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Increased plasma levels epithelial cell-derived neutrophil-activating peptide 78/CXCL5 in periodontitis patients undergoing supportive therapy

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Abstract

Aim: To investigate the influence of cigarette smoking on plasma epithelial cell-derived neutrophil-activating peptide-78 (CXCL5/ENA-78) and interleukin-6 (IL-6) in supportive therapy periodontitis patients.

Materials and Methods: Plasma concentrations of CXCL5/ENA-78 and IL-6 were evaluated in 167 systemically healthy subjects (54 smokers and 113 non-smokers) divided into four groups: non-smokers with periodontitis ($n = 90$), smokers with periodontitis ($n = 49$), healthy non smokers ($n = 23$) and healthy smokers ($n = 5$).

Results: Clinical probing depth (CPD) of smokers with periodontitis were significantly greater than those of non-smoking patients ($p < 0.05$). Although clinical attachment loss (CAL) and the number of deep sites affected were greater in the smokers with periodontitis, these differences were not significant. Periodontitis patients had significantly higher plasma IL-6 and ENA-78 than healthy subjects ($p < 0.05$). There was no significant difference in IL-6 between smokers and non-smokers with periodontitis but CXCL5/ENA-78 concentrations were significantly greater in smokers with periodontitis ($p = 0.006$). Plasma CXCL5/ENA-78 correlated with CPD, CAL and tobacco consumption (all $p < 0.05$).

Conclusion: Plasma CXCL5/ENA-78 concentrations are a good systemic indicator of the inflammatory process and disease severity in subjects with periodontitis and in addition are potential indicator of inflammatory effects of cigarette smoking. Further studies are required to elucidate the biological mechanisms underlining this increase in CXCL5/ENA-78

Key words: CXCL5/ENA-78; IL-6, smoking; periodontitis

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Conflict of interest and source of funding statement

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Periodontitis is a chronic inflammatory disease resulting from complex interactions between the immune system and microorganisms (Sanz & van Winkelhoff 2011), which can be modified by environmental and acquired risk factors (Zee 2009).

Tobacco smoking is a major contributing factor to many diseases including periodontitis (Tonetti 1998, Iribarren et al. 1999, Tomar & Asma 2000). More recent epidemiological studies indicate that smokers are on average four times more likely to have periodontitis than non-smokers, although significant variation on the magnitude of the risk is clearly observed in the literature (Tonetti 1998, Tomar & Asma 2000, Bergstrom 2003, Bergstrom 2004, Johnson & Hill 2004).

Smoking is associated with a reduced effect of periodontal therapy and greater

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disease progression as measured by clinical parameters such as periodontal pocket formation, increased probing depth, loss of attachment, furcation involvement, hypermobility, bone loss and reduced dentition (Bergstrom & Floderus-Myrhed 1983, Bergstrom & Eliasson 1987, MacFarlane et al. 1992, Persson et al. 1998, Machtei et al. 1999).

Although the relationship between smoking and increased periodontal disease is strong, the mechanism is not entirely clear. The microflora is likely to play a major role and while contradictory reports exist (Machuca et al. 2000, Mager et al. 2003) as to the influence of smoking, a general consensus is emerging that smoking can alter the microflora (Haffajee & Socransky 2001, van Winkelhoff et al. 2001, Kubota et al. 2011). Smoking is believed to have a negative effect on the host defense by perturbing functions of fibroblasts, macrophages, neutrophils and epithelial cells (Raulin et al. 1988, Walz et al. 1997, Kinane & Chestnutt 2000, Güntsch et al. 2006, Mehta et al. 2008). For example, lower IgG2 concentration have been seen in smokers, which might reduce the phagocytic function of neutrophils and the possibility of lower numbers of these cells in the gingival crevice of smokers (Pauletto et al. 2000, Güntsch et al. 2006) contribute to the impaired host response in smokers (Preber et al. 1992, Stoltenberg et al. 1993, Barbour et al. 1997, van Winkelhoff et al. 2001).

Neutrophils are fundamentally important in providing host defence against bacterial infection and loss of neutrophil function greatly predisposes individuals to diseases such as periodontitis (Nussbaum & Shapira 2011). Neutrophils are a key source of interleukin-6 (IL-6) during infection (Riedemann et al. 2004). IL-6 is a pro-inflammatory cytokine which plays a key role in periodontal disease and is linked to inflammation, B lymphocyte activation, antibody production, cell migration and bone resorption (Garlet 2010) and is generally a good indicator of an inflammatory response. IL-6 and its soluble receptors may have an important role in differentially modulating C–X–C motif chemokines expression, including ENA-78 and influencing neutrophil recruitment (McLoughlin et al. 2004).

ENA-78 also called C–X–C motif chemokine 5 (CXCL5), is a mature product of the CXCL5 gene. This chemoattractant and activator of neutrophils is mainly expressed by monocytes, epithelial cells and platelets (Walz et al. 1997, Damås et al. 2000). In the presence of various extracellular proteases ENA-78 undergoes post translational modifications that make it more effective than IL-8, but can reduce its chemotactic activity (Nufer et al. 1999).

Many host-derived molecules have been investigated for their role in soft tissue destruction and bone degradation in periodontitis (Buduneli & Kinane 2011, Preshaw & Taylor 2011). This has greatly increased the knowledge of the relationship between host response to infection and the disease process. The influence of particular risk factors such as diabetes or smoking has also been investigated (Buduneli & Kinane 2011). While, the influence of smoking on circulating levels of many mediators, including IL-8 (Iho et al. 2003) has been shown, an analysis of the potent neutrophil specific chemoattractant ENA-78 has not.

We hypothesize that the circulating levels of CXCL5/ENA-78 and IL-6 are elevated in patients with periodontitis and correlate with the severity of the disease, but are uncertain as to the effect smoking will have on circulating levels of CXCL5/ENA-78 and IL-6. Thus, the aim of this study is to investigate the influence of cigarette smoking on plasma levels of CXCL5/ENA-78 and IL-6 in patients with chronic adult periodontitis.

Materials and Methods

Study design

Ethical approval and consent

The Glasgow Dental Hospital and School Ethics Committee approved the conduct of this study. All patients included in this study had received periodontal treatment at least 3 years before commence and were receiving supportive therapy from their general practitioner. The 28 healthy control subjects (23 non-smokers and five smokers) were drawn from relatives of the patients and from staff at the Dental School. Written informed consent was obtained from all those wishing to participate.

Clinical examination

All patients had a thorough clinical periodontal examination with full-mouth six-point charting using the PCP12 probe (Hu-Friedy). The following data were obtained: number of teeth, number of pockets, loss of clinical attachment and sites with pocket $\geqslant 5$ mm, clinical probing depth (CPD), and clinical attachment loss (CAL) from the cemento-enamel junction and bleeding on probing after 30 s.

Inclusion and exclusion criteria

At the time of recruitment, patients were included in the periodontitis group if they had clinical signs of chronic adult periodontitis. All participants were systemically healthy, and apart from periodontitis had no other known disease. Patients were excluded if they had surgical periodontal therapy within the previous 12 months and if they were currently taking or had previously taken antibiotics or any other medication during the past 6 months. Females who were pregnant at the time of the study or in the previous year were also excluded. Non-smokers and smokers who smoke greater than 10 cigarettes per day and had at least 10 pack years of cigarette consumption were included in this study this was to ensure a clear distinction between the non-smokers and the smokers in the study. To be included in the periodontitis groups a minimum number of 16 teeth with at least four molars in different quadrants, two periodontal pockets >5 mm in depth and a minimum of 5 mm attachment loss were considered as the border line for recruitment. The healthy control subjects had no history of periodontitis, had no periodontal pockets >2 mm or discernable attachment loss.

Blood samples and measurements

Blood was collected between 09:00 and 11:00 hours (to minimize diurnal variations in biochemical parameters) from a peripheral vein in an anticoagulant tube. This was centrifuged (200 *g*) and stored in aliquots at -70°C. Plasma samples were assayed using commercially available enzyme-linked immunosorbent assay (ELISA) kits for IL-6 (Invitrogen, Paisley, UK) and CXCL5/ENA-78 (R&D Systems, Abingdon, UK); which also detects ENA-74, and ENA-70 post-translationally modified versions of ENA-78; following the manufacturer's recommendations. To identify the lowest dilution that did not interfere with

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either ELISA, six surplus plasma samples were titrated and the optimal dilution determined. The results of this test indicated that dilutions of 1/5 were suitable and this was used subsequently for both ELISAs. In addition to duplication, several measurements were repeated to reassure reproducibility of the assay. The optical density of each well was read by using a microplate reader [FluoStar Optima (BMG LABTECH Ltd., Aylesbury, UK) or Dynex Technologies MRII (East Grinstead, UK)] set at 450 nm. Smoking status for the patients was confirmed by cotinine assay (Cozart, Abingdon, UK).

Statistical analyses

The periodontitis patients were originally recruited for a related study after an *a priori* statistical power calculation had been performed. For this study, an additional power calculation was performed after patient enrolment. Statistical power calculations indicated that at least three of the four patient groups were large enough for a robust statistical analysis. The diminutive size of the healthy smoker group ($n = 5$) meant that it was unwise to include it in the statistical analysis. Nevertheless, it has been included for comparison. The Kolmogorov–Smirnov test indicated that the data were not normally distributed. Because one of the groups included in the full analysis had only 23 participants it was decided to proceed with non-parametric statistics. All statistical differences between the groups were determined by using the non-parametric ANOVA (Kruskal–Wallis test) and the Mann–Whitney *U*-test. The Bonferroni corrections were then used to adjust the p value (p value $\times$ 2) to allow for two comparisons between three groups. The Spearman correlation analysis was performed to investigate relationships between the biochemical and clinical parameters. The Bonferroni correction of the correlation analysis was made by multiplying the p value by 10. A more conservative modification was rejected as several parameters are covariates.

To adjust for the age or tobacco consumption (pack year) of the patients a series of partial correlations were performed on $\log_{10}$ transformations of the non-normal distributed biochemical data. The suitability of this analysis was confirmed by performing a Kolmogorov–Smirnov test on the $\log_{10}$ transformed data. Differences were considered statistically significant with a Bonferroni modified $p < 0.05$.

Results

Demographic data

With the exception of the small group of healthy smokers ($n = 5$, three male and two female subjects) the gender make up of the other three groups was similar (χ^2 analysis $p > 0.05$) (Table 1). The healthy patients were significantly younger than the periodontitis groups (median = 34 years). The median age of the smokers with periodontitis (42 years) was significantly less ($p = 0.001$) than that of the non-smokers with periodontitis (47 years) (Table 1) as was the age of the healthy non-smokers.

Cotinine assay

The results of the cotinine assay have been published for the many of these patients (Lappin et al. 2007). In brief, there was a clear distinction between the levels of this metabolite in smokers and all self-reported non-smokers $n = 113$ ($p < 0.0001$) (Fig. 1). However, the cotinine analysis was performed on 47 of the 54 smokers.

Clinical data

Patients with periodontitis naturally have significantly less teeth and greater CPD, CAL (Table 1) the number of teeth or sites with CPD $\geqslant$ 5 mm or CAL $\geqslant$ 5 mm and BOP (Table 2) than the healthy group ($p < 0.001$), but there was no significant difference between the smokers and non-smokers with periodontitis in CAL (Table 1) the number of teeth or sites with CPD $\geqslant$ 5 mm or CAL $\geqslant$ 5 mm (Table 2). Despite the lower median age of the smokers with periodontitis, the smokers with periodontitis had significantly greater median probing depths ($p = 0.0011$). In contrast the proportion of sites bleeding on probing was significantly less in the smokers with periodontitis than in the non-smokers with periodontitis ($p = 0.037$) (Table 2).

Plasma concentrations of IL-6

Significantly higher levels of IL-6 ($p < 0.001$) were observed in the non-smokers with periodontitis than in the healthy non-smokers (Fig. 2). The highest levels were observed in the plasma of the non-smokers with periodontitis, but there was no significant difference in the plasma concentrations of IL-6

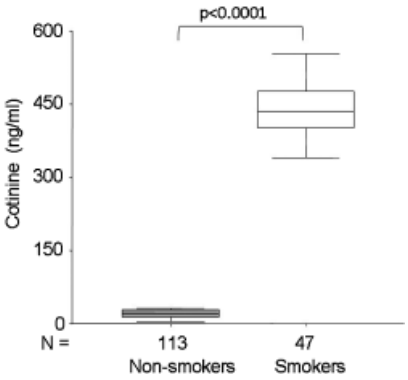

Fig. 1. Plasma concentrations of cotinine in non-smokers and smokers. Statistically significant differences were observed in smokers compared with non-smokers.

Table 1. Details of the patient groups

Group	Median (IQR)				
	age	number of teeth	CPD	CAL	pack year
Non-smokers with periodontitis ($n = 90$; 38M, 52F)	47* (41–55)	25† (23–27)	2.68† (2.48–2.88)	3.23† (2.84–3.83)	0 (0.0–0.0)
Smokers with periodontitis (n = 49; 22M, 27F)	42* (37–51)	24 (23–26)	2.82† (2.68–3.14)	3.48 (3.10–4.07)	21 (19.6–29.8)
Healthy non-smoker ($n = 23$; 11M, 12F)	34* (28–40)	28† (27–30)	1.85† (1.75–2.02)	2.16† (2.05–2.32)	0 (0.0–0.0)
Healthy smoker (n = 5; 3M; 2F)	34 (32–35)	30 (30–32)	1.58 (1.38–1.84)	1.85 (1.85–2.06)	20 (20–20)

*Significant differences in age between non-smokers with periodontitis and smokers with periodontitis and between non-smokers with periodontitis and healthy non-smokers.
†Significant differences in CPD between non-smokers with periodontitis and smokers with periodontitis and in number of teeth, CPD and CAL between non-smokers with periodontitis and healthy non-smokers.
IQR, interquartile range; CAL, clinical attachment loss; CPD, clinical probing depth.

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Table 2. The number of teeth with clinical probing depth (CPD) >5 mm, and clinical attachment loss (CAL) >5 mm and the percentage of sites (%) bleeding on probing (BOP)

Group	Median (IQR)				
	teeth CPD > 5 mm	teeth CAL > 5 mm	sites CPD > 5 mm	sites CAL > 5 mm	sites BOP
Non-smokers with periodontitis	5	4	6.5	6.5	0.33*
($n = 90$; 38M,52F)	(3.0–7.0)	(2.0–7.0)	(3.0–11.8)	(3.0–12.0)	(0.23–0.44)
Smokers with periodontitis	6	6	9	9	0.28*
($n = 49$; 22M, 27F)	(3.0–8.0)	(4.0–8.0)	(3.0–12.0)	(4.0–12.0)	(0.18–0.36)
Healthy non-smoker	0	0	0	0	0.18*
($n = 23$; 11M,12F)	(0–0)	(0–0)	(0–0)	(0–0)	(0.01–0.25)
Healthy smoker	0	0	0	0	0
($n = 5$; 3M; 2F)	(0–0)	(0–0)	(0–0)	(0–0)	(0.00–0.10)

*Significant differences in BOP between non-smokers with periodontitis and smokers with periodontitis and between non-smokers with periodontitis and healthy non-smokers.
IQR, interquartile range.

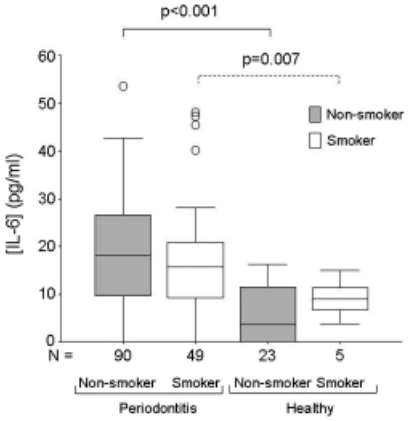

Fig. 2. Plasma concentrations of IL-6 in non-smokers with periodontitis, smokers with periodontitis, healthy non smokers and healthy smokers. Outliers (O) are shown by the symbols. Statistically significant increases in IL-6 were observed in non-smokers with periodontitis compared with healthy non-smokers (also in smokers with periodontitis compared with healthy smokers). The concentration of IL-6 was greater in non-smokers with periodontitis compared with smokers with periodontitis.

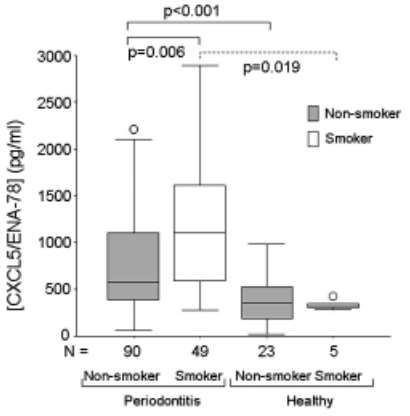

Fig. 3. Plasma concentrations of CXCL5/ENA-78 in non-smokers with periodontitis, smokers with periodontitis, healthy non-smokers and healthy smokers. Outliers (O) are shown by the symbols. Statistically significant increases in CXCL5/ENA-78 were observed in smokers with periodontitis compared with non-smokers with periodontitis and in non-smokers with periodontitis compared with healthy non-smokers (also in smokers with periodontitis compared with healthy smokers).

between the non-smokers with periodontitis and smokers with periodontitis. Although the small group of five healthy smokers appeared to have a higher median concentration of IL-6 than the healthy non-smokers this result was not statistically significant (Fig. 2).

Plasma concentrations of CXCL5/ENA-78

Smokers with periodontitis had significantly elevated plasma levels of ENA-78 ($p = 0.0062$) compared with non-smokers with periodontitis (Fig. 3). The control group of healthy non-smokers had significantly lower levels of the chemokine ($p < 0.001$) than the non-smokers with periodontitis and they had lower median ENA-78 concentrations than smokers with periodontitis ($p = 0.019$).

Correlations

The CPD correlated with the all the clinical parameters: CAL ($p = 0.001$), BOP ($p = 0.002$), number of pockets >5 mm ($p < 0.001$), number of sites with CAL > 5 mm ($p < 0.001$) as well as with ENA-78 ($p < 0.001$) (Table 3a). The CAL correlated with all the clinical markers ($p < 0.001$) with the exception of BOP. CAL also correlated with IL-6 ($p = 0.009$) and ENA-78 concentrations ($p = 0.003$). The number of sites with >5 mm loss of attachment also correlated with IL-6 concentrations ($p = 0.046$). While IL-6 concentrations showed a positive correlation with the age of the non-smoker patients ($p < 0.001$) and with the age of all the patients collectively ($p = 0.001$), ENA-78 concentrations did not ($p = 0.195$ and 0.105, respectively).

In contrast, ENA-78 had a positive correlation with the age of the smokers ($p = 0.033$), as did IL-6 ($p = 0.044$). In addition, ENA-78 concentrations correlated with the tobacco consumption (represented as pack years) of the patients ($p < 0.001$) but IL-6 showed no such relationship ($p = 0.136$).

In addition the correlation between $\log_{10}$ [ENA-78] and pack years was stronger ($p < 0.0032$) when the adjusted for the age of the patients ($r = 0.398$ age adjusted, $r = 0.275$, otherwise) (Table 3b).

Post hoc statistical power calculation (power = 85%) indicates that a sufficient number of smokers were included in the study to support the results of the correlation analysis.

Discussion

Our aim was to investigate the effect of smoking, a major periodontal risk factor on circulating levels of ENA-78 and IL-6 in patients with chronic adult periodontitis. This study was conducted in smokers and non-smokers who were either healthy or consisted of a cohort of chronic adult periodontitis patients undergoing supportive therapy. To our knowledge this is the first study to compare the levels of ENA-78 on

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Table 3. (a) Spearman correlations investigating the correlations in non-smokers with periodontitis, smokers with periodontitis and all subjects. (b) Partial correlations correcting for (1) pack year, (2) age

Group	Parameters	Pack year	IL-6	ENA-78	CAL ⩾ 5 mm	CPD ⩾ 5 mm	BOP	CAL	CPD
Non-smokers			1.0000	0.3006	1.0000	1.0000	0.8028	**0.0032**	0.5155
Smokers	**Age**	**<0.0001***	**0.0459**	**0.0332**	0.5596	1.0000	**0.0266**	1.0000	1.0000
All subjects		1.0000	**0.0010**	0.1049	**<0.0001**	**0.0001**	1.0000	0.37121	0.2059
Non-smokers			0.0862	1.0000	**0.0110**	0.0250	1.0000	1.0000	
Smokers	**CPD**	0.9648	1.0000	1.0000	**0.0026**	**0.0012**	1.0000	**<0.0000**	
All subjects		**0.0007**	0.2211	**<0.0001**	**<0.0001**	**<0.0001**	**0.0025**	**<0.0001**	
Non-smokers			0.5452	0.2187	**0.0002**	**<0.0001**	1.0000		
Smokers	**CAL**	1.0000	1.0000	1.0000	**0.0038**	**0.0027**	1.0000		
All subjects		**0.0414**	**0.0091**	**0.0033**	**<0.0000**	**<0.0000**	1.0000		
Non-smokers			**0.0104**	1.0000	1.0000	0.6773			
Smokers	**BOP**	0.9689	1.0000	1.0000	0.0804	0.1188			
All subjects		0.4502	1.0000	1.0000	**0.0034**	**0.0015**			
Non-smokers			1.0000	0.5059	**<0.0000**				
Smokers	**CPD ⩾ 5 mm**	1.0000	1.0000	1.0000	**<0.0001**				
All subjects		0.7126	**<0.0001**	0.0006	**<0.0002**				
Non-smokers			1.0000	0.1276					
Smokers	**CAL ⩾ 5 mm**	1.0000	1.0000	1.0000					
All subjects		0.1788	**0.0455**	0.1276					
Non-smokers			1.0000						
Smokers	**[ENA-78]**	**0.0009**	1.0000						
All subjects		**0.0007**	0.2770						
Non-smokers									
Smokers	**[IL-6]**	0.7278							
All subjects		0.1361							

Correction	Correlation	IL-6	ENA-78
(1) Pack year	**Age**	**0.0010**	0.1280
(2) Age	**Pack year**	0.2259	**0.0032**

*Statistically significant correlations are shown in bold text. The p value shown represents the Bonferroni corrected value and statistically significance is $p < 0.050$.

IL-6, interleukin-6; CAL, clinical attachment loss; CPD, clinical probing depth; BOP, bleeding on probing.

smokers and non-smokers with and without periodontitis.

Although, seven of the smokers were missing from the cotinine analysis, this is unlikely to have an impact on the results, of more concern would have been misreporting non-smokers.

Tappia et al. (1995) found that smokers have higher levels of IL-6 in plasma than the non-smokers. Our data do not necessarily contradict this finding since we found higher median IL-6 concentrations of healthy smokers ($n = 5$) compared with the healthy non-smokers ($n = 23$) although this difference was not statistically significant. As IL-6 levels also correlated with the age of the patients, the possibility that the younger age of smokers in comparison with non-smokers masked smoking related differences in IL-6 levels cannot be excluded.

The GCF levels of IL-6 were not affected by smoking (Bostrom et al. 1999, Erdemir et al. 2004) While Kamma et al. (2004) showed in patients with aggressive periodontitis that GCF IL-6 was not affected by smoking. The results of the present investigation appear to support these studies.

While the present study was not designed to test the relationship between ENA-78 and the age of the patients, we found that ENA-78 did not correlate with the age of the non-smoker subjects, an observation previously noted by Dimberg et al. (2007). In contrast, ENA-78 concentrations did appear to correlate with the age of the smokers. However when tobacco consumption (pack year) and an age adjusted analyses were performed this correlation between ENA-78 and the age of smokers was lost, whereas the correlation between levels of ENA-78 and pack years was strengthened. A *post hoc* statistical power calculation indicated that there were enough subjects in the investigation to justify this conclusion. This result is supported by similar findings for circulating levels of IL-8 in smokers (Iho et al. 2003).

Nevertheless, several studies have suggested that tobacco smoking adversely affects host immunity and increases the possibility of infection (Sopori 2002, Arcavi & Benowitz 2004). It is believed that several components of cigarettes have the ability to alter the function of the immune system cells. Acrolein, for example, is a toxic unsaturated aldehyde, which affects neutrophil functions (Finkelstein et al. 2001, Sopori 2002, Arcavi & Benowitz 2004). Although it is well documented that smoking significantly increases the number of peripheral blood neutrophils, the migration of these cells into the periodontal tissues is reduced (Mehta et al. 2008).

Higher plasma chemokine levels, e.g., IL-8 and ENA-78, concentrations may contribute to the increased numbers of neutrophils in the blood stream. However, we are unable to state whether this is true for the smoker patients in this study. Neutrophils play a key role in the first line defence against infection

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(Li et al. 2002, Nussbaum & Shapira 2011). However, their hyperreactivity could contribute to tissue damage (Weiss 1989, Gustafsson et al. 2000, Nussbaum & Shapira 2011). Smoking promotes neutrophil induced inflammation an essential feature of chronic bronchitis, emphysema and periodontitis (Kornman et al. 1997, Thatcher et al. 2005).

The CXCL5/ENA-78 ELISA detects the secreted form of ENA-78 according to the manufacturer, and the protease modified forms: ENA-74 and ENA-70, which have enhanced chemokine activity (Nufer et al. 1999). The secretion of ENA-78 is mainly mediated by the c-Jun N-terminal kinase and nuclear factor kappa B pathways (Thiefes et al. 2005). TLR4 activation, p38 and JNK kinases, regulate the expression of ENA-78 (Jeyaseelan et al. 2005). Thatcher et al. (2005) showed that the C–X–C motif chemokine receptor (CXCR2) has an essential effect in the inflammation associated with smoking; that neutrophil infiltration and tissue destruction could be diminished by the inhibition of CXCL5 receptor CXCR2. Cacalano et al. (1994) found that there was a deficiency in neutrophils recruitment in mice that lack CXCR2 although the CXCR2 knocked out mice have normal neutrophil killing activity.(Brown et al. 2003) Furthermore, Brown et al. (2003) showed that CXCR2 knockout mice have reduced severity of arthritis with considerably less tissue destruction . In contrast, Yu et al. (2007) showed that CXCR2 double knockout mice exhibit fragile alveolar bone and exhibit considerable bone loss when infected with *Porphyromonas gingivalis*. In a more recent knockout study, expression of ENA-78 in periodontal tissues appeared to be restricted to the epithelium of gingival papillae at the interface between the host immune cells and the periodontal pathogens (Hernandez et al. 2010).

To our knowledge, few studies have compared the expression of ENA-78 in smokers and non-smokers. In gastritis, ENA-78 mRNA expression was increased in smokers infected with *Helicobacter pylori* (22 smokers *versus* 24 non-smokers) where it was concluded that the increased expression could have an effect in magnifying the severity of the infection and the disease (Shimoyama et al. 2001). While some investigations support the findings of the present study, others conflict. In bronchoalveolar lavage fluid (BALF), there were no significant differences in ENA-78 levels between smokers and non-smokers with or without emphysema (Tanino et al. 2002). ENA-78 levels were lower in BALF of smokers and showed no differences in bronchoalveolar lavage leucocyte-conditioned medium of smokers and non-smokers (Morrison et al. 1998).

The increased levels of ENA-78 in smokers, augmented apparently by the amount of tobacco consumed, may have other consequences as ENA-78 has been linked to squamous cell carcinomas. Overexpression of ENA-78 appears to enhance the proliferation and invasion of squamous cell carcinomas and suppression of ENA-78 inhibits this process (Miyazaki et al. 2006). An obvious source of plasma ENA-78 are platelets (Damås et al. 2000) but further investigation is required to determine the effect of smoking on chemokine release.

In conclusion plasma ENA-78 concentrations are a good systemic indicator of the altered inflammatory process in cigarette smokers and disease severity in subjects with periodontitis irrespective of their smoking habits. Further studies are needed to elucidate the biological mechanism underlining role of ENA-78 in the disease process and the precise mechanism involved in increasing the circulating levels of this chemokine.

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Clinical Relevance

Scientific rationale for the study: Circulating levels of epithelial cell-derived neutrophil-activating peptide 78 a product of the chemokines CXCL5 gene are implicated in carcinoma and many inflammatory diseases including periodontitis. As smoking is a major risk factor for the severity and progression of chronic adult periodontitis and cancer, it was decided to study the impact of smoking on plasma of CXCL5 gene products in periodontitis patients.

Principal findings: Not only were plasma concentrations higher in smokers with periodontitis than in their non-smoker counterparts, but concentrations of this chemokines correlated with the amount of tobacco consumed.

Practical implications: This study provides further evidence for the negative effects smoking has on oral and general health.

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