Effect of Cyclosporin A therapy and periodontal parameters on the severity of gingival overgrowth in renal transplant patients

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ABSTRACT

Objectives

The aim of this study was to evaluate in adult renal transplant patients with CsA-induced gingival overgrowth a potential correlation between salivary and whole blood CsA concentrations and the gingival overgrowth (GO) and its relationship with periodontal parameters.

Materials and methods: Thirty three renal transplant patients (RTP) with GO were assessed for blood and salivary CsA levels using the monoclonal FPIA technique. The Wilcoxon signed rank test was used to compare differences in blood and salivary CsA. The association between GO severity and the other variables was evaluated using Spearman's correlation.

Results: No significant correlation was observed between blood and salivary CsA levels. There was no significant correlation between severity of GO and either blood CsA or salivary CsA concentrations. A significant association was detected between severity of GO and all periodontal parameters.

Conclusion: It can be concluded that salivary CsA concentrations cannot be used instead of blood levels as an indicator of CsA bioavailability. The lack of significant correlation between blood/ salivary CsA levels and the severity of GO could indicate that drug metabolism is not crucial in GO expression and that periodontal variables can be more predictive of GO severity.

Key Words

Cyclosporin A, gingival overgrowth, plaque index, renal transplants, whole blood levels, salivary level, monoclonal, FPIA, MERI.

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INTRODUCTION

Cyclosporin A (CsA) has been extensively used as the immunosuppressant of choice in renal transplant patients. The use of CsA is associated with a number of side effects, including nephropathy, hypertension, hepatotoxicity, neurotoxicity, thromboembolic complications and gingival overgrowth (GO).[1,2]

The gingival changes associated with CsA are esthetically disfiguring and often have psychological sequelae in affected individuals. Occasionally, overgrowth can be so severe that it interferes with mastication, occlusion and speech.3 In addition to being disfiguring and uncomfortable for affected individuals, moderate to severe forms of GO impair oral hygiene, may lead to increased accumulation of micro-organisms and possibly compromise the general health of patients.[4,5]

The incidence of CsA-induced GO differs greatly among studies. It has been reported to vary between 8% and 85% depending on the criteria used.[5,6,7] Because of the narrow therapeutic index and significant intra- and interindividual variability in the pharmacokinetic characteristics

of CsA, the therapeutic concentrations of this drug must be routinely monitored to avoid renal graft rejection and/or adverse effects including nephrotoxicity, hepatotoxicity, and neurotoxicity.[2] Assessment of CsA whole blood level is the usual approach used for therapeutic drug monitoring in renal transplant patients to provide therapeutic guidelines. Ideally, monitoring of CsA whole blood levels should include repeated sampling and determination of the area under the time-concentration curve over a period of four hours after administration (AUC0-4h) to better target the therapeutic window, thus avoiding underdosing or overdosing.[8] However even a 4-hour AUC is not practical in clinical practice. As an alternative, the single blood CsA concentration measurement two hours after administration (C2) has been shown to correlate better with AUC0-4h than trough levels (C0), i.e. CsA blood level before morning dose, and to reflect better the maximal immunosuppressive effect of CsA.[8,9,10] In fact, the maximal immunosuppressive effect of CsA would be obtained about two hours following CsA microemulsion intake.[10] Finally, single serum C2 measures are easier to obtain as part of the patient's ongoing medical care.

Salivary CsA monitoring has been suggested as an alternative means of therapeutic drug monitoring in children and patients with difficult venous access.[11] Measurement of salivary concentrations offers the potential advantage of substituting repeated blood sampling with noninvasive salivary sampling.

It is generally believed that the pharmacological action of a highly protein-bound drug such as CsA is related to the concentration of the unbound drug in plasma.[12] Since only the unbound portion of a CsA is capable of diffusing across the capillaries of salivary glands; it is conceivable to believe that the salivary concentration will better reflect the unbound and pharmacologically active fraction of the drug than the serum or whole blood concentration.[11] It has been speculated that saliva monitoring is ideal for measuring neutral lipophilic compounds such as CsA. The availability of a saliva-based method is therefore essential to measure the salivary concentration of CsA and to explore its correlation with the total CsA concentration.[12] The specific aims of the present study were to:

- compare the results of salivary concentrations with those of whole blood CsA 2 hours after the morning dose (C2);
- evaluate potential correlation between whole blood or salivary CsA concentration and severity of GO and
- evaluate potential correlation between severity of GO and periodontal parameters including plaque index, gingival index and papillary bleeding index.

MATERIALS AND METHODS

Thirty-three renal transplant patients, aging between 20 and 50 years (17 females and 16 males) were selected for the study from a patient population attending the renal transplant outpatient clinics at Rizk hospital in Beirut, Lebanon. The patients had to fulfill the following inclusion criteria:

- medication with CsA therapy for at least 6 months prior to the study with a daily oral dose ranging between 1 mg to 8 mg/kg;
- stable systemic condition with no graft rejection;
- no intake of any medication known to produce GO;
- presence of at least 6 anterior teeth in both upper and lower arches; and
- presence of GO in at least 6 teeth.

Smokers were excluded from the study.

A written informed consent was obtained prior to initiating data collection. A full drug history was recorded and dental and periodontal evaluations were carried out by two trained dental surgeons two hours after CsA administration (C2). Evaluation calibration was based on pre-study double assessment of 15 consecutive cases examined by these two examiners. The clinicians were blinded to patients identity and medical history.

The following periodontal parameters were assessed at all teeth present in the mouth excluding second and third molars:

- plaque index (PI): recorded at the buccal, lingual/palatal and interproximal surfaces using the plaque index of Löe and Sillness.[13]
- gingival inflammation (GI): recorded at the buccal, lingual/palatal and

interproximal surfaces using the gingival index of Sillnes & Löe .[14]

- papillary bleeding: recorded using the papillary bleeding index (PBI) of Saxer and Mühlemann.[15]
- severity of gingival overgrowth (GO): using the semi-quantitative index developed by Aas.[16] (The following criteria were applied:

* Grade 1: mild GO covering less than 1/3 of the crown length;
* Grade 2: moderate GO covering 1/2 of the crown length;
* Grade 3: severe GO covering 2/3 or more of the crown length.

A mean index was assigned to each tooth and a mean value for all teeth was calculated for each patient.

Blood samples of 2 ml were collected in the Immunopathology Laboratory at Rizk Hospital at the same time of the dental examination, 2 hours after the CsA morning dose. The whole blood levels of CsA were determined on the same day of collection with the monoclonal FPIA kit (TDx®/TDxFLx® Cyclosporin Monoclonal, Abbott Diagnostics®, Wiesbaden, Germany) using a random and continuous access immunoassay analyzer.[17,18,19] All patients were instructed to clean their teeth before the session of saliva sampling. To measure the CsA levels in saliva, a method developed and patented under the name of MERI (Middle East Research Institute, Beirut, Lebanon) drug extraction solution, was used.[20,21,22,23] Two ml of saliva were collected from the patients at (C2). The unstimulated saliva was collected by asking patients to accumulate their saliva over 4-5 minutes and spit it into glass vials. The salivary sample was then centrifuged for 10 minutes at 4,000 rpm. The supernatant was discarded and the pellet dissolved in 200 µl of MERI solution. Then, 150 µl of the saliva/ MERI solution was added to 350 µl of lysine/precipitation reagent and centrifuged for 10 minutes at 10,000 rpm to determine CsA salivary concentration.

Statistical analysis

Summary statistics were obtained for the variables (blood CsA, salivary CsA, GO, PI, GI, PBI) by calculating the mean and the standard deviation. For variables that do not follow a normal distribution the Wilcoxon rank sum test was used to compare the GO, PI, GI and PBI between the genders. Wilcoxon signed rank test was used to compare the differences in blood and salivary CsA. Moreover, Pearson's correlation coefficient was computed to assess the linear association between blood and salivary CsA. Spearman's correlation was used to assess the association between severity of gingival overgrowth and other variables.

RESULTS

A total of 33 subjects were recruited. There were 17 (52%) females and 16 (48%) males. In table 1, a summary of several variables, mentioned above, are presented. The results showed a significant difference between blood (565.02 µg/L) and salivary (182.95 µg/L) CsA levels ($P<0.0001$) when exploring the potential correlation between salivary and serum CsA concentrations (table 1). Additionally, there was no significant correlation between these two variables ($r=.066$, $p=0.725$). The difference between genders was not statistically significant as shown in table 1. No significant correlation was observed between the severity of gingival overgrowth and either blood CsA or salivary CsA. - P values calculated were 0.554 and 0.302, respectively (table 2). The results of comparing CsA levels in blood among subjects with "high GO" (GO scores > 1) vs "low GO" (GO < 1) are summarized in table 3. It can be seen there was no significant difference in blood CsA when comparing the 2 groups. It is worthy to note that salivary CsA was marginally ($P=0.055$) significantly higher in the "high GO" group (table 3, Fig. 1). Regarding our objective to evaluate the gingival overgrowth (GO) and its relationship with periodontal parameters, we obtained a significant correlation between severity of GO and all 3 periodontal indices, i.e. PI, GI, and PBI (table 4).

DISCUSSION

The aim of this study was to investigate a potential correlation between salivary

CsA concentration and whole blood therapeutic CsA level. Our results showed no correlation between the above mentioned variables (r=0.066, P=0.725). In addition, no statistically significant differences were evidenced between the two genders relative to the correlation between blood and salivary CsA levels. These data are in agreement with the low correlation reported by McGaw et al between serum CsA levels and the corresponding values of CsA in both parotid and submandibular saliva.[24]

Furthermore our results showed that there was no significant correlation between severity of GO and CsA blood levels (P=0.554). Statistical data in the present study do not support the concept of previous studies[8,25,26] which concluded that blood levels of CsA remain an important risk factor in the development of GO. Moreover, no correlation was found between salivary CsA concentrations and severity of GO (P=0.302). However when patients were divided into two groups namely low GO (GO score ≤ 1) and high GO group (GO score > 1), it was noted that people in the low GO group had a low variability of CsA salivary concentration of 77.07 with a mean value of 103.26. Conversely people in the high GO group exhibited a variability in the GO salivary concentration (262.01). As a result we found a marginally statistically difference (P=0.055) between the two groups. King et al.[27] reported a lack of correlation between unstimulated salivary

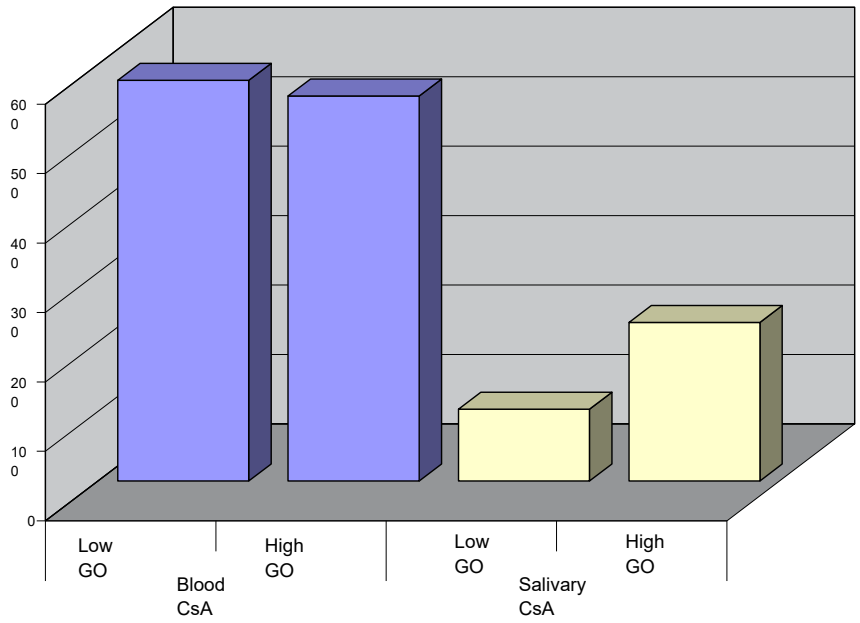

Fig 1. Blood and Saliva CsA in low and high GO groups.

CsA levels and GO while in other studies a positive correlation has been found between CsA concentrations in stimulated saliva and the extent of GO.[9,10,28] In our study unstimulated saliva were measured and the discrepancy of all these findings may be explained by the fact that dental plaque may act as a reservoir for CsA, which is then released in saliva.[29] Other factors may have also affected the CsA concentration in saliva (the way salivary CsA concentration is measured, the timing, the patient's mouth condition). The results suggest that high levels of CsA may be contributing to GO. However, this could be considered only as a pilot study. To increase the statistical power of future studies, larger samples are needed both in high and low GO groups.

Salivary and serum CsA concentrations were assessed 2 hours after the morning dose (C2) since several authors have suggested a clinical benefit of CsA monitoring using the CsA at C2, which correlates better with AUC than Co.[9,10,30] A significant correlation was observed between the severity of GO and the periodontal parameters. These findings are in agreement with previous reports where inter-individual variation in the severity of GO was predominantly related to local factors.[7,8,10,26] Plaque buildup and gingival inflammation appear to exacerbate the expression of drug-induced GO.[31] They do not support the claims of Afonso et al. who suggested that CsA-induced GO may vary according to the individual sensitivity of each patient and may or not be correlated with other periodontal local factors.[32] Histologically, gingival hyperplasia is associated with an increase in the deposition of intercellular matrix, in the percentage of inflammatory cells and in the degree of tissue vascularization.[33]

This inflammatory response is increased by the presence of dental plaque suggesting that the hyperplasia can represent a response to bacterial toxins.[34] When subjects were classified in "high GO" and "low GO" groups, data showed that plaque index, gingival index and papillary bleeding index were significantly greater in the "high GO" group. The results of the present study and those of the abovementioned reports highlight the primary role of gingival inflammation in the pathogenesis and severity of GO.[35] Such observations however are not conclusive as to whether the plaque-induced changes are the "cause" or the "result" of GO.[36] Furthermore, attention to plaque control and removal of plaque retentive factors does improve gingival health in organ transplant patients, but these measures alone fail to prevent the development of GO, or its recurrence following surgery.[6] Further studies are needed to elucidate the effect of periodontal therapy on the severity of GO in long-term follow-up studies.[8] Finally GO seems to be a complex multi-factorial event in which free CsA is an important factor but that it is also dependent on other factors such as periodontal parameters.

In the present study, males had a tendency to express higher severity of GO associated with greater periodontal indices. This result could be explained by the fact that males tended to have poorer plaque control than females. Vescovi et al suggested that males are at greater risk of developing this adverse effect than females.[26]

Whether it relates to existing periodontal factors, pharmacological variables or hormonal co-factor is to be determined.

Table 1: Summary of the variables and gender.

Variable	Overall Mean (SD)	Female Mean	Male Mean	P-value
Blood CsA (μg/L)	565.02 (262.42)	556.54 (267.42)	573.51 (259.16)	0.857† <.001*
Salivary CsA (μg/L)	182.95 (222.79)	150.07 (182.20)	219.51 (265.58)	0.391†
Gingival Overgrowth	1.46 (.51)	1.37 (.53)	1.55 (.49)	0.128†
Plaque Index	1.73 (.74)	1.70 (.86)	1.77 (.61)	0.958†
Gingival Index	1.97 (.66)	1.76 (.74)	2.19 (.49)	0.110†
Papillary Bleeding Index	0.80 (.38)	0.63 (.48)	0.97 (.09)	0.086†

Comparing the genders
**Comparing blood CsA to Salivary CsA*

Table 2: Correlation between severity of gingival overgrowth and blood/salivary CsA concentrations.

	Correlation with GO Severity (r)	P-value
Blood CsA	0.110	0.554
Salivary CsA	0.191	0.302

Table 3: Comparison of blood and salivary CsA levels between high GO (n=23) and low GO (n=10) groups.

	Low GO Mean (SD)	High GO Mean (SD)	P-value
Blood CsA (μg/L)	576.57 (271.87)	554.03 (264.25)	0.832
Salivary CsA (μg/L)	103.26 (77.07)	227.87 (262.01)	0.055

Table 4: Correlation between severity of GO and all 3 periodontal parameters.

	Correlation with GO Severity (r)	P-value
Plaque Index	0.609	<.0001
Gingival Index	0.660	<.0001
Papillary Bleeding Index	0.642	<.0001

CONCLUSION

This study failed to establish a correlation between CsA saliva and blood concentrations. As

it is, it cannot replace the blood monitoring for CsA follow-up. However, the improved approach for CsA measurement in saliva found a marginal correlation when high and low GO groups were compared with salivary CsA levels. Therefore, the study could be considered as a pilot study for future investigations with larger sample sizes.

The most accurate parameters associated with GO severity seem to be the periodontal indices. Therefore, it is reasonable to suggest that improved oral hygiene prior to renal transplantation may at least minimize the severity of GO, reflecting the removal of the inflammatory component of this condition.

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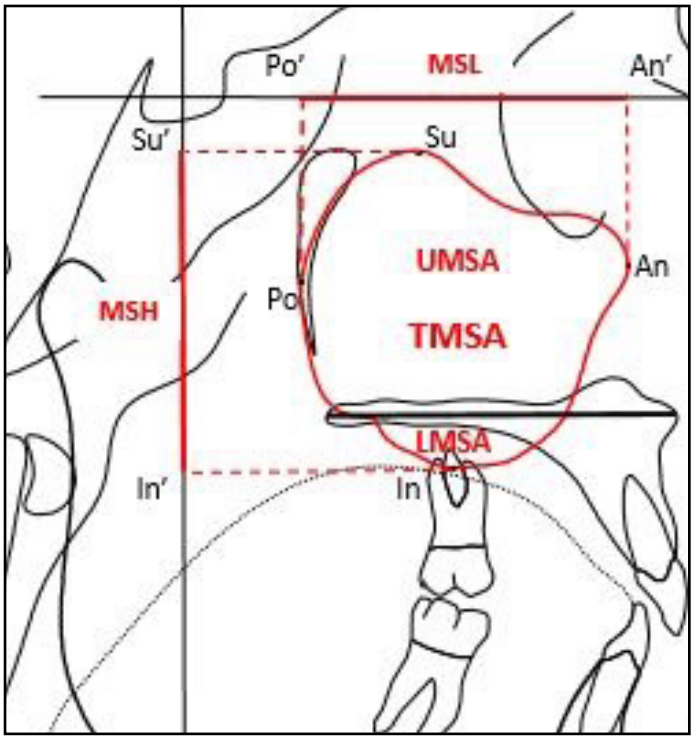
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