved 30 renal transplanted patients whom received Cyclosporine A as an immunosuppressant after transplantation in order to reduce the receiver's immune response against the donor's tissue or graft rejection. Demographic patterns and total white blood cells and other serum parameters were evaluated in order to determine the effect of CsA within specific therapeutic regimen. Total WBC was significantly reduced in patients, Urea and creatinine were increased remarkably. Serum GPT, K⁺ and Ca⁺ were also significantly changed. Results showed that CsA might has a potential impact on decreasing the WBC counts and increasing the triglyceride, cholesterol and blood glucose and alters the liver function. The side effects of the drug were considered as a risk for hyperlipidemia and alters the liver enzymes activity.

Key word: Cyclosporine A, WBC, Renal Function, Liver Function, Renal Transplantation

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Introduction

Cyclosporine A (CsA) is a compound widely used as an immunosuppressive drug, particularly, in case of heart, liver and kidney transplantation to prevent rejection of transplanted organ. Toxic effects of CsA have been demonstrated in a variety of organs of experimental animals, including kidney, liver, hematopoietic systems, the lymphoid system, the alimentary tract, and skin (1).

Cyclosporine A (CyA) is a lipophilic cyclic undecapeptide originally derived from the filamentous fungus *Tolypocladium inflatum*. Despite its effectiveness, there are several problems and limitations in the treatment of CyA: these include transplant rejection, opportunistic infection, and renal toxicity (2).

Dose adjustments of these drugs are required for their effectiveness and safety; the narrow therapeutic range of a drug from a maximum effect to a minimum toxicity demands a strict dosage. Therefore, to avoid adverse effects and to ensure its effectiveness, dosage individualization and therapeutic drug monitoring (TDM) mainly based on the plasma concentrations are required (3). CyA also has a narrow therapeutic range and requires TDM. However, CyA is extensively affected by many factors such as transplant organ, time after transplantation, presence of various disease states, concurrent use of drugs, race, age, and gender, resulting in a large individual variability (2, 4). Therefore, the current strategy of CyA treatment demands frequent dose adjustment for TDM following empirical initial dosage adjusted for TBW, which would not be considered optimal (2).

As indicated by experimental studies, immunosuppressive drugs like CsA and tacrolimus can lead to diabetes by decreasing insulin production and release, reducing peripheral insulin sensitivity, and being β -cell toxic (5). These 2 immunosuppressive drugs have also been the focus in various comparative studies which have revealed a significantly

higher incidence of PTDM as a result of tacrolimus therapy compared to CsA therapy. Other risk factors including a history of diabetes in the family, obesity, human leukocyte antigen type, race, and age have also been reported to be involved with the incidence of PTDM (4). It should be noted that because PTDM has not uniformly been defined, classifying patients with varying degrees of glucose tolerance is difficult, which, in turn, complicates evaluating the importance of different risk factors affecting long-term outcomes (6).

As suggested by research studies, different protocols of immunosuppressive drugs need to be employed for different patients. For example, patients with delayed graft function, a higher level of preformed antibodies, and previous transplants, or young patients should be given higher doses of immunosuppression (7). In addition, patients receiving kidneys from older donors are likely to be less tolerant to immunological assault and other aggression, while those with kidneys received from well-matched donors may need fewer immunosuppressive drugs. It has also been shown that acute rejection may happen during the first months after transplantation (induction phase) and decline later on (maintenance phase). As a result, the highest level of immunosuppressive drugs should be administered during the early period followed by decreased levels for long-term therapy (8).

Materials and Method

Thirty patients of renal transplantation and 30 healthy individuals were included in this study. A questionnaire form was filled for each of them. 7 ml of blood was taken from the participants and divided into two aliquots. 2 ml to the heparinized tube for the hematological study and 5 ml to the gel tube to obtain serum for further parameters. Blood samples were also obtained on which biochemical examinations were conducted so as to collect the required data on the subjects' random blood

sugar (RBS), white blood cell count (WBC), creatinine level, and blood urea nitrogen (BUN). Cholesterol, triglyceride, liver function test and serum electrolytes. Quantitative data was given as mean \pm standard deviation (SD). The independent sample t test was employed to compare the parameter means (age, BMI, BUN, creatinine, WBC, RBC, cholesterol, triglyceride, K⁺, Na⁺, and Ca⁺) between the two studied groups (case and control). A P value 0.05 was considered as the statistically significant level. SPSS 22.0 was used to analyze the data.

Results

Results of the present study showed different age groups of the patients with the mean 33.8 years. The ratio of male was higher than female, 55.6% and 44.4% respectively. The BMI of the case group was almost within normal range 25.33, table 1.

Cyclosporine A was used as immunosuppress drug for the renal transplanted patients with two different maintenance phases of dosage in order to give the best result, at scheduled period. First week 10mg /Kg/Day and then reduced to 8mg/Kg/Day for the next weeks, table 2.

Impact of the CsA was evaluated on total white blood cell counts, and some other physiological parameters were measured to show the effect of the immunosuppressive drug. Total WBC count was significantly decreased in patient, $p=0.031$. Lymphocytes and monocytes were decreased non significantly, $p>0.05$. While the number of granulocytes was increased but the difference was not significant. Renal function parameters (Urea and Creatinine) were changed in patients in comparison to control group. But were significantly increased. Serum cholesterol and triglyceride were remarkably increased in patients $p<0.05$.

Cholestrol and triglyceride were significantly increased in patients, $p<0.05$. Regarding the liver enzymes, the ALP and GOT were increased non significantly. While the GPT

was significantly increased. Total serum bilirubin, direct and indirect bilirubin were increased non significantly in patients. Serum electrolytes K⁺ and Ca⁺ were increased remarkably, $p<0.05$, while the differences in Na⁺ and Cl⁺ were not significant, $p>0.05$, table 3.

Discussion

Elsayed et al., reported that chronic treatment of rats with CsA induced nephrotoxicity by increasing Kidney function parameters. The relationship between anemia and chronic kidney disease is thoroughly documented (9). Also, anemia is common after treatment with cyclosporine in the liver transplanted patients which may be due to inhibition of erythropoietin production induced by cyclosporine. Also, erythropoietin production was reduced when cyclosporine added to the culture medium of the human Hep3B cell line(10). In activated T cells, cyclosporine has the ability to block the transcription of cytokine genes, through the inhibition of the phosphatase activity of calcineurin, which subsequent activation of NFAT transcription factor and regulates nuclear translocation. Also, cyclosporine blocks the JNK, NFkB, and p38 signaling pathways (11). Tacrolimus and cyclosporine have several effects on the growth factors and production of cytokines. Both immunosuppressive drugs are impairing the production of IFN gamma, interleukin 2, and interleukin 3(12).

Cyclosporine exerts its main pharmacological effect as an immunosuppressive by binding to a member of intracellular protein known as immunophilin, forming a complex that interferes with signalling pathway important for the clonal expansion of lymphocytes. It blocks proliferation of T-cell by inhibiting the phosphatase activity of a Ca²⁺ activated enzyme at nanomolar concentration (13). The compound binds to the cyclophilins and the cyclophilin-drug complex binds and inhibits the calcineurin When intracellular Ca²⁺-levels

rise calcineurin dephosphorylates the NFATc in the cytoplasm, which migrate to the nucleus to form complexes with nuclear partners, and induce transcription of genes including those for IL2, CD25, CD40 ligand, Fas ligand and interferon (IFN) (14). In the previous study, cyclosporine A treatment induced a decrease in WBCs count, and lymphocytes %. On the other hand, chronic treatment of rats with CsA induced a significant increase ($p < 0.05$) in neutrophils % after 1, 2 & 4 weeks as compared with controls. Also, monocytes % was a significantly increase ($p < 0.05$) in rats treated with CsA for 1 & 2 weeks when compared with controls (14). These changes are in accordance with the previous studies which reported that the immunosuppressive agent, mycophenolate mofetil, caused leucopenia with a lymphopenic effect (15). Bone marrow is a source of blood cells involved in immune activity because it is the site of proliferation and turnover of blood cells. It is the organ most affected by immunosuppressive drugs, which may decrease the ability of bone marrow to produce new blood cells leads to leucopenia (16).

CsA is known to reduce renal blood flow by afferent arteriole vasoconstriction. This may occur even after one dose of CsA and some degree of renal vasoconstriction probably occurs in all CsA-treated subjects (17). The vasoconstriction may produce a dose-dependent rise in serum creatinine, as well as clinical manifestations of a reduced glomerular filtration rate, hypertension, hyperkalemia, tubular acidosis, and increased reabsorption of sodium and oliguria. These acute hemodynamic effects are functional, dose-related, and reversible (18). The potential mediators of cyclosporine-induced vasoconstriction, including activation of renal sympathetic nerves, endothelin-1, angiotensin II, reduced nitric oxide production, or alteration of the prostaglandin-thromboxane cascade (19).

However, novel clinical data indicate that CsA-induced chronic nephropathy can occur

independently of acute renal dysfunction, cyclosporine dosage, or blood concentration. Although the exact mechanism of chronic CsA nephrotoxicity is not fully understood, several factors have been implicated in the pathogenesis of immunosuppressive-induced nephrotoxicity (20).

It is well known that CsA treatment induces intrarenal vasoconstriction and enhances vascular reactivity to various contractile agonists that is thought to become permanent and irreversible after 3 months. CsA-induced alteration of GFR has been studied primarily in liver, renal, and cardiac transplant recipients (21). In those patients, CsA-induced nephrotoxicity may lead to end-stage renal failure and dialysis. However, transplantation is a condition often associated with multifactorial decline in renal function. Most studies were descriptive and retrospective, evaluated renal function on the basis of plasma creatinine, which is a notoriously inaccurate marker of renal function, did not include any value (either by measurement or calculation) of the GFR, and did not report pathologic data corroborating with biologic evidence of renal failure (22).

Experimental studies and clinical observations reveal that CsA can lead to drug-induced liver injury (DILI). In CsA induced liver injury, functional and morphological changes are observed. The functional changes include elevated serum levels of liver transaminases and alkaline phosphatase, cholestasis, hyperbilirubinemia, increased production of bile salts, and impaired secretion of lipids. The morphological changes observed in experimental animals receiving CsA include impaired trabecular structure, hepatic sinus congestion and widening, activation of the Kupffer cells, passive congestion and oedema of portal tracts, mild mononuclear cell infiltrations within portal tracts, and degenerative changes in the hepatocytes including their focal necrosis (23).

Drug-induced liver injury can develop in the form of acute drug-induced hepatitis or cholestatic hepatitis. Toxic effects of drugs can cause degenerative changes in the hepatocytes, including their necrosis (paracetamol, bendazac, CsA, carbon tetrachloride, and ethionine), steatosis (tetracycline and ticlopidine), or cholestasis (methapyrilene and naphthyl isothiocyanate) (24). Three major mechanisms of drug-induced liver injury have been implicated: (1) direct cell injury, (2) inhibition of mitochondrial betaoxidation and the mitochondrial respiratory chain, and (3) immunologic reactions. The symptoms of toxic action of drugs observed in histopathological examination include hepatocyte degeneration or necrosis, inflammatory infiltrates in the portal tracts, Kupffer cells, or stellate cell activation. The mechanisms explaining drug-induced liver injury include mitochondrial damage and oxidative stress (25).

Long-term treatment with CsA is associated with hyperlipidemia and an increased risk of atherosclerosis. The mechanisms by which cyclosporin A causes hyperlipidemia are unclear. Cell and animal studies have pointed to various mechanisms that may mediate CsA-induced hyperlipidemia (4).

Hyperlipidemia is observed in about 60% of kidney, liver, cardiac and bone marrow transplants after treatment with CsA. There are multiple factors potentially contributing to hyperlipidemia in these patients, such as post-transplantation obesity, multiple drug therapy and diabetes. The concurrent use of steroids in particular, makes it hard to establish a direct contribution of CsA to dyslipidemia in humans, as corticosteroids are known to exacerbate hyperlipidemia in transplant recipients (26).

Only a few studies have directly compared the combination of CsA therapy with low dose prednisolone with other immune suppressing strategies in combination with low dose steroids (27, 28). In general, these studies indicate that CsA treatment can independently lead to elevated plasma triglyceride and

cholesterol levels in humans and that these effects are reversible upon cessation of immunosuppression therapy (26).

Similar to our results, as it shows the effect of cyclosporine A on ALP and ALT besides bilirubin levels. With low doses of cyclosporine A (100 and 50 mg/day) our patient's laboratory findings were normal, except for mild hyperbilirubinemia. After stopping cyclosporin A, serum bilirubin levels returned to normal. Is this a dose-dependent effect of cyclosporine A (23).

Conclusion

Regarding the results of the present study. The most dependent candidate of immunosuppressant Cyclosporine A was usable as much as gives the best results within the therapeutic regimen determined here. The side effects of the CsA was considered as a risk for hyperlipidemia and alters the liver enzymes activity.

References:

1. Mukherjee, S., & Mukherjee, U. (2009). A comprehensive review of immunosuppression used for liver transplantation. *Journal of transplantation*, 2009, 701464. <https://doi.org/10.1155/2009/701464>
2. Kokuhu, T., Fukushima, K., Ushigome, H., Yoshimura, N., & Sugioka, N. (2013). Dose adjustment strategy of cyclosporine A in renal transplant patients: evaluation of anthropometric parameters for dose adjustment and C0 vs. C2 monitoring in Japan, 2001-2010. *International journal of medical sciences*, 10(12), 1665–1673. <https://doi.org/10.7150/ijms.6727>
3. Kang, J. S., & Lee, M. H. (2009). Overview of therapeutic drug monitoring. *The Korean journal of internal medicine*, 24(1), 1–10. <https://doi.org/10.3904/kjim.2009.24.1.1>
4. Ismael, I., Azeez, S. (2019). Immunosuppressive Drugs and Kidney

- Post-transplant Diabetes Mellitus. *Hospital Practices and Research*, 4(2), 50-56. doi: 10.15171/hpr.2019.09
5. Penfornis A, Kury-Paulin S. Immunosuppressive drug-induced diabetes. *Diabetes Metab*. 2006 Dec;32(5 Pt 2):539-46. doi: 10.1016/s1262-3636(06)72809-9. PMID: 17130815.
 6. Bansal N. (2015). Prediabetes diagnosis and treatment: A review. *World journal of diabetes*, 6(2), 296–303. <https://doi.org/10.4239/wjd.v6.i2.296>
 7. Mahmud, N., Klipa, D., & Ahsan, N. (2010). Antibody immunosuppressive therapy in solid-organ transplant: Part I. *mAbs*, 2(2), 148–156. <https://doi.org/10.4161/mabs.2.2.11159>
 8. Kalluri, H. V., & Hardinger, K. L. (2012). Current state of renal transplant immunosuppression: Present and future. *World journal of transplantation*, 2(4), 51–68. <https://doi.org/10.5500/wjt.v2.i4.51>
 9. Elsayed ASI, Bayomy MFF, Azab AE. Effect of acute and chronic treatment of Cyclosporine A on liver and kidney functions in rats. *J Appl Pharm Sci*. 2016;6(03):116–119.
 10. Bardet V, Junior AP, Coste J, Lecoq-Lafon C, Chouzenoux S, Bernard D, Soubrane O, Lacombe C, Calmus Y, Conti F. Impaired erythropoietin production in liver transplant recipients: the role of calcineurin inhibitors. *Liver Transpl*. 2006 Nov;12(11):1649-54. doi: 10.1002/lt.20898. PMID: 17058250.
 11. Pan, M. G., Xiong, Y., & Chen, F. (2013). NFAT gene family in inflammation and cancer. *Current molecular medicine*, 13(4), 543–554. <https://doi.org/10.2174/1566524011313040007>
 12. Howell J, Sawhney R, Testro A, Skinner N, Gow P, Angus P, Ratnam D, Visvanathan K. Cyclosporine and tacrolimus have inhibitory effects on toll-like receptor signaling after liver transplantation. *Liver Transpl*. 2013 Oct;19(10):1099-107. doi: 10.1002/lt.23712. PMID: 23894100.
 13. Russell RG, Graveley R, Coxon F, Skjodt H, Del Pozo E, Elford P, Mackenzie A. Cyclosporin A. Mode of action and effects on bone and joint tissues. *Scand J Rheumatol Suppl*. 1992;95:9-18. doi: 10.3109/03009749209101478. PMID: 1475634.
 14. Elsayed ASI, Jbirea JM, Azab AE. Effect of acute and chronic cyclosporine a treatment on haematological data in male albino rats. *J Appl Biotechnol Bioeng*. 2018;5(6):350-357
DOI: 10.15406/jabb.2018.05.00164
 15. Khalil, M., Khalil, M., Khan, T., & Tan, J. (2018). Drug-Induced Hematological Cytopenia in Kidney Transplantation and the Challenges It Poses for Kidney Transplant Physicians. *Journal of transplantation*, 2018, 9429265. <https://doi.org/10.1155/2018/9429265>
 16. Boes, K. M., & Durham, A. C. (2017). Bone Marrow, Blood Cells, and the Lymphoid/Lymphatic System. *Pathologic Basis of Veterinary Disease*, 724–804.e2. <https://doi.org/10.1016/B978-0-323-35775-3.00013-8>
 17. Yoon, H. E., & Yang, C. W. (2009). Established and newly proposed mechanisms of chronic cyclosporine nephropathy. *The Korean journal of internal medicine*, 24(2), 81–92. <https://doi.org/10.3904/kjim.2009.24.2.81>
 18. Pazhayattil, G. S., & Shirali, A. C. (2014). Drug-induced impairment of renal function. *International journal of nephrology and renovascular disease*, 7, 457–468. <https://doi.org/10.2147/IJNRD.S39747>
 19. Kohan, D. E., Inscho, E. W., Wesson, D., & Pollock, D. M. (2011). Physiology of endothelin and the kidney. *Comprehensive Physiology*, 1(2), 883–919. <https://doi.org/10.1002/cphy.c100039>

20. Sereno, J., Rodrigues-Santos, P., Vala, H., Rocha-Pereira, P., Alves, R., Fernandes, J., Santos-Silva, A., Carvalho, E., Teixeira, F., & Reis, F. (2014). Transition from cyclosporine-induced renal dysfunction to nephrotoxicity in an in vivo rat model. *International journal of molecular sciences*, *15*(5), 8979–8997. <https://doi.org/10.3390/ijms15058979>
21. Takeda A, Uchida K, Haba T, Tominaga Y, Katayama A, Yoshida A, Oikawa T, Morozumi K. Chronic cyclosporin nephropathy: long-term effects of cyclosporin on renal allografts. *Clin Transplant*. 2001;15 Suppl 5:22-9. doi: 10.1034/j.1399-0012.2001.0150s5022.x. PMID: 11791791.
22. Sandilands, E. A., Dhaun, N., Dear, J. W., & Webb, D. J. (2013). Measurement of renal function in patients with chronic kidney disease. *British journal of clinical pharmacology*, *76*(4), 504–515. <https://doi.org/10.1111/bcp.12198>
23. Agnieszka Korolczuk, Kinga Caban, Magdalena Amarowicz, Grażyna Czechowska, Joanna Irla-Miduch, "Oxidative Stress and Liver Morphology in Experimental Cyclosporine A-Induced Hepatotoxicity", *BioMed Research International*, vol. 2016, Article ID 5823271, 9 pages, 2016. <https://doi.org/10.1155/2016/5823271>
24. David, S., & Hamilton, J. P. (2010). Drug-induced Liver Injury. *US gastroenterology & hepatology review*, *6*, 73–80.
25. Russmann, S., Kullak-Ublick, G. A., & Grattagliano, I. (2009). Current concepts of mechanisms in drug-induced hepatotoxicity. *Current medicinal chemistry*, *16*(23), 3041–3053. <https://doi.org/10.2174/092986709788803097>
26. Maaike Kockx and Leonard Kritharides (October 3rd 2012). Cyclosporin A-Induced Hyperlipidemia, Lipoproteins - Role in Health and Diseases, Sasa Frank and Gerhard Kostner, IntechOpen, DOI: 10.5772/47866. Available from: <https://www.intechopen.com/books/lipoproteins-role-in-health-and-diseases/cyclosporin-a-induced-hyperlipidemia>
27. Srinivas, T. R., & Meier-Kriesche, H. U. (2008). Minimizing immunosuppression, an alternative approach to reducing side effects: objectives and interim result. *Clinical journal of the American Society of Nephrology : CJASN*, *3* Suppl 2(Suppl 2), S101–S116. <https://doi.org/10.2215/CJN.03510807>
28. Lee, J.M., Kronbichler, A., Shin, J.I. *et al*. Current understandings in treating children with steroid-resistant nephrotic syndrome. *Pediatr Nephrol* **36**, 747–761 (2021). <https://doi.org/10.1007/s00467-020-04476-9>.

Tables

Table 1: Demographic distributions of patients and control

Characteristics		Groups		P value
		Case N=30	Control N=30	
Age		33.8±3.9	35.3±2.2	0.690
Sex	Male	17/30 (55.6%)	15/30 (50%)	0.901
	Female	13/30 (44.4%)	15/30 (50%)	0.824
BMI		25.33±2.2	24.88±1.35	0.726

Table 2: cyclosporine A and the therapeutic regimen

Immunosuppressive Drug	Maintenance Phase	Period
CsA dosage	10 mg/kg/day	1-6 days
CsA dosage	8-9 mg/kg/day	2-5 weeks

Table 3: WBC counts, renal function, Cholesterol, triglyceride, liver function and serum electrolytes in studied group

Characteristics	Groups		P value
	Case N=30	Control N=30	
WBC *10 ³ /mL	6.33±1.21	7.56±0.19	0.031
Lym *10 ⁹ /L	1.244±0.5269	1.512±0.352	0.9
Mon *10 ⁹ /L	0.5154±0.269	0.6112±0.149	0.9
Gran *10 ⁹ /L	7.177±3.349	4.344±0.698	0.8
Urea mg/dl	132.11±12.53	45.13±3.87	0.001
Creatinine mg/dl	6.14±0.88	1.04±0.09	0.001
Triglyceride mg/dl	174.4 ± 12.45	102.3 ± 6.436	0.001
Cholesterol mg/dl	200.8 ± 12.093	171.1 ± 9.111	0.025
Blood Glucose mg/dl	128.8 ± 6.560	95.36 ± 2.260	0.011
ALP IU/L	95.58±51.82	88.84±14.61	0.5
GPT IU/L	65.42±26.86	36.00±5.99	0.008
GOT IU/L	51.04±21.82	38.40±7.76	0.6
TSB mg/dl	1.077±0.369	0.588±0.247	0.8
Direct TSB mg/dl	0.4808±0.304	0.2964±0.072	0.6
Indirect TSB mg/dl	0.6769±0.117	0.3680±0.124	0.9
K ⁺ mM/L	8.981 ± 0.2130	4.832 ± 0.1791	0.021
Na ⁺ mM/L	132.2 ± 1.484	136.1 ± 1.797	0.117
Ca ⁺ mM/L	6.813 ± 0.4193	1.180 ± 0.0264	0.001
Cl ⁺ mM/L	108.0±9.163	102.3±8.335	0.7