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Original Article

Urinary excretion of N-Acetyl Glucosaminidase (NAG) as a marker of tubular injury in cyclosporin A associated nephrotoxicity in rat and human

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Abstract

The possible role of NAG in the diagnosis of early reversible renal tubular damage caused by cyclosporin A (CyA) was examined. A trial was also done to elucidate any possible correlation in chronic CyA nephrotoxicity. We first tested the effects of different doses of CyA (10,20,40, and 80 mg/kg) on urinary NAG level, serum creatinine (Cr), sodium (Na), potassium (K), creatinine clearance (CC), FENa, FEK, and histopathology of the rat kidney. Secondly, we determined blood CyA trough level in rat and human renal allograft recipient and was correlated to NAG. We found that oral 10 and 20 mg/kg/day CyA produced no tubular toxicity nor drop in CC while 40 mg/kg doses resulted in a significant increase in Cr. with tubular vacuolation of low score in 33% of rats. On the other hand, higher doses of 80 mg/kg/day of CyA produced tubular vacuolation of higher score in 83% of rats. A positive correlation was found between plasma K and CyA doses. NAG was found to be elevated significantly before the appearance of definite pathogenic changes starting from 20 mg/kg/day. A strong positive correlation was observed between NAG and different doses of CyA. CyA trough level was found neither to be correlated to tubular toxicity nor to urinary NAG level. NAG was found to be increased in vast majority of renal allograft recipient on CyA therapy with no correlation to either CyA trough level or CyA dose. We can conclude that urinary NAG is superior to conventional methods used to diagnose acute CyA toxicity as they appear in the urine before any pathological changes become evident. It is of no diagnostic value in chronic CyA nephrotoxicity.

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Introduction

Urinary NAG (N-acetyl-β- D- glucosamininidase), a tubular enzyme, can be used as a reliable diagnostic marker of nephrotoxicity [1].
To date nephrotoxicity has been the most significant limiting factor in the clinical use of CsA. About 25% of patients with autoimmune disorders and as many as 80-90% of transplant recipients manifest some evidence of impaired renal function related to CyA treatment [2,3].

Aim of work

The present study was designed to investigate the possible role of N-Acetyl-β- D- Glucosamininidase in diagnosis of early reversible renal tubular damage caused by the immunosuppressive agent cyclosporin A. A trial was also done to elucidate any possible correlation between cyclosporin blood level and its nephrotoxic effect.

Material and methods

Animals: 30 male albino rats, weighing 200-250 gm were divided into 5 groups, each group of 6 were housed in a separate cage and had free access to a standard diet and water. The rats were acclimatized for 1 week till used. Thereafter, the 1st 4 groups received Cyclosporin A (in a vehicle of 90% olive oil and 10% ethanol) orally by a gavage at a dose of 10, 20, 40 and 80 mg/Kg / day for 10 consecutive days. An equivalent volume of olive oil in ethanol was given to the 5th (control) group.

Patients:

- 21 kidney transplant recipients were receiving cyclosporin A at a dose of 5 mg/Kg/day during their follow up after the operation in Cairo University Hospital.
- 12 healthy subjects of both sexes free from any disease were included as control.

Methods:

On the 9 th day of treatment, the animals were placed individually in metabolic cages and urine was collected for 24 hours. On the 10 th day, blood samples were drawn from the retro-orbital plexus under ether anesthesia. After collecting blood, the animals were killed by decapitation and kidney tissues were processed for histologic examination.

In patient and control groups 24 hours urine was collected and a blood sample was obtained from each subject (before next Cy A dose in patients).

Urine volume of each sample was measured and then samples were used to measure N-Acetyl-β- D-Glucosamininidase (NAG) [Akira Noto and associates, 1983], creatinine, protein, sodium and potassium levels. Urinary NAG estimation was done specrophotometrically using Boehringer Manheim Biochemica Kits. FENa and FEK (Fractional excretion of sodium and potassium) were calculated.

Blood samples were used to estimate serum electrolytes, creatinine and blood Cyclosporin A trough level.

Statistical analysis using mean ± standard error of the mean and the Duncan's multiple range test was then performed.

Results

Results are summarized in tables 1-2 and figures I - XVI.

S.creatinine and urea are significantly higher in rats receiving 40 mg/kg CyA ($p<0.05$). The highest level of urinary NAG is seen in rats receiving 80 mg/kg CyA ($p<0.05$). Urine volume, FENa and FEK are significantly lower in rats receiving 40 or 80 mg/kg CyA ($p<0.05$). Creatinine clearance is significantly lower in rats receiving 40 mg/kg CyA ($p<0.05$). There is no significant difference in urine protein between the different groups of rats.

Cytoplasmic vacuolation of proximal tubular epithelium is only observed in rats receiving 40 mg/kg [low score, 33.3%] or 80 mg/kg [high score, 83%].

A postive correlation was found between plasma potassium and CyA Dose. NAG was found to be elevated significantly before the appearance of definite pathologic changes starting from 20 mg / kg / day. A strong positive correlation was observed between NAG and different doses of CyA.

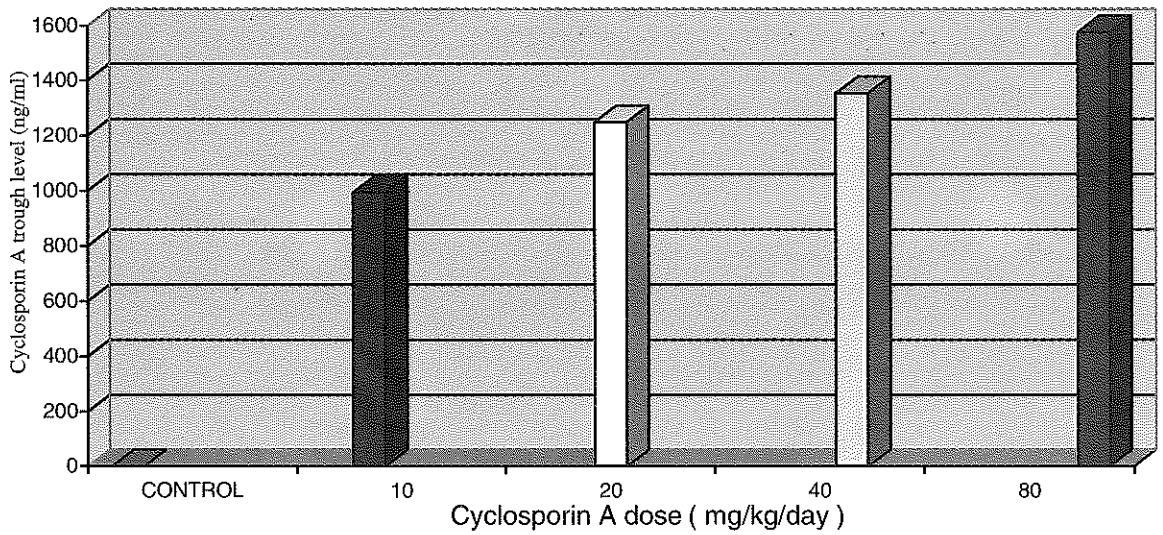

Fig. I. Cyclosporin A trough level in relation to its dose in different groups of rats

Table 1. Different laboratory parameters studied in different groups of rats

ITEM	*Group* ←	*Control*	*10 mg/Kg*	*20 mg/Kg*	*40mg/Kg*	*80mg/Kg*
Blood Cyclosporin A Trough level (ng/mL)	mean±SD	0	995±37	1250±56	1355±41	1575±57
	Dt	d	c	b	b	a
Plasma Creatinine (mg/dL)	mean±SD	0.77± 0.05	0.78± 0.04	0.79± 0.04	0.997±0.04	0.67±0.03
	Dt	bc	bc	b	a	c

Plasma urea (mg/dL)	mean±SD	35.8± 1.8	36.5± 1.17	36.6± 1.22	56± 1.23	34.3± 1.89
	Dt	b	b	b	a	b
Total Plasma Protein (g/dL)	mean±SD	6.46± 0.03	6.33± 0.04	6.3± 0.04	6.3± 0.04	4 ± 0.11
	Dt	a	a	a	a	b
Urinary NAG level (U/L)	mean±SD	5.53± 0.22	6.68± 0.31	10.48± 0.73	15.4± 1.05	26.6± 2.8
	Dt	d	cd	c	b	a
Urine Volume (mL/24h)	mean±SD	6 ± 0.4	6 ± 0.3	6 ± 0.3	4 ± 0.1	4 ± 0.2
	Dt	a	a	a	b	b
Urine Creatinine (gm/L)	mean±SD	83 ± 2	83 ± 1.8	75 ± 2.7	87 ± 1.6	80 ± 1.3
	Dt	a	a	b	a	b
Creatinine Clearance (mL/min)	mean±SD	0.46 ± 0.03	0.44± 0.04	0.41±0.02	0.25±0.01	0.37±0.01
	Dt	a	ab	ab	c	b
Urinary Sodium (mEq/L)	mean±SD	64 ± 0.84	65 ± 1.92	56 ± 2.79	30 ± 0.87	27 ± 1.84
	Dt	a	a	b	c	c
Plasma Sodium (mEq/L)	mean±SD	141 ± 0.84	141 ± 0.95	142 ± 0.73	142 ± 0.77	141 ± 0.61
	Dt	a	a	a	a	a
Sodium Clearance (µL/min)	mean±SD	1.92 ± 0.14	1.88 ± 0.12	1.72± 0.07	0.64± 0.03	0.6± 0.05
	Dt	a	a	a	b	b
Fractional Excretion of Sodium [FENa] (%)	mean±SD	0.42± 0.02	0.43± 0.03	0.41± 0.01	0.24± 0.01	0.15± 0.01
	Dt	a	a	a	b	c
Urinary Potassium (mEq/L)	mean±SD	32± 2.4	31± 1.7	29± 1.5	23± 0.8	22± 1.1
	Dt	a	a	a	b	b
Plasma Potassium (mEq/L)	mean±SD	4.7± 0.07	4.9± 0.1	5 ± 0.1	6 ± 0.1	7 ± 0.2
	Dt	c	c	c	b	a
Potassium Clearance (µL/min)	mean±SD	28 ± 2.2	25 ± 1.8	26 ± 2	11 ± 0.5	10 ± 0.8
	Dt	a	a	a	b	b
Fractional Excretion of Potassium [FEk] (%)	mean±SD	6 ± 0.3	6 ± 0.4	6.2 ± 0.3	4.5 ± 0.2	2.5 ± 0.2
	Dt	a	a	a	b	c
Urinary Protein (mg/dL)	mean±SD	6.5 ± 0.5	6 ± 0.5	5.5 ± 0.4	6 ± 0.6	6.3 ± 0.5
	Dt	a	a	a	a	a

Dt = Duncan's multiple range test (means with dissimilar letters are significantly different at $p<0.05$).

Table 2. Urinary NAG and plasma creatinine levels in control and kidney transplantation recipients under CyA treatment

Parameter	Control (Mean ± S.D.)	Tx (Mean ± S.D.)	*P*
Urinary NAG level (U/L)	4.17 ± 0.37	14.5 ± 1.04	<0.01
Plasma creatinine (mg/dL)	1.03 ± 0.09	1.49 ± 0.11	<0.05

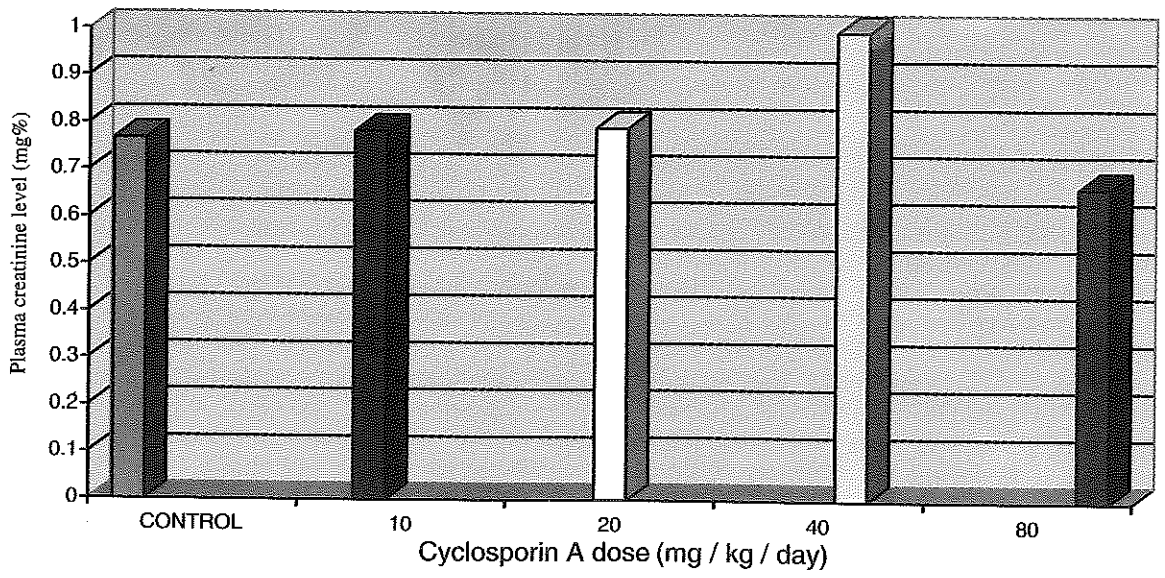

Fig. II. Plasma creatinine level in relation to different doses of CyA in different groups of rats

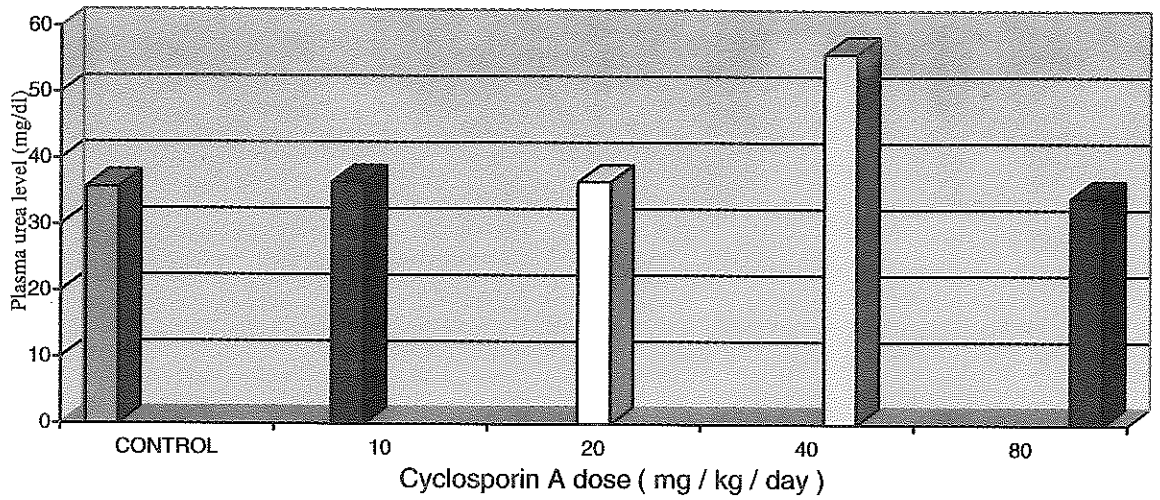

Fig. III. Plasma urea level in relation to different doses of CyA in different groups of rats

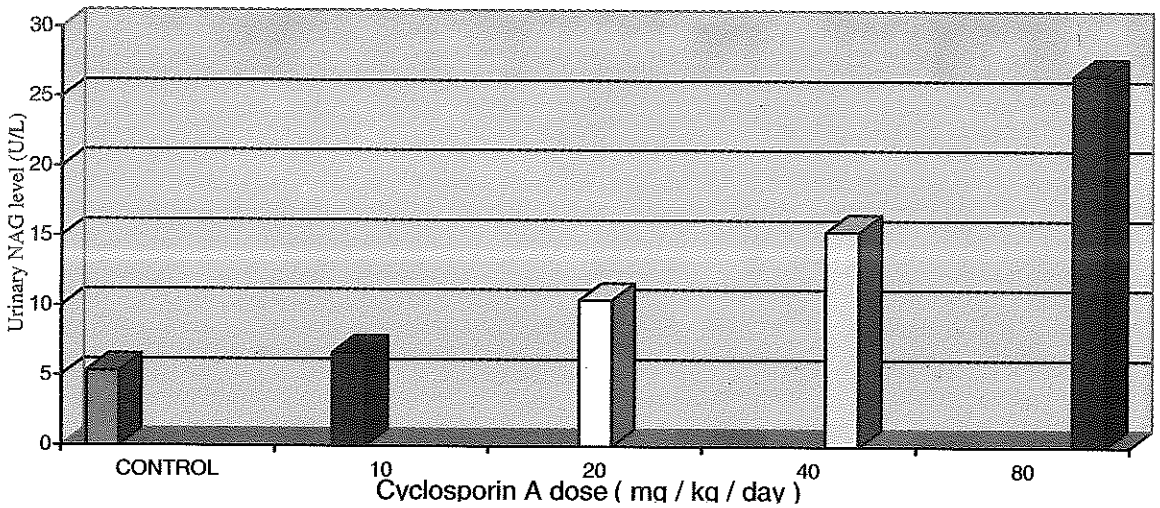

Fig. IV. Urinary NAG level in relation to different doses of CyA in different groups of rats

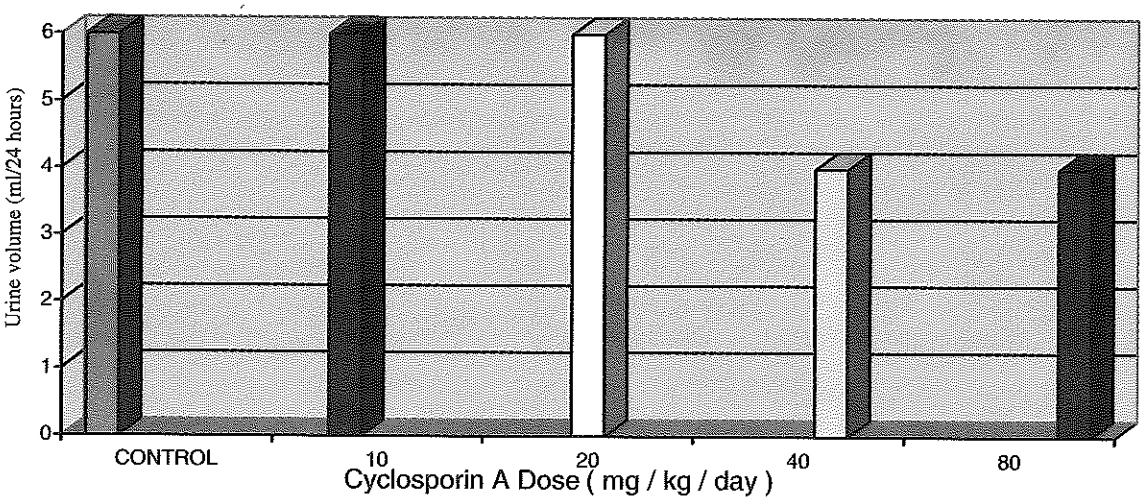

Fig. V. Urine volume in relation to CyA dose in different groups of rats

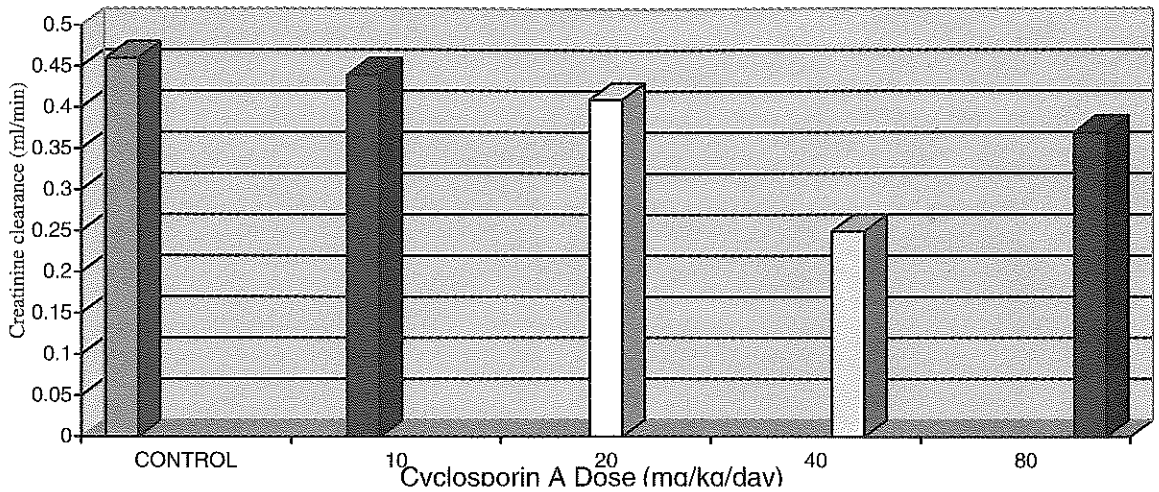

Fig. VI. Creatinine clearance in different groups of rats with different doses of CyA

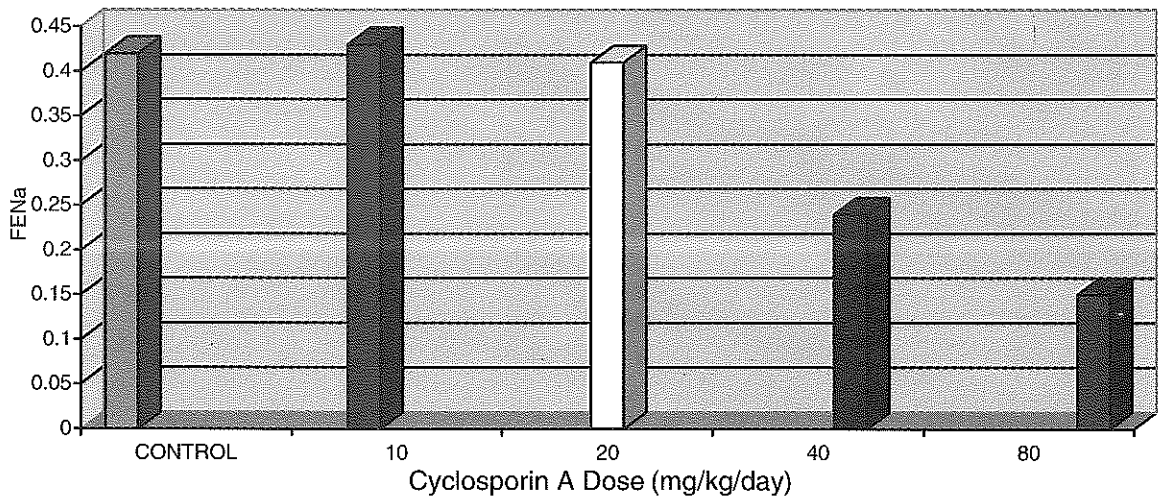

Fig. VII. Fractional excretion of sodium in different groups of rats

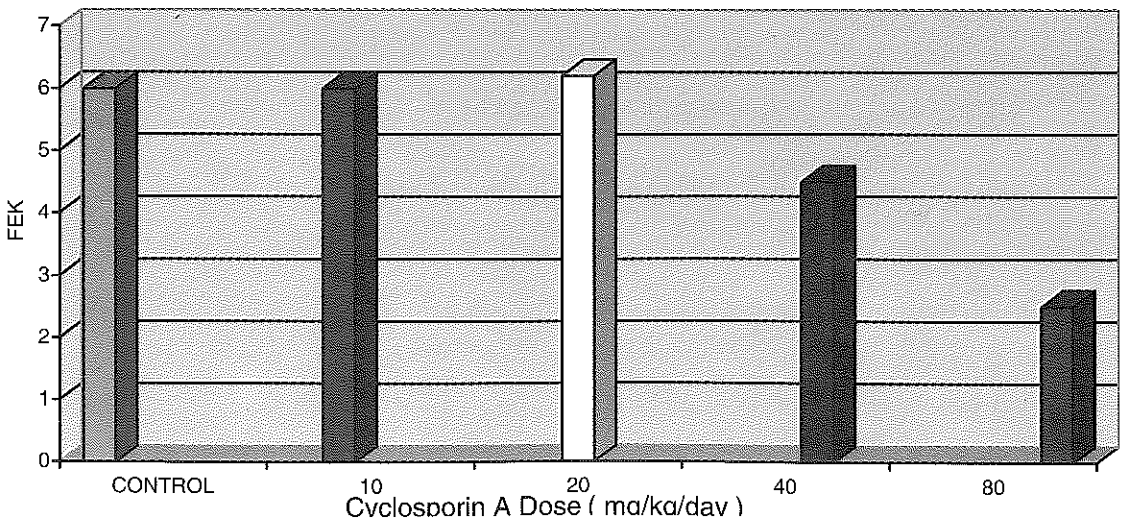

Fig. VIII. Fractional excretion of potassium in different groups of rats

CyA trough level was found neither to be correlated to tubular toxicity nor to urinary NAG levels.

Urinary NAG and serum creatinine are significantly higher in transplanted patients compared to the normal control cases ($p<0.01$ and <0.05 respectively). There was no correlation between NAG and either CyA trough level or CyA dose.

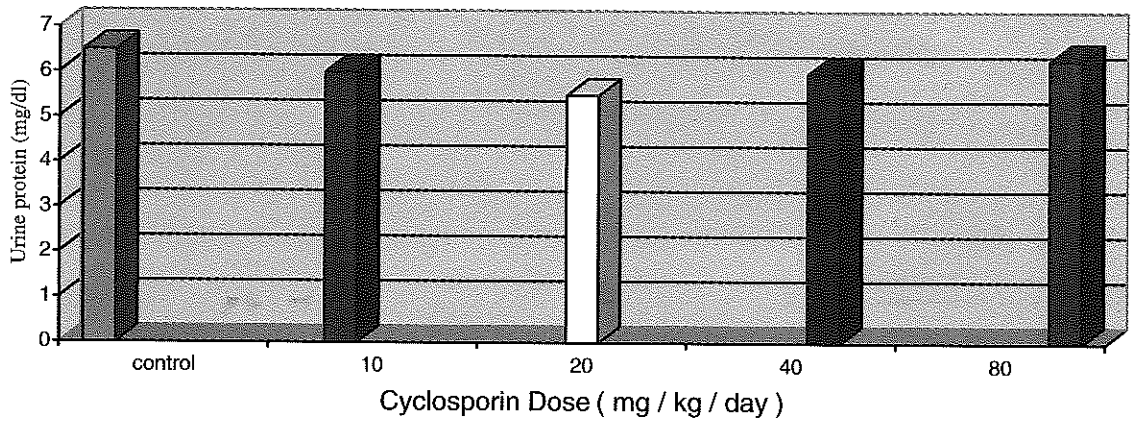

Fig. IX. Urine protein in different groups of rats

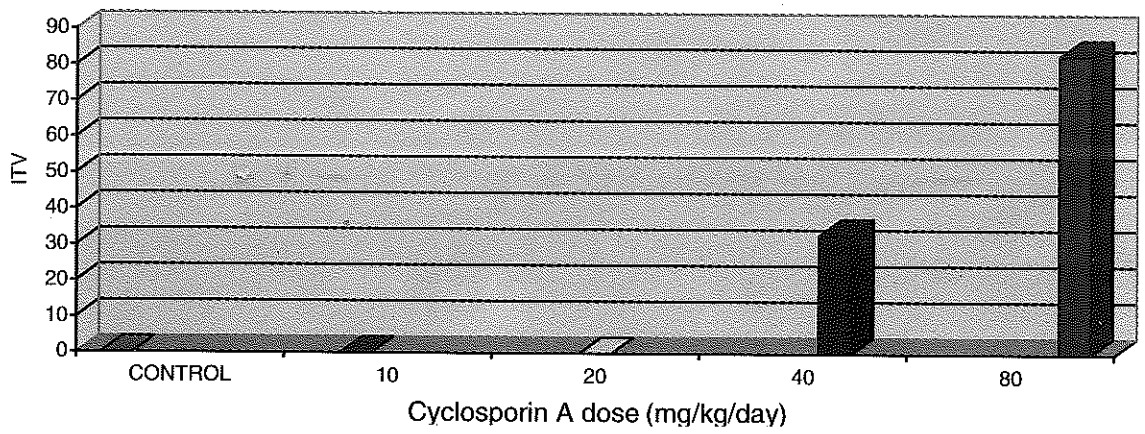

Fig. X. Incidence of tubular vacuolation in different groups of rats

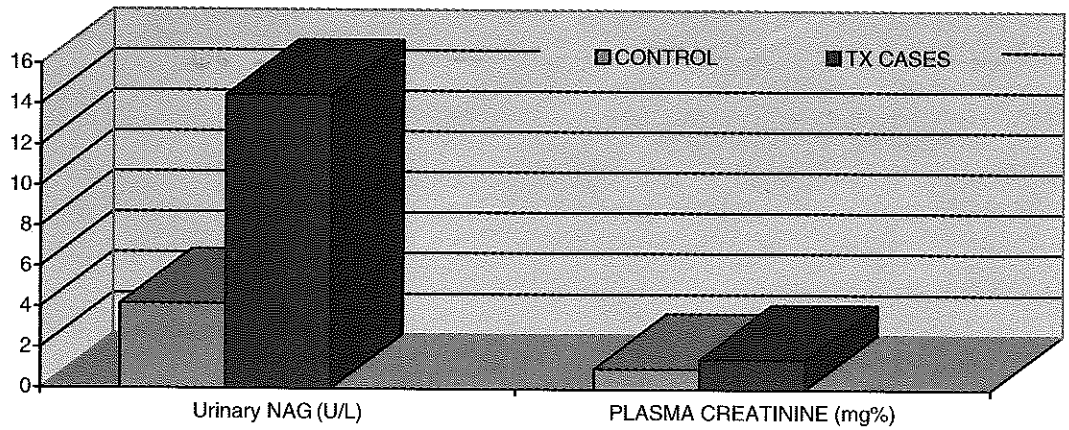

Fig. XI. Urinary NAG and plasma creatinine levels in transplanted patients and normal control subjects

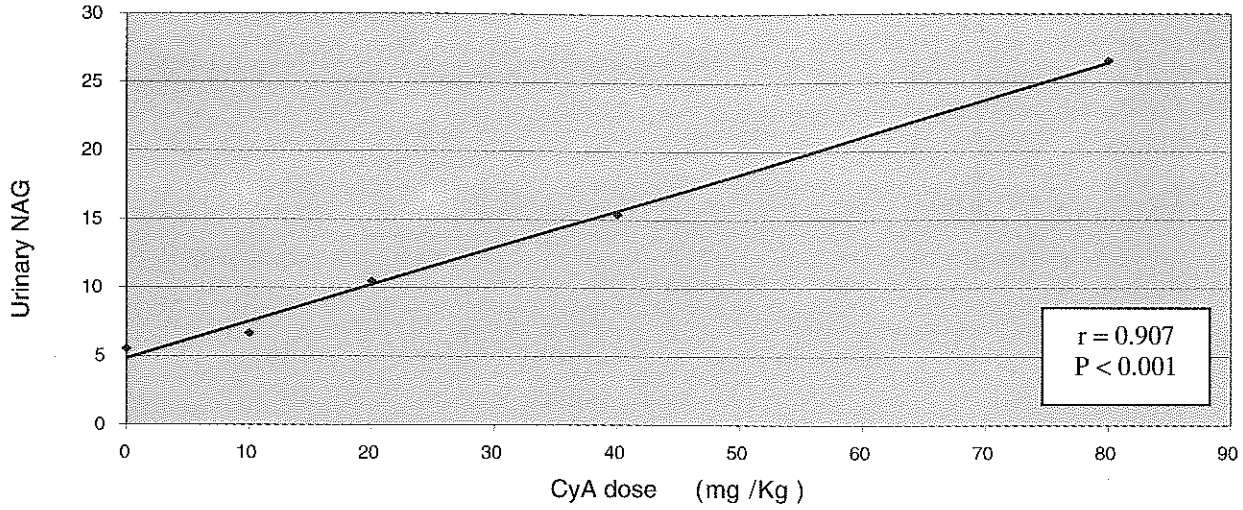

Fig. XII. Correlarion between CyA dose and urinary NAG

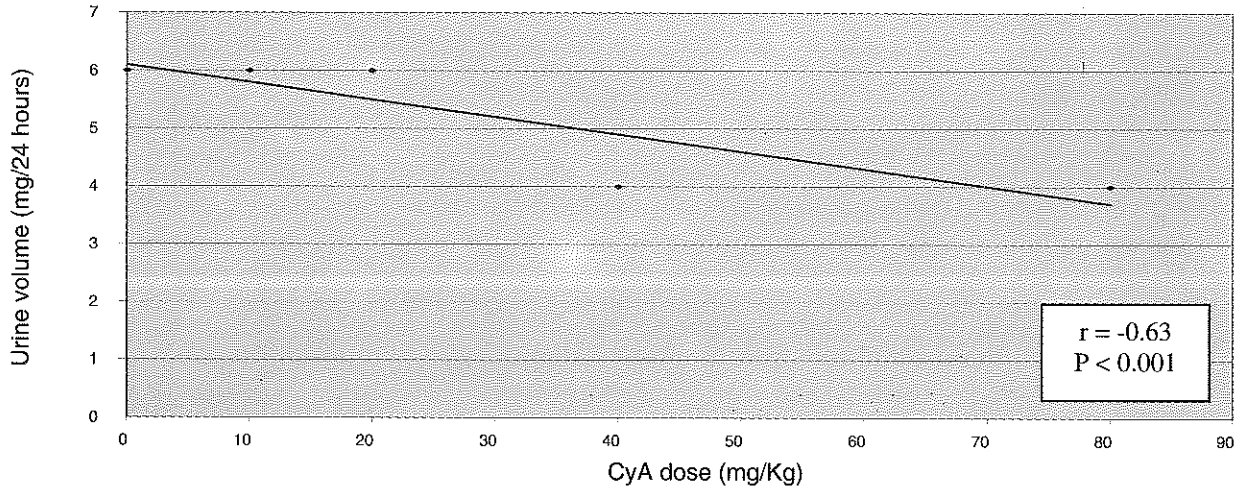

Fig. XIII. Correlation between CyA dose and urine volume in rats

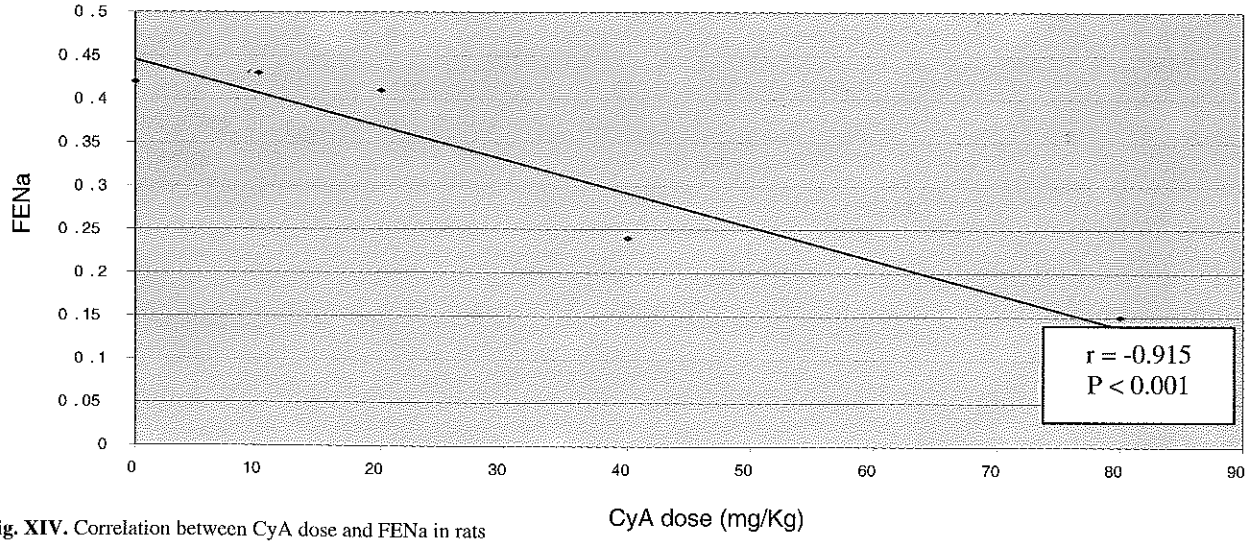

Fig. XIV. Correlation between CyA dose and FENa in rats

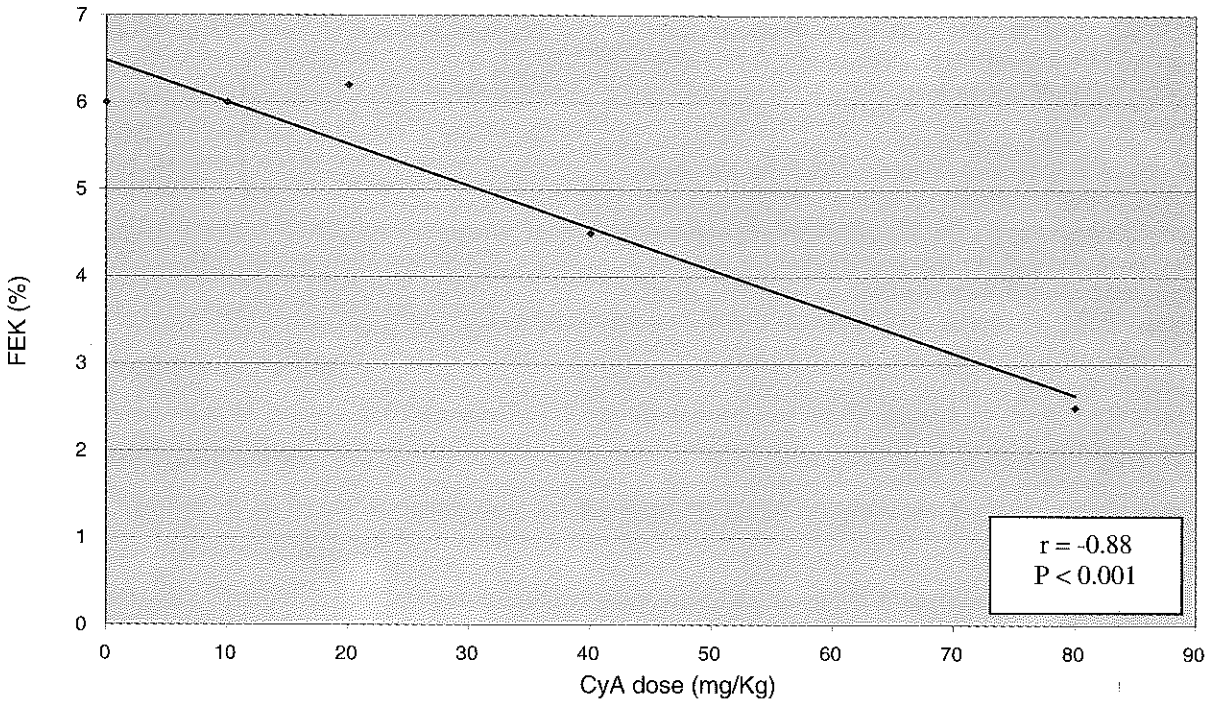

Fig. XV. Correlation between CyA dose and FEK in rats

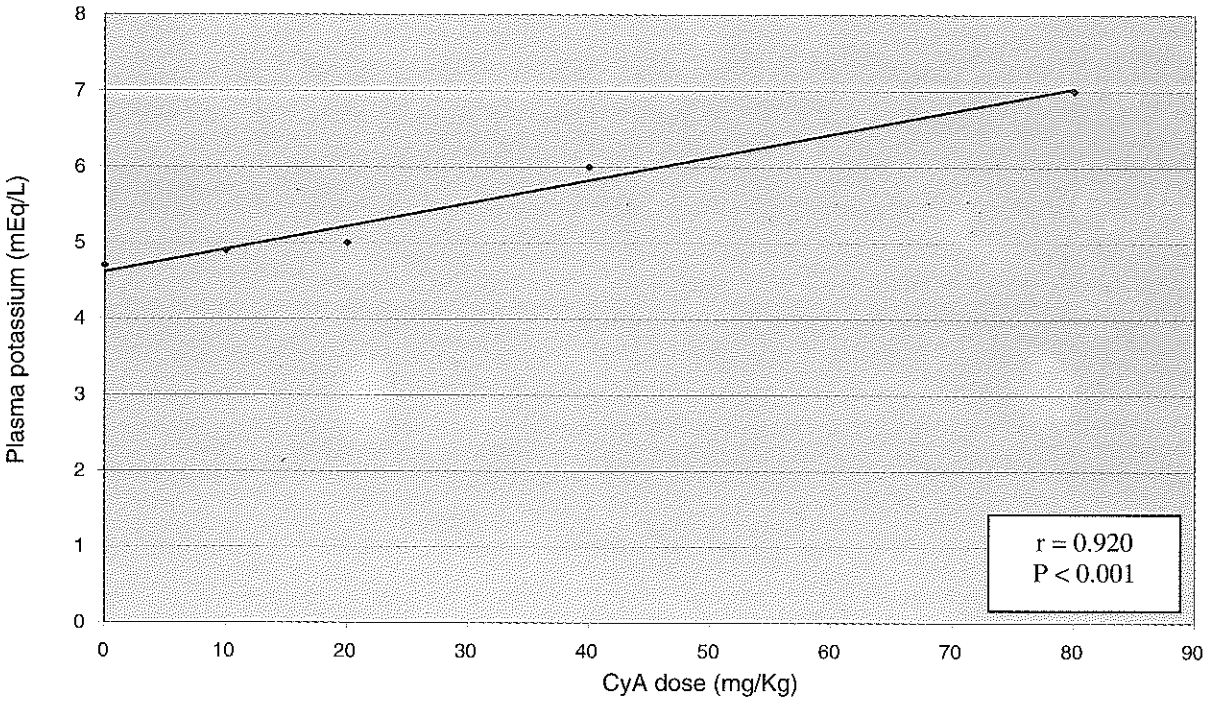

Fig. XVI. Correlation between CyA dose and plasma potassium

Discussion

Although cytoplasmic vacuolations are not seen except with the higher CyA doses (20 and 40 mg / kg), urinary NAG level is significantly elevated before the appearance of definite pathologic changes. NAG level increases significantly following the administration of CyA in a dose of 20 mg / kg reaching a maximum at a dose of 80 mg / kg and shows a strong positive correlation with CyA dose.

On the other hand, the conventional methods used for the assessment of kidney functions as plasma creatinine and creatinine clearance are unreliable, being within the normal range in rats with remarkable pathologic changes.

Although the pathologic changes are dose dependent, there is no clear definite correlation between these changes and CyA trough level. This finding casts great doubts the value of trough level of CyA for monitoring its toxicity.

Moreover, the increased urinary NAG in the vast majority of renal transplant recipients precludes its use as a good indicator of chronic CyA nephrotoxicity.
In conclusion, we can say that urinary NAG is superior to other conventional methods used to diagnose acute CyA nephrotoxicity as they appear in the urine before any pathological changes become evident. It is of no diagnostic value in chronic CyA nephrotoxicity.

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