CORRELATION OF BLOOD LEVELS OF CYCLOSPORINE AND IT'S METABOLITES AND LOCAL FACTORS WITH GINGIVAL OVERGROWTH IN IRANIAN RENAL ALLOGRAFT PATIENTS

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Abstract — Forty renal allograft patients with three months under immunosuppression by cyclosporine were examined for their gingival overgrowth and it's correlation with several parameters including the trough levels of blood cyclosporine and it's metabolites measured by the fluorescence polarization immunoassay technique. No correlation was found between the scores of gingival overgrowth and both the age of patients and duration of cyclosporine therapy. Also, there was no correlation between the scores of gingival overgrowth and the levels of dental plaque. Our findings confirm the effective role of gingival inflammation as a local predisposing factor and also suggest the potential toxic action of cyclosporine metabolites on development of gingival overgrowth or it's accentuation.
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Key words: Cyclosporine metabolites; gingival overgrowth; Iranian renal allograft

INTRODUCTION

Cyclosporine (Cs), a selective immunosuppressant, has been considered as the first choice in many organ transplantation procedures as well as many immunologic disorders (1). Because of large intersubject variability in the pharmacokinetics parameters, unpredictable bioavailability and narrow therapeutic range, Cs monitoring has proven to be a valuable aid in the successful therapeutic use of the drug (2). However, transfer of the drug from blood to saliva has been observed (3) and we have previously reported some adverse effects of Cs on secretory function and ultrastructural changes in the morphology of rat salivary glands (4,5,6). The incidence of Cs-induced gingival overgrowth (GO) has been reported as 25% to 81% by different authors (1). The significance of GO in addition to its unwanted effect on patients, appearance is that it can impair dental hygiene and may lead to the development of foci of oral sepsis that are potentially serious in an immunosuppressed person (3). In the other hand, some controversy on biological significance of Cs metabolites in terms of immunosuppressive (7,8) and nephrotoxic actions (2) or association with neurologic toxicity has been developed (9). So, this study was undertaken to investigate a correlation, if any, between Cs blood levels and its metabolites and local background factors with the incidence and severity of GO in a population of Iranian renal allograft patients.

MATERIALS AND METHODS

In this study forty patients were followed in our nephrology clinic following renal transplantation. All patients were under immunosuppression with Cs for a minimum of 3 months. Each patient underwent a routine dental examination to identify possible foci of oral sepsis or any necessary dental treatment. As a part of the dental examination, a periodontal assessment was performed and scores of the levels of dental plaque and gingivitis by established semiquantitative indices (10,11) were recorded. Upper and lower full mouth alginate impressions were taken for each patient. Gingival overgrowth was assessed on the plaster study casts by the method described by Seymour and coworkers (12). In order to avoid the missing of GO in posterior segments, the method was used for posterior as well as anterior segments of both jaws and the degree of overgrowth was expressed as a percent. The casts of each patients were scored by 2 independent examiners in addition to one of the authors. Prior to scoring, the examiners were taught for accurate determination of the score. In over 90% of the areas scored, the examiners concurred with the author. A total mean score of GO

$\frac{\text{The author}}{\text{two examiners}}$ was recorded for each patient. At the time of the dental examination, blood samples were obtained

from each subject before the morning dose of Cs. Blood levels of Cs and its metabolites were determined by means of the fluorescence polarization immunoassay technique and its specific reagents (Abbott laboratories, IL, USA) according to the manufacturer's instructions (13). Each sample underwent two separate assays: One assay for quantitative measurment of Cs (Monoclonal) and the other for measurement of Cs plus its metabolities (Polyclonal) in whole blood for each patient, the number of months of Cs therapy was also recorded. Because of the established effect of nifedipine on development of GO (10,14), concurrent use of this drug was also recorded. The patients were divided into five groups with respect to age and duration of Cs therapy. The patients were divided in another way into three groups with respect to the findings of monoclonal and polyclonal drug assays being upper, within or lower than the proposed therapeutic range (13). For each group the mean value of the GO ± SE was calculated separately. In addition, the mean value of GO was calculated for each sex and for the patients with and without the concurrent use of nifedipine. Comparisons of the mean GO between the groups were by one-way analysis of variance for the parameters of age, duration of Cs therapy, and its assay findings. Comparisons of the mean GO between the sexes and also between the patients with and without concurrent use of nifedipine were made by t-test. Relationships between the scores of GO and both the levels of dental plaque and gingivitis were determined by calculation of Pearson correlation coefficients. A p-value of less than 0.05 was accepted as statistically significant.

RESULTS

The patient sample was composed of 25 males and 15 females ranging from 13 to 55 years (Mean: 33 Years). The degree of GO ranged from zero to 29.2%, from which the degree of ten patients was more than 5% with evidence of GO on their study casts. This gingival response ranged from enlargement of few dental papillae to involvement of marginal gingiva with lobulations extending to cover some portions of the crown of the involved teeth (Fig. 1). The mean score of GO for males and females were (4.90 ± 1.45) and (4.40 ± 1.34) respectively. The comparison of means revealed a t=0.403, indicating no significant difference in the degree of GO between sexes.

In this study 21 patients had been maintained on concurrent therapy with nifedipine for at least 1 month. The mean scores of GO in the patients with or without concurrent use of nifedipine were (5.38 ± 1.42) and (3.97 ± 1.51) respectively. The comparison of means revealed no significant difference in the degree of GO between these two groups. In addition no significant difference in the mean scores of GO was found between the divided groups according to parameters of age and duration of Cs therapy (Table 1).

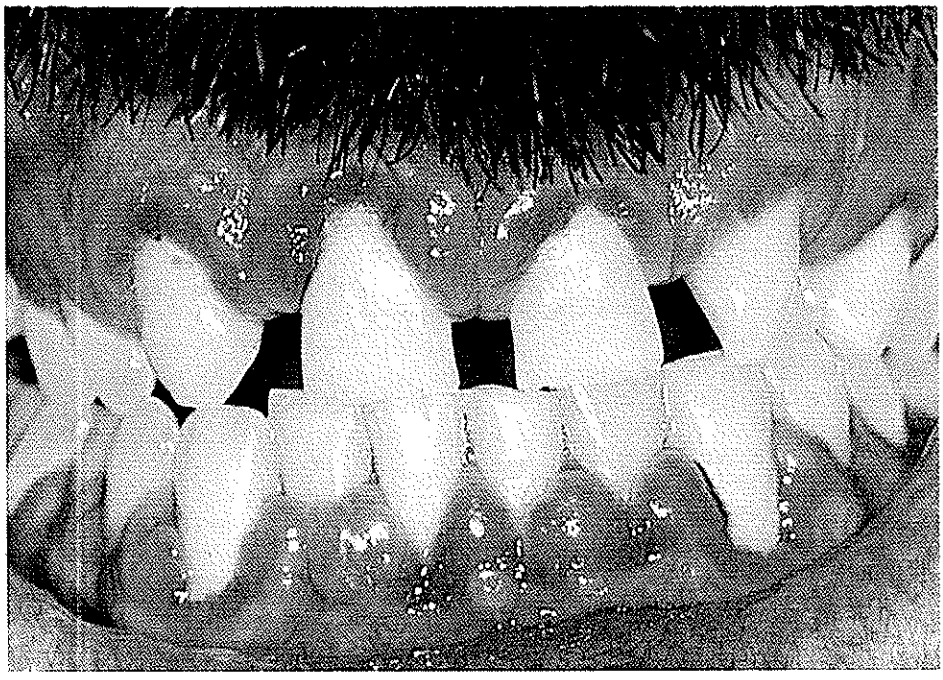

Fig. 1. Enlargement of few dental papillae and marginal gingiva with lobulations extending to cover some portions of crown of involved teeth

The scores of dental plaque and gingivitis ranged from 0.81 to 3.79 and zero to 2.208 respectively. No correlation was found between dental plaque and GO (r=-0.02). However, a significant positive correlation was found between the scores of gingivitis and GO (r= 0.65, P<0.001).

In recording the blood levels of Cs alone (Monoclonal assay) and Cs plus its metabolites (Polyclonal assay), the levels of one patient were too low to be detected by our method and so in this part of study 39 cases were considered. In monoclonal assay of blood samples, the levels of Cs ranged from 27.3 to 334.6 ng/ml and in polyclonal assay, the levels of Cs plus it's metabolites ranged from 107.5 to 1288 ng/ml. No significant difference in the mean scores of GO was found between three groups with the monoclonal and polyclonal findings of upper, within, and lower ranges (Table 2).

However, as the scores of Cs-induced GO could be influenced by the gingival enlarging effect of nifedipine (10,14), the patients with concurrent use of this drug were excluded. The repeated analysis for this new set of patients (With sole usage of Cs, n=19) revealed no significant difference between the mean scores of GO between three groups with respect to monoclonal findings. However, a significant difference was found between the mean score of GO between the patients with polyclonal findings of upper and those within the therapeutic range (Table 3).

Table 1. Mean scores of gingival overgrowth in the divided groups based on age and duration of Cs therapy and results of comparisons between groups

Age (year)			Duration of Cs therapy (month)		
Groups	n*	Mean GO±SE	Groups	n	Mean GO ± SE
10-19	5	3.08 ± 1.00	0-9	6	2.33 ± 0.66
20-29	10	5.52 ± 2.31	10-19	16	5.12 ± 1.27
30-39	15	5.16 ± 2.11	20-29	9	7.20 ± 3.43
40-49	6	3.96 ± 2.11	30-39	5	4.61 ± 3.58
50-59	4	4.18 ± 2.07	40-49	4	1.19 ± 0.39
F = 0.15 , P = 0.96 NS			F = 0.83 , P = 0.52 NS		

* Number of patients per each group ; SE = Standard error ; NS = Not Significant

Table 2. Frequency distribution of the patients and their mean scores of gingival overgrowth in the divided groups based on the therpeutic range of each assay and results of comparisons between groups

Assay		Monoclonal (Cs)		Polyclonal (Cs + metabolites)	
Therapeutic Range (Th.R)		100-300 ng/ml		150-400 ng/ml	
Number of cases and mean Go/group	> Th.R	n = 3	5.48	n = 9	8.76
	within Th.R	n = 23	5.81	n = 28	3.60
	< Th.R	n = 13	2.69	n = 2	2.61
Comparison of mean GO between groups		F = 0.95 NS		F = 2.36 NS	

NS = Not significant

Table 3. Frequency distribution of the patients with sole usage of Cs, (n=19) in the divided groups based on the therapeutic range of each assay and results of comparisons between groups

Assay		Monoclonal (Cs)		Polyclonal (Cs + metabolites)	
Therapeutic Range (Th.R)		100-300 ng/ml		150-400 ng/ml	
Number of cases and mean Go/group	> Th.R	n = 2	6.80	n = 4	10.95
	Within Th.R	n = 10	4.32	n = 13	2.03
	< Th.R	n = 7	2.67	n = 2	, 2.62
Comparison of mean GO between groups		F = 0.31 N.S.		F = 3.69*	

NS = Not significant ; * P < 0.05

DISCUSSION

The most consistent finding in this study was the positive significant correlation between the scores of gingivitis and GO which is in agreement with the results of other similar studies (4,15,16). However, as there were some patients with high levels of dental plaque and gingival inflammation without evidence of GO, the exact causative role of gingival inflammation in development of GO can not be proved. The nonsignificant difference in scores of GO in the patients with combined use of Cs and nifedipine and those with Cs alone can be related to interference of other factors such as duration of Cs therapy, blood levels of Cs and its metabolites, and especially gingival index, the role of which has been found to be more important than other factors, i.e. in patients with combined use of Cs and nifedipine, the level of oral hygiene was found to be better than patients with sole usage of Cs. This problem indirectly could neutralize the possible reinforcing action of nifedipine on gingival enlarging effect of Cs. However, the precise role of nifedipine in GO of Cs-treated patients needs to be investigated in more controlled conditions.

In searching for the role of Cs with its highly variable pharmacokinetics aspects on GO, the most reliable parameter seemed to be its blood level (2). But in our study no correlation was found between this parameter and GO and also no critical value was found beyond which increasing the risk or severity of GO. However, in considering the potential role of Cs metabolites for development of GO, after ruling out the misleading effects of nifedipine on ultimate scores of GO by limiting the data to the patients with sole usage of Cs, a significant difference in the mean scores of GO was found between the patient with polyclonal findings within and those with findings upper than therapeutic range suggesting the importance of therapeutic range in providing a low chance for development of GO in the patients. Also, the relatively low incidence of GO in our study can be largely due to our many drug monitoring findings within or lower than the therapeutic range.

The results of our study shows that the possible role of Cs metabolites on the development of GO when polyclonal findings exceeded the therapeutic range. Meanwhile, this emphasizes the critical role of drug monitoring in predicting the gingival complication in addition to its major use in maintaining patients in a stable systemic condition. However, the high cost of polyclonal assays of Cs seems to be a limiting factor for it's common application in patients' follow-up and prediction of some drug related complications. It should be noted that although our method for measurement of Cs with or without its metabolities was precise and relaible, it seems that more clarification of the role of these parameters on GO necessitates more controled and longitudinal studies. However, aside from our conclusive findings, we had some patients with low degrees of GO associated with high levels of dental plaque and gingival inflammation, high drug monitoring findings, long duration of therapy or concurrent use of nifedipine. This paradox may be related to a still undefined important parameter, such as individual susceptibility of patients (1,3). The nature of this susceptibility as an important factor in gingival responsiveness to adverse effects of Cs and its metabolities, remains to be investigated.

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