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Time as a factor in the identification of subjects with different susceptibility to plaque-induced gingivitis

Leonardo Trombelli[1], Chiara Scapoli[1,2], Giorgio Calura[1] and Dimitris N. Tatakis[1,3]

[1]Research Center for the Study of Periodontal Diseases, University of Ferrara, Ferrara, Italy; [2]Department of Biology, University of Ferrara, Ferrara, Italy; [3]Section of Periodontology, College of Dentistry, The Ohio State University, Columbus, Ohio, USA

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Abstract

Objectives: The purpose of this study was to assess whether identification of subjects with different susceptibility to plaque-induced gingival inflammation is dependent on the length of time of de novo plaque accumulation.

Methods: Retrospective analysis of data obtained from a recently reported (*J Clin Periodontol* **31**, 239, 2004) randomized split-mouth localized experimental gingivitis trial involving 96 healthy non-smokers. Gingival and plaque index, gingival crevicular fluid volume (GCF), angulated bleeding score, and the derived parameter cumulative plaque exposure (CPE) were recorded at days 0, 7, 14, and 21. The primary outcome variable to express severity of inflammation was GCF and each subject was a statistical unit. Based on subject distribution of GCF-day 21 residuals after standardization for CPE-day 21, two sub-populations (upper and lower distribution quartiles) were selected. They were, respectively, defined as ''high responders'' (HR) ($n = 24$) and ''low responders'' (LR) ($n = 24$) and characterized by significantly different severity of gingivitis to similar amounts of plaque deposits. The same analysis was repeated at days 7 and 14. Prevalence of HR and LR was compared between days using the χ^2 [ML] test.

Results: For both day 7 and day 14, the quartile distribution of LR and HR was statistically significant ($p = 0.02$). Fifty percent of LR and 71% of HR presented a consistent level of susceptibility to plaque-induced gingival inflammation even after only 7 and/or 14 days of plaque accumulation.

Conclusions: These findings support the concept that the subject-based susceptibility to plaque-induced gingival inflammation is an individual trait, only partly related to the length of time of exposure to plaque.

Key words: disease susceptibility; gingivitis; time factor

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The aetiologic role of dental bacterial plaque in gingivitis was definitively demonstrated with the experimental gingivitis model (Löe et al. 1965, 1967, Theilade et al. 1966). Evidence suggested early on that the onset and severity of the gingival inflammatory response to plaque accumulation might differ significantly among individuals, with such differences essentially ascribed to differences in plaque accumulation rates (quantitative plaque differences) and/or differences in plaque species present (qualitative plaque differences) (Löe et al. 1965, Theilade et al. 1966). However, a review of the subsequent experimental gingivitis literature indicates that susceptibility to plaque-induced gingivitis may differ significantly among subjects, in the absence of differences in plaque deposits (Tatakis & Trombelli 2004).

The reported significant differences in gingival inflammatory response under quantitatively and/or qualitatively almost identical plaque accumulation (Abbas et al. 1986, Lie et al. 1995, Trombelli et al. 2004a) suggest that the level of the gingival tissue response to plaque accumulation may be an individual trait (Abbas et al. 1986, Tatakis & Trombelli 2004), dependent on host-related factors, possibly genetic in origin (Tatakis & Trombelli 2004, Scapoli et al. 2005). An immediate implication of such a tenet is that a subject's gingival inflammatory response will be consistently high or low relative to the level of plaque exposure; on this topic,

the results of a limited number of studies appear conflicting. Although some studies have reported that a percentage of participants have consistently high or low inflammatory response to de novo plaque accumulation when repeatedly tested (Watts 1978, van der Weijden et al. 1994a), others report that there is little, if any, agreement between individual subject responses in repeated experimental gingivitis trials (Shearer et al. 2005). It should be pointed out that all aforementioned studies have used gingival bleeding as the primary outcome variable for quantification of inflammation (Watts 1978, van der Weijden et al. 1994a, Shearer et al. 2005); gingival bleeding assessment is not without challenges, as documented in several studies (Watts 1978, van der Weijden et al. 1994b,c, Müller & Barrieshi-Nusair 2005), and susceptibility to gingival bleeding upon mechanical stimulation (i.e. upon probing) may be related to anatomical characteristics (Trombelli et al. 2004b, Müller & Kononen 2005). At this point, the question of the consistency of the individual gingival response to de novo plaque accumulation remains to be resolved.

From the original experimental gingivitis studies of Löe et al. (1965) it was evident that time of exposure to de novo plaque accumulation was a determining factor for the level of gingival inflammation, a fact explained by the time-dependent increase in plaque deposits. However, when differences in the individual gingival response to plaque are detected in the absence of quantitative plaque differences (Trombelli et al. 2004a), the role of time as a factor in the identification of subjects with different susceptibility to plaque-induced gingivitis has not been explored. In other words, does this subject-specific susceptibility become manifest only when plaque deposits are experimentally left undisturbed for 21 days, or is it a host-related trait that can consistently be anticipated at earlier plaque exposure intervals? It was hypothesized that subjects with different susceptibility to gingival inflammation could be consistently identified in less than 21 days of de novo plaque accumulation. Therefore, the aim of the present study was to assess whether the identification of subjects with different susceptibility to plaque-induced gingival inflammation is dependent on the length of time of de novo plaque accumulation.

Material and Methods

Experimental design and study population

The study design was approved by the local ethics committee and was found to conform to the requirements of the ''Declaration of Helsinki'' as adopted by the 18thWorld Medical Assembly in 1964 and subsequently revised (www.wma.net/e/policy/17-c_e.html). All participants provided written informed consent.

The overall experimental design has been previously described (Tatakis & Trombelli 2004, Trombelli et al. 2004a), and the clinical analysis of the examined population, consisting of 96 systemically and periodontally healthy non-smokers, 46 males (mean age: 23.9 ± 1.7) and 50 females (mean age: 23.3 ± 1.6), has been detailed (Trombelli et al. 2004a). Briefly, a randomized split-mouth localized experimental gingivitis clinical trail was conducted in volunteers. In each subject one maxillary quadrant was randomly assigned as ''test'' (experimental gingivitis) and the contralateral quadrant as ''control''.

Clinical parameters

The following clinical parameters, defined in detail previously (Trombelli et al. 2004a), were obtained in the order listed below from the selected sites: gingival index (GI), plaque index (PlI), gingival crevicular fluid volume (GCF), angulated bleeding score (AngBS), and the derived parameter cumulative plaque exposure (CPE). CPE represents the area under the curve (AUC) of subject-specific PlI over a specific period of time (7, 14, or 21 days) (Trombelli et al. 2004c). All clinical parameters were recorded at days 0, 7, 14, and 21 by two trained and calibrated examiners with good to excellent intra- and inter-examiner agreement, as measured by the k coefficient (Trombelli et al. 2004a).

Statistical analysis

General

The subject was regarded as the statistical unit. For each clinical parameter, the recordings from the six selected sites for either test and control quadrants were added and divided by six to give the mean value for each subject. Therefore, for each parameter at each observational period, the subject was represented by a single test and a single control value. Kolmogorov–Smirnov goodness of fit tests were computed for each variable to assess whether the variables were normally (Gaussian) distributed. Data were expressed by either median and inter-quartile range (IR) for non-parametric variables, or mean $\pm$ standard deviation (SD) for parametric variables.

Identification of HR and LR

The statistical procedure to identify HR and LR on day 21 among the 96 individuals who completed the experimental gingivitis trial has been reported in detail in a previous paper (Trombelli et al. 2004a). Briefly, we first determined which clinical parameter of gingival inflammation showed the highest correlation with plaque-related variables, i.e. PlI and CPE. Correlation analysis showed that GCF-day 21 presented the highest correlation with both PlI-day 21 and CPE-day 21 compared with GI and AngBS. Therefore, GCF was chosen as the primary outcome variable to express the severity of plaque-induced gingival inflammation. Then, GCF-day 21 was standardized according to CPE-day 21, and residuals of GCF-day 21 on CPE were calculated. On the basis of subject distribution of GCF-day 21 residuals after standardization for CPE-day 21, two sub-populations were selected on the basis of upper and lower quartiles of this GCF-residual distribution. These sub-populations were, respectively, defined as HR ($n = 24$) and LR ($n = 24$). They were characterized by significantly different severity of gingivitis to similar amounts of plaque deposits. The HR group comprised 13 males and 11 females (mean age: 24.1 ± 1.6), and the LR group comprised 11 males and 13 females (mean age: 23.4 ± 1.9) (Trombelli et al. 2004a).

Retrospective analysis of HR and LR

To assess whether and to what extent the identification of HR and LR was dependent by the length of time of de novo plaque accumulation (i.e. 7, 14, or 21 days), we proceeded as follows.

First, a linear regression analysis was performed between GCF-day 7, and either PlI-day 7 or CPE-day 7. Again, GCF was chosen as the primary outcome variable to express the severity of plaque-induced gingival inflammation due to the highest correlation with plaque-related clinical parameters. The same analysis was performed between GCF-day 14, and either PlI-day 14 or

CPE-day 14. Comparison between the two regression analyses showed that the variability observed for the outcome variable (GCF) was significantly better explained by CPE than PlI at both day 7 and day 14 (data not shown).

Then, GCF-day 7 and GCF-day 14 were standardized according to CPE-day 7 and CPE-day 14, respectively, and residuals of GCF on CPE were separately calculated for each observation interval, i.e. day 7 and day 14. On the basis of subject (Gaussian) distribution of GCF residuals after standardization for CPE, three sub-populations were identified for each (day 7, day 14) observation interval: the first (lower) quartile ($N = 24$), the two central (second and third) quartiles ($N = 48$), and the fourth (upper) quartile ($N = 24$). In essence, for day 7 and day 14, respectively, we discriminated three sub-populations of subjects with low level (first lower quartile), high level (fourth upper quartile), and moderate level (second and third central quartiles) of gingival inflammation to similar rates of plaque accumulation.

Finally, the prevalence of HR and LR, as discriminated on standardized GCF on CPE at day 21, in each of the three sub-populations (lower, central, and upper quartiles), as identified on standardized GCF on CPE at days 7 and 14, respectively, was calculated and analysed.

Comparisons were performed by using the χ^2 [ML] and the unpaired *t*-test for dichotomous and parametric variables (i.e. GCF residuals), respectively. The level of significance was set at 5%.

Results

At day 7, seven (29.2%) LR presented a low level, 13 (54.2%) LR a moderate level, and four (16.6%) LR a high level of gingival inflammation. Among HR, 10 (41.7%) were highly inflamed, 13 (54.2%) showed moderate inflammation, and one (4.1%) had a low level of gingival inflammation. Quartile distribution of LR and HR was statistically significant (χ^2 [ML] = 7.72, $p = 0.02$).

At day 14, 10 (41.7%) LR presented a low level, 10 (41.7%) LR a moderate level, and four (16.6%) LR a high level of gingival inflammation. In contrast, 13 (54.2%) HR presented a high level, seven (29.2%) showed a moderate level, and four (16.6%) a low level of gingival inflammation. Quartile distribution of LR and HR was statistically significant (χ^2 [ML] = 8.21, $p = 0.02$).

Tables 1 and 2 show in greater detail the distribution of LR and HR, respectively, with respect to the level of inflammation (low, moderate, and high) as presented at day 7 and day 14. Among LR, five subjects always presented a low level of inflammation, two subjects presented a low level of inflammation at day 7 and moderate at day 14, and five subjects presented a moderate level of inflammation at day 7 and low at day 14. Six LR presented a high level of inflammation at day 7 and/or day 14. Among HR, six subjects always presented a high level of inflammation, four subjects presented a high level of inflammation at day 7 and moderate at day 14, and seven subjects presented a moderate level of inflammation at day 7 and high at day 14. four HR presented a low level of inflammation at day 7 and/or day 14.

Statistical analysis showed no significant differences in GCF residuals between LR individuals who presented a low level of gingival inflammation at day 7 and/or day 14 ($N = 12$), and LR individuals who did not ($p > 0.05$). Similarly, no significant differences were found in GCF residuals between HR individuals who presented a high level of gingival inflammation at day 7 and/or day 14 ($N = 17$) and HR individuals who did not ($p > 0.05$).

Table 1. Distribution of LR subjects with respect to the level of gingival inflammation (low, moderate and high) as assessed at day-7 and day-14

Day 7	Day 14	N
Moderate	Moderate	6
Moderate	Low	5
Low	Low	5
Low	Moderate	2
High	Moderate	2
Moderate	High	2
High	High	2

LR, low responders.

Table 2. Distribution of HR subjects with respect to the level of gingival inflammation (low, moderate and high) as presented at day-7 and day-14

Day 7	Day 14	N
Moderate	Moderate	3
Moderate	High	7
High	High	6
High	Moderate	4
Moderate	Low	3
Low	Low	1

HR, high responders.

Discussion

In the present study, data from a reported randomized split-mouth localized experimental gingivitis trial involving 96 healthy non-smokers (Trombelli et al. 2004a) were retrospectively analysed. The gingival inflammatory response of the originally identified 48 subjects, 24 subjects exhibiting a low inflammatory response (LR) and 24 subjects manifesting a high inflammatory response (HR) to similar plaque accumulation after a 21-day experimental period, was retrospectively analysed to determine whether and to what extent their susceptibility to plaque-induced gingivitis was consistent after 7 and/or 14 days of de novo plaque accumulation. The results indicate that (1) a significant prevalence of LR and HR subjects can be detected among subjects who presented with low and high level, respectively, of gingival inflammation to similar plaque accumulation at earlier observation intervals and (2) 50% of LR and 71% of HR presented a consistent susceptibility to plaque-induced gingival inflammation even after 7 and/or 14 days of plaque accumulation. Overall, these findings seem to support the concept that the subject-based susceptibility to plaque-induced gingival inflammation is an individual trait that is only partly related to the amount and rate of accumulation of plaque deposits.

In several respects, the results of the present study appear to be in agreement with others' and our own previous findings. The existence of subjects who can be consistently identified as having a high or low susceptibility to plaque-induced gingival inflammation, as reported here, is in agreement with the results of van der Weijden et al. (1994a). They found, among 45 study participants, 10 subjects who consistently exhibited greater than average gingival inflammation, representing a ''susceptible'' group, and six subjects who were consistently below average, representing a ''resistant'' group. Similarly, our finding that consistency in susceptibility pattern was higher for HR subjects (71%) than for LR subjects (50%) parallels their results in terms of the proportion of consistently responding ''susceptible'' and ''resistant'' subjects (van der Weijden et al. 1994a). The

present results provide additional support for our previous findings, where the identified HR group – when compared with the LR group – had a significantly greater gingival inflammatory response after either 7 or 14 days of de novo plaque accumulation (Trombelli et al. 2004a, b), significantly higher GCF levels even in areas of the dentition where ideal plaque control was maintained (Trombelli et al. 2004a, c), and significantly higher GCF levels in areas of the dentition where ideal plaque control was re-established after implementation of a therapeutic regimen (Trombelli et al. 2004c). Collectively, our present and past observations support the notion of a subject-specific susceptibility to plaque-induced gingival inflammation.

Our results contrast with those of Shearer et al. (2005), who reported a limited, if any, intra-individual agreement between subject gingival inflammatory responses to plaque following repeated experimental gingivitis trials. The discrepancy between the present results and the findings reported by Shearer et al. (2005) could be attributed to the sizeable number of methodological differences between the studies. The fundamental, and most significant in our estimation, difference is that in our studies the determination of subject susceptibility to plaque-induced inflammation (GCF levels) is based on an approach that accounts for the level of exposure to the aetiologic agent (CPE), by standardizing the individual GCF response on the basis of CPE. In contrast, Shearer et al. (2005) analysed subject variability in gingival inflammatory response (gingival bleeding) without accounting for the individual level of exposure to the aetiologic factor (PlI). Coupled with the fact that their data demonstrate little, if any, within-subject consistency in the level of plaque accumulation between trials, their methodological approach explains why they found no consistency in the subject-based gingival inflammatory responses in repeated trials.

The landmark studies of Löe and colleagues showed that time of exposure to de novo plaque accumulation was a determining factor for the development of gingival inflammation, a fact explained by the time-dependent increase in plaque deposits (Löe et al. 1965, Theilade et al. 1966). However, when the gingival inflammatory response of an individual is standardized for the level of exposure to plaque (Trombelli et al. 2004a), the role of time as a factor in characterizing the subject-based susceptibility to plaque-induced inflammation merits independent examination. The results of the present study, i.e. the considerable consistency (50% of LR and 71% of HR) in the subject-based susceptibility to plaque-induced gingival inflammation regardless of time of plaque exposure, suggest that time per se has a limited role in determining the subject-based susceptibility to gingivitis; this supports the concept of susceptibility to plaque-induced gingival inflammation as an individual trait. Additional studies, with the same HR and LR subjects participating in repeat experimental gingivitis trials, would help bolster or refute this conclusion.

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Address:
Leonardo Trombelli
Research Center for the Study of Periodontal Diseases
University of Ferrara
Corso Giovecca 203
44100 Ferrara
Italy
E-mail: l.trombelli@unife.it

Clinical Relevance

Based on previous findings supporting the existence of an individual susceptibility to plaque-induced gingival inflammation, we designed a retrospective study to assess whether the identification of subjects with different gingivitis susceptibility is dependent on the length of timeF of de novo plaque accumulation. The considerable consistency observed in the subject-based susceptibility to plaque-induced gingival inflammation, regardless of time of plaque exposure, suggests that time per se has a limited role in determining subject-based susceptibility to gingivitis. These results reinforce the concept that susceptibility to plaque-induced gingival inflammation may be a subject-based trait.