

Effect of Cyclosporine on the Relevant Biochemical Parameters in Renal Transplantation Patients in Mosul/Iraq

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الخلاصة

السيكلوسبورين هو العمود الفقري لكبت المناعة في مرضى زرع الكلى. ومع ذلك، فإنه يؤدي إلى آثار جانبية متعددة، وهي في معظمها تعتمد على الجرعة. في هذا الصدد، وترتبط جودة وظائف الكلى مما لا شك فيه مع مستويات دواء السيكلوسبورين. تم جمع 44 عينة مصل دم من متلقي زرع الكلى و 28 عينة دم لأشخاص اصحاء، ثم تم قياس اليوريا والكرياتينين والصوديوم والبوتاسيوم، والكوليسترول والدهون الثلاثية و HDL-C و LDL-C و VLDL-C والكلوتاثيون والمالونديالدهيد في مصل الدم. أظهرت الدراسة حدوث زيادة في نسبة الكوليسترول والدهون الثلاثية و HDL-C و LDL-C و VLDL-C في مصل مرضى زرع الكلى بشكل ملحوظ بالمقارنة مع الاصحاء، كما اظهرت النتائج أيضا انخفاض تركيز الكلوتاثيون و زيادة تركيز المالونديالدهيد في المرضى زرع الكلى مقارنة مع الاصحاء. وقد لوحظ ان الكوليسترول والدهون الثلاثية و HDL-C و LDL-C و VLDL-C والمالونديالدهيد كان لهم ارتباط ضعيف مع جرعة السيكلوسبورين في حين اظهر الكلوتاثيون علاقة عكسية ضعيفة مع جرعة السيكلوسبورين.

الكلمة المفتاح: زرع الكلى، السيكلوسبورين، الكلوتاثيون.

ABSTRACT

Cyclosporine (sandimmune) is the backbone of immunosuppression in renal transplantation. However, it leads to multiple side effects, most of which are dose-dependent. In this respect, the quality of renal functions is undoubtedly linked to cyclosporine drug levels.

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Blood sample from 44 kidney-transplant recipients and 28 healthy control were collected, then serum Urea, Creatinine, Sodium (Na⁺), Potassium(K⁺), Cholesterol, Triglyceride, HDL-c, LDL-c, VLDL-c, Glutathione GSH and Malondialdehyde MDA were estimated. The current study showed that serum Cholesterol, TG, HDL-c, LDL-c and VLDL-c were significantly increased in renal transplantation patients comparing with the control group, The result also has been revealed that GSH concentration was decreased in renal recipient patients while MDA was increased renal recipient patients as comparing with the control group. On other hand Cholesterol, Triglyceride, HDL-c, LDL-c, VLDL-c, and MDA exhibited a weak correlation with dose of cyclosporine while GSH exhibited reciprocal correlation with CsA dose.

Key word: renal transplantation, cyclosporine, lipid profile

INTRODUCTION

Renal transplantation is an established method of renal replacement therapy in patients with end stage renal failure⁽¹⁾.

Cyclosporine A (CsA) is a powerful immunosuppressive agent which is now widely used in all organ transplantation such as the kidney and liver. The agent was marketed initially as Sandimmune (SIM) in 1984, its use has considerably improved graft and patient survival after renal transplantation⁽²⁾. The clinical usage of CsA is however restricted due to both functional and structural changes in the kidney of transplant patients and experimental animals⁽³⁾. CsA treatment is associated with increased plasma bile acid concentrations and cholestasis in humans as well as in animal models⁽⁴⁾. The incidence of post-transplant hyperlipidemia has been studied primarily in patients managed on cyclosporine A (CsA)-based immunosuppressive regiment⁽⁵⁾. Hyperlipidemia is observed in about 60% of kidney, liver, cardiac and bone marrow transplants after treatment with CsA⁽⁶⁾. Reported changes in serum lipid levels include elevation of triglycerides and total cholesterol. Hyperlipidemia is associated with significant morbidity and mortality rates in transplant recipients⁽⁷⁾. CsA-induced renal dysfunction, in turn, causes an increase in serum creatinine concentration and a decrease in creatinine clearance. These changes are dose-dependent and are usually reversible after short-term CsA treatment⁽⁸⁾. Also, Antioxidants, such as α -tocopherol, ascorbate, lazaroids, superoxide dismutase and catalase have been shown to diminish CsA-induced renal toxicity⁽⁹⁾. The mechanisms and the role of oxidative stress in CsA toxicity is unknown. Free radicals may be derived directly from CsA or its metabolites⁽¹⁰⁾. Some authors have found that significant increases in MDA concentration and reduced glutathione in recipient patients in treatment of CsA^(11, 12).

The aim of the current research is to study the effect of Cyclosporine on the lipid profile and the biochemical parameters related to renal functions and show the correlation between cyclosporine and glutathione and MDA.

MATERIALS AND METHODS

Subjects

A total of 44 renal transplant patients (M: 29, F: 15) who visit Ibn-Sina Hospital were included in this study, their ages ranged from (22-57) years, and their transplant duration ranged from (24- 360) months . All the studied patients were under cyclosporine A (CsA) treatment with starting dose of 15.6 - 46.5 $\mu\text{l}/\text{kg}$ body weight taken orally / day and gradually reduced according to the physician instructions. While 28 adult individuals were taken from the same population who were apparently healthy were used as a control group.

Determination of renal function tests

Serum creatinine and urea concentrations were determined colorimetrically as described by Bartles *et al.* ⁽¹³⁾ and Patton and Crouch⁽¹⁴⁾, respectively, using commercially available diagnostic kits (Randox, UK; Biomerieux , France respectively).The sodium and potassium in the serum samples were analyzed using flame emission spectrophotometric method ¹⁵. Briefly 1 to 100 dilution of serum was made with deionized water in universal container, mixed and aspirated into the flame analyzer at a wavelength of 589 nm for sodium and 765 nm for potassium having calibrated the machine with a standard solution containing 140 mmol L^{-1} Na and 4.0 mmol L^{-1} of deionized water.

Determination of lipid profile

The lipid profile estimated from the serum was total cholesterol triglycerides, high density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) after 12 hours of fasting. Total cholesterol was assayed by Tindar's reaction ^(16, 17) using commercial kits from Fortress Diagnostics Ltd; Antrim. Serum triglycerides level was determined as described by Fossati and Prencipe ⁽¹⁸⁾, Cole *et al.*, ⁽¹⁹⁾ using commercial kit (Fortress Diagnostics Ltd; Antrim). HDL-c was determined as described by Grove, ⁽²⁰⁾ using commercial kit (Fortress Diagnostics Ltd; Antrim).

VLDL-c was calculated using the formula ^(21, 22) :

$$\text{VLDL-c (mmol/L)} = \frac{\text{Triglycerides}}{2.2}$$

The serum LDL-c level was calculated according to Friedwald's formula^(23, 24).

$$\text{LDL-c (mmol/L)} = \text{TCh- (HDL-c + VLDL-c)}$$

Determination of glutathione and malondialdehyde

Glutathione is determined in serum by utilizing Ellman`s reagent ⁽²⁵⁾. An equal volume 150 µl of serum and 4% sulfosalicylic acid were mixed, centrifuged (314 xg) at 4 °C for 5 minutes. To 150 µl of supernatant, 4.5ml of 0.1 mmol Ellman`s reagent (5,5-dithiobis 2-Nitrobenzoic acid (DTNB)) was added in phosphate buffer of pH 8.0 (Prepared by mixing of 0.6 mol KH₂PO₄ and 0.08 mol K₂HPO₄) , then the absorbance at 412 nm was measured.

The level of serum MDA concentration was determined by using the method described by (Guidet , *et al*) ⁽²⁶⁾. To 150 µl serum sample the following was added: 1 ml trichloroacetic acid (TCA) 17.5%, 1 ml of 0.6% thiobarbituric acid, mixed well by vortex, incubated in boiling water bath for 15 minutes, and then allowed it to be cooled (Figure 1).

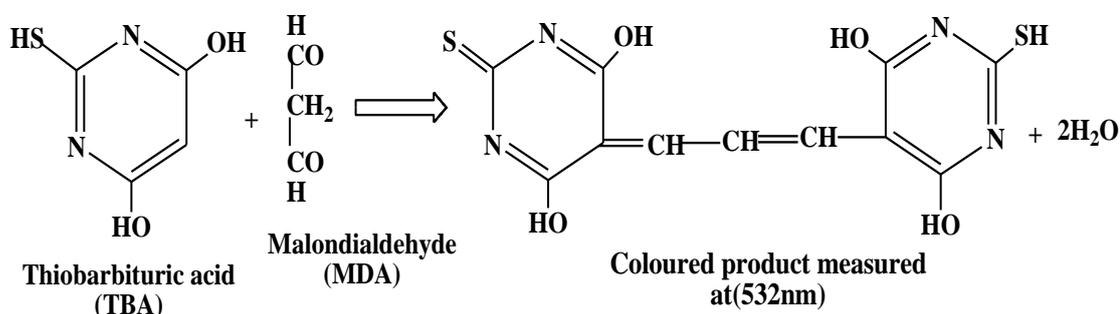


Figure (1): Reaction of malondialdehyde with thiobarbituric acid.

Then 1 ml of 70% TCA was added, and let the mixture to stand at room temperature for 20 minutes, centrifuged at 314 xg for 15 minutes, and the absorbance of the supernatant was measured at 532 nm.

Statistical analysis

All data were analyzed using the statistical package for social sciences SPSS version 14 software for windows 7. The results were expressed as mean ± standard deviation (mean ± SD). One way ANOVA-test was used to compare parameters between dosage of CsA, malondialdehyde and GSH in different studied groups.

RESULTS

Forty four patients with renal transplant and 28 healthy controls were included in the study; the characteristics of those patients and controls are shown in [Table 1](#).

Table 1: Demographic data of the renal transplantation patients and control group

Characteristics	Control group	patients group
	Mean \pm SD	Mean \pm SD
Age(year)	35.14 \pm 8.59	36.3 \pm 13.49
Sex (male/female)	18/10	29/15
Weight(kg)	66.14 \pm 9.68	69.1 \pm 9.02
Dose (μ l/kg/day)	-----	20 \pm 6
Smoking/ non smoking	5/23	3/41

Table 2 demonstrates the mean of lipid profile in renal transplantation patients and controls. Serum Total Cholesterol, TG, HDL-c, LDL-c and VLDL-c were significantly increased in renal-transplantation patient's comparing with the control group (Table 2).

Table 2: Lipid profile in renal transplantation patients and control group

Parameters (mmol/l)	Control group	Patients group
	Mean \pm SD	Mean \pm SD
TC	4.55 \pm 0.40	5.45 \pm 1.23
TG	1.42 \pm 0.28	1.80 \pm 0.57
HDL-c	1.58 \pm 0.47	1.35 \pm 0.47
LDL-c	2.30 \pm 0.58	3.25 \pm 0.94
VLDL-c	0.64 \pm 0.15	0.80 \pm 0.16

TC: Total Cholesterol, TG: Triglyceride, LDL-C: Low Density Lipoprotein- cholesterol, HDL-C: High Density Lipoprotein- cholesterol, and VLDL-C: Very Low Density Lipoprotein- cholesterol

Table 3 showed the clinical parameters of renal function tests, the results showed that serum urea, creatinine, Na⁺, and K⁺ significantly increased in recipient patients comparing with control (Table 3).

Table 3: Renal function tests in renal transplantation patients and control group

Parameters (mmol/l)	Control group	Patients group
	Mean \pm SD	Mean \pm SD
Urea	4.69 \pm 0.51	6.54 \pm 3.38
Creatinine	96.05 \pm 11.59	106.9 \pm 31.68
Na ⁺	140.43 \pm 1.96	140.75 \pm 2.92
K ⁺	4.27 \pm 0.33	4.29 \pm 0.50

The present study has revealed that GSH concentration was decreased (13.42 μ mol/l) while MDA was increased in serum of renal recipient patients (6.60 μ mol/l) as comparing with control group (18.05 μ mol/l). see Table 4.

Table 4: GSH and MDA in renal transplantation patients and control group

Parameters (μ mol/l)	Control group	Patients group
	Mean \pm SD	Mean \pm SD
GSH	18.05 \pm 0.46	13.42 \pm 1.45
MDA	5.16 \pm 0.32	6.60 \pm 0.56

GSH: Glutathione, MDA: Malondialdehyde

Our study showed that total cholesterol, Triglyceride, HDL-c, LDL-c, VLDL-c, and MDA had a weak correlation to the dose of cyclosporine (Table 7).

Table 7: dose correlation with lipid profile, GSH, and MDA

Parameters	Dose
TC	0.049
TG	0.287
HDL-c	0.118
LDL-c	0.054
VLDL-c	0.286
GSH	-0.376
MDA	0.098

TC: Total Cholesterol, TG: Triglyceride, LDL-C: Low Density Lipoprotein- cholesterol, HDL-C: High Density Lipoprotein- cholesterol, and VLDL-C: Very Low Density Lipoprotein- cholesterol, GSH: Glutathione, MDA: Malondialdehyde

DISCUSSION

The present study highlighted the hyperlipidemia, GSH and MDA also its correlation with CsA. The overall number of patients who did their renal transplantation was higher in male than in females in our study. It has been observed that kidney function tests (Creatinine, Urea, Na⁺ & K⁺) were normal comparing to control which differs from other study that showed abnormalities of the electrolytes in patients with renal trans-plantation⁽²⁷⁾, suggested that abnormal transport of electrolytes in renal tubules in transplant kidneys might be responsible for these electrolytes disturbances⁽²⁸⁾. Some studies showed that CsA is associated with electrolyte disturbances; for example, long-term treatment with CsA increases the fractional excretion of sodium⁽²⁹⁾. Also with Gado *et al.*⁽³⁰⁾ who documented that the serum creatinine and urea are elevates in renal-transplantation patients, serum creatinine level was slightly increased compared to the healthy control these results are in agreement with the results of other studies conducted by Wissman *et al.*, 1996, Moroni *et al* 2006^(31,32). Who found that the cyclosporine nephrotoxicity is dose - dependent and the low doses of cyclosporine did not significant changes renal function. A recent study showed the existence of dyslipidemia in renal transplant patients⁽³³⁾. It has been observed that lipid profile altered in transplanted patients and the levels of total cholesterol and triglyceride HDL-c, LDL-c and VLDL-c significantly increased in all patients under cyclosporine regimen as compared with healthy controls. The role of cyclosporine in post-transplant hyperlipidemia is still unclear although some workers had implicated in its causation⁽³⁴⁾. Others indicated that, cyclosporine contributes to hypercholesterolemia in renal transplant patients⁽³⁵⁾. In the present study, it has been reported that cyclosporine had weak correlation with lipid profile levels in agreement with other

studies showing weak correlation between cyclosporine level and lipid profiles, suggested may due to the long duration of treatment by cyclosporine rather than its level may affect the lipid profile in post-transplant patients⁽²⁵⁾, and could explain the discrepancy between results, on other hands disagreement with Awad *et al.*⁽³⁶⁾ and Kuster *et al.*⁽³⁷⁾ who reported that no correlation between TG and total cholesterol levels with cyclosporine dose. In addition, it has been observed that MDA significantly increase in renal recipients with increasing dosage that harmony with Sawicka *et al.*⁽¹¹⁾ who documented that significant increase in MDA concentration after CsA doses of 450–750 ng/ml. In children after liver transplantation, CsA concentrations in plasma of 70–120 ng/ml did not cause statistically significant increases in MDA levels compared to control⁽³⁸⁾. In research on patients with rheumatoid arthritis treated with low doses of CsA it was observed that 2.5 –3.5 mg/kg b.w. of CsA did not increase MDA⁽³⁹⁾.

Glutathione is a small peptide composed of three amino acids, (cysteine, glutamic acid and glycine). It is an important antioxidant and plays a very important role in the defense mechanism of tissues against ROS⁽⁴⁰⁾. In the present study, it has been observed that GSH decreased with increasing dosage in renal recipients which compatible with Mostafavi-Pour *et al.*,⁽⁴¹⁾ who documented that decreased in GSH levels in CsA treated patients suggested a possible direct interference of the drug with the intracellular homeostasis of glutathione⁽⁴²⁾. Other study in rats showed that CsA had an effect on lipid peroxidation; it caused significant decrease in the antioxidant status that are GSH and catalase enzyme activity⁽⁴³⁾.

CONCLUSIONS

In conclusion, hyperlipidemia is a common problem after renal transplantation. CsA has a weak correlation with lipid profile and MDA and it has been reciprocal relationship with GSH.

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