

Association of rs4073 polymorphism in interleukin 8 gene and periodontitis susceptibility: a meta-analysis with two statistical models used

Associação do polimorfismo rs4073 no gene da interleucina 8 e periodontite: uma metanálise com dois modelos estatísticos utilizados

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RESUMO

Periodontite é uma doença infecciosa resultante da resposta inflamatória do corpo com destruição dos tecidos de suporte do dente. Embora a periodontite seja uma doença multifatorial, fatores genéticos estão envolvidos na resposta do hospedeiro pela produção de mediadores inflamatórios como as citocinas. Este estudo teve por objetivo avaliar a o polimorfismo rs4073 no gene da interleucina 8 e o risco de desenvolvimento de periodontite com o desenvolvimento de uma metanálise. Para isso uma revisão da literatura nas bases de dados PubMed, Medline e Cochrane Library para artigos publicados anteriormente a Outubro de 2014, os resumos foram avaliados e selecionados aqueles que trouxessem associação entre periodontite e o polimorfismo e a extração dos dados feita por três examinadores calibrados. Os cálculos da metanálise foram obtidos por meio do software estatístico Review Manager versão 5.2 com cálculo do índice Odds Ratio (OR) e Funnel Plots com $P < 0.05$. Nove estudos com 1.780 pacientes e 2.170 controles compuseram a metanálise onde o alelo A não foi associado ao risco de desenvolvimento de periodontite quando comparado ao alelo T por meio do modelo estatístico de Efeitos Aleatórios (OR= 1,09, 95% CI: 0,80, 1,47. $P = 0,58$). O polimorfismo rs4073 no gene de IL-8 está envolvido em outras doenças inflamatórias com o alelo A relacionado ao aumento dos níveis de IL-8, particularmente por estimulação de lipopolissacarídeos. Em conclusão, o alelo A não foi associado ao risco de desenvolvimento de periodontite e o alelo T foi sugerido como fator de proteção contra a doença.

Descritores: Doença periodontal. Citocinas. Inflamação. Alelos.

ABSTRACT

Periodontitis is an infectious disease resultant from inflammatory response with destruction of supporting tissues of teeth. Although periodontitis is a multifactorial disease, genetic factors also are involved with the host response by production of inflammatory mediators as cytokines. This study aimed to assess the rs4073 polymorphism in interleukin 8 and the risk of development of periodontitis with conducting a meta-analysis. Thereunto a review in literature was performed in PUBMED, Medline and Cochrane Library databases for published articles before October of 2014, abstracts were evaluated and selected those which brought association between polymorphism and periodontitis, the data extraction was performed by three calibrated examiners. The calculations of the meta-analysis were obtained through statistical software Review Manager version 5.2 with calculation Odds Ratio (OR) and Funnel plots with $P < 0.05$. Nine studies with 1,780 patients with periodontitis and 2,170 controls composed the meta-analysis with the A allele was not associated to development of periodontitis when compared to T allele by Random-effects statistical model (OR= 1.09, 95% CI: 0.80, 1.47. $P = 0.58$). The rs4073 polymorphism in IL-8 gene is involved in others inflammatory diseases with A allele related to increased levels of IL-8, particularly when stimulated by lipopolysaccharide. In conclusion A allele was not associated to risk of development of periodontitis and T allele is suggested as protective factor against disease.

Key words: Periodontal disease. Cytokines. Inflammation. Alleles

INTRODUÇÃO

Periodontitis is a multifactorial infectious disease caused by inflammatory response against accumulation of plaque in dental surface¹ with alveolar bone destruction around the tooth root surfaces and eventual loss of the tooth². This condition is caused by a chronic and mixed infection of Gram-negative bacteria and Gram-positive bacteria that reaches about 10% of adult population and 30% of individuals over the age of 50 years³.

Although microorganisms play an important role in development of periodontitis, the presence of inflammatory cells such as T and B lymphocytes and others in periapical lesions suggests that immune response is also involved in the pathogenesis of the disease⁴. As result from a complex interaction between bacteria and the host's immune and inflammatory response with secretion of cytokines⁵ such as tumor necrosis factor- α , interleukin 6, interleukin 1 α and interleukin 17^{6,7}.

IL-8 also involved in inflammation by neutrophil and monocyte recruiting⁸ and is associated in different inflammatory diseases such as rheumatoid arthritis, gastritis and lung disease⁹ by promotion of angiogenic responses in endothelial cells and potentiates the infiltrating neutrophils¹⁰. In other hand IL-8 levels is increased in blood when stimulated by lipopolysaccharide from periodontopathogens which can initiate a number of host-mediated destructive processes¹¹.

The development of periodontitis involves many factors including genetic variations that give susceptibility to disease and studies like that carried out by Houshmand et al.¹² relating the polymorphism rs4073 in IL-8 and risk of periodontitis developing. However the results are contradictory and not reached a conclusion about the influence this polymorphism in pathogenesis of periodontitis.

Therefore, the aim of this meta-analysis was elucidate the association between rs4073 polymorphism in IL-8 (-

251A/T) and the risk of development of periodontitis and assessing this polymorphism as a possible genetic marker for the disease.

MATERIAL AND METHODS

Search strategy

A systemic search of literature was conducted in PubMed (National Center for Biotechnology, National Library of Medicine), Medline database (US National Library of Medicine, Bethesda, Maryland) and Cochrane Library for articles published up October 2014 to identify all studies on the association between IL-8 gene polymorphism and periodontitis. We used the key words: polymorphism, IL-8, periodontitis, rs4073 polymorphism and IL-8 gene.

Three authors screened all citations and abstracts listed to identify the studies which contained sufficient and clear information on association of the IL-8 (-251 A/T) polymorphism and periodontitis. References of retrieved articles were screened to trace additional relevant studies.

Inclusion criteria

Articles were included in current meta-analysis if the studies met all the following criteria: (1) Evaluation of the association of IL-8 gene polymorphism and periodontitis; (2) Studies are case control design; (3) Genotype frequency documented; (4) Diagnosis of periodontitis confirmed through radiographic findings and clinical manifestations; (5) The allele distributions in study meet the Hardy-Weinberg equilibrium (HWE).

Data extraction

Two investigators independently reviewed all studies and extracted the data using a standardized form. Data were collected on the authors, year of publication, study design (case, control), number of cases and controls with AA, AT or TT genotypes, allele distributions and type of periodontitis.

Statistical analysis

The statistical analysis of data was carried out with the Review Manager version 5.2 software (RevMan, Nordic Cochrane Centre, The Cochrane Collaboration, 2012).

The HWE analysis for genotype distribution among cases and controls was carried out using Pearson's goodness-of-fit chi-squared test. The chi-squared based Q statistic test (I^2) was used to assess the presence of heterogeneity. When heterogeneity was not significant ($I^2 < 50\%$, $P > 0.05$) the Fixed-effects model was used to estimate the pooled Odds Ratio (OR), when heterogeneity was significant ($I^2 > 50\%$, $P < 0.05$) and the studies that case this value could not excluded the Random-effects model was used. Both methods the P value < 0.05 was considered statistically significant. Funnel plots were used to examine heterogeneity and the publications bias of reported associations and all of the data in studies were dichotomous data expressed as OR with 95% of confidence intervals (CI) to assess the association between rs4073 IL-8 gene polymorphism and periodontitis.

RESULTS

Based on search strategy, a total of eleven studies focusing the topic association of IL-8 gene polymorphism (-251 A/T) with periodontitis were detected^{4,12-21}. Nevertheless two studies were excluded^{15,20} because they carried others analyzes but not by polymerase chain reaction-restriction fragment length polymorphism or associated to the polymorphism studied with other factor, respectively. Thus, nine studies which involved 1,780 patients with periodontitis and 2,170 controls met the inclusion criteria and were selected in this meta-analysis.

Table 1 shows the baseline characteristics of including studies in this meta-analysis. The genotype frequencies of all the studies in case and control groups followed HWE.

The frequency of A allele in periodontitis patients and control was

44.60% and 43.80%, respectively. The meta-analysis presented in these results was composed by four studies^{12,14,17,19}. Overall, the pooled analysis showed heterogeneity more than 50% ($I^2 = 86\%$, $P < 0.001$). This was caused by five studies^{4,13,18,16,21}. This event was detected by Funnel plots in which studies were outlier in graphic. Thus, after excluding these studies, the heterogeneity of the meta-analysis decreased to be unremarkable ($I^2 = 16\%$, $P = 0.31$).

Meta-analysis using Fixed-effects as statistical model showed A allele is associated to group with periodontitis owing to its frequency has been significantly higher for the pooled cases (patients with periodontitis) than for the controls. Allele T was significantly associated to control group (OR = 0.82, 95% CI: 0.70, 0.95. $P = 0.01$) and AA genotype frequency was significant in patients with periodontitis (OR = 1.36, 95% CI: 1.02, 1.82. $P = 0.04$).

In other hand, when considered the studies that caused elevated heterogeneity the meta-analysis, with Random-effects statistical model used, evidenced A allele was not associated to patients with periodontitis.

A allele frequency was not significant associated to aggressive periodontitis (as showed in Table 2). The meta-analysis showed heterogeneity caused by the study of Kim et al.¹⁷ which did not present heterogeneity in overall meta-analysis and was excluded. The meta-analysis in chronic periodontitis also brought increased heterogeneity. It was composed by two studies, thus the statistical method used was the Random-effects, A allele was not associated to risk of disease (OR = 1.24, 95%CI: 0.84, 1.83. $P = 0.28$) with $I^2 = 68\%$ ($P = 0.08$).

Table 1 Characteristics of studies focusing on IL-8 (-251 A/T) polymorphism and periodontitis

AUTHOR	STUDY DESIGNE	SIMPLE SIZE	AGE (YEARS)	AA	AT	TT	A	T	FORM OF DISEASE
AMAYA et al., 2012	Case	63	32,8 ± 10,4	6	29	28	41	85	Chronic
	Control	57	35,4 ± 11,6	10	32	15	52	62	
ANDIA et al., 2011	Case	181	44,1 ± 8,8	21	135	25	177	185	Chronic
	Control	108	37,2 ± 12,8	13	57	38	83	133	
ANDIA et al., 2012	Case	76	29,2 ± 5,6	11	50	15	72	80	Agressive
	Control	108	37,2 ± 12,8	13	57	38	83	133	
HOUSHMAND et al., 2012	Case	107	36,4 ± 3,7	40	55	12	135	79	Agressive, chronic
	Control	199	35,2 ± 5,3	10	120	69	140	258	
KHOSROPANAH et al., 2013	Case	227	NO DATA	41	101	82	183	265	Chronic
	Control	40	REPORTED	12	17	11	41	39	
KIM et al., 2009	Case	276	43,4 ± 10,5	56	146	66	258	278	Agressive
	Control	224	35,3 ± 10,4	36	120	64	192	248	
LI et al., 2012	Case	122	54,4 ± 7,8	35	27	60	97	147	Chronic
	Control	532	36,9 ± 9,7	121	264	147	506	558	
LINHARTOVA et al., 2013	Case	278	47,9 ± 8,7	79	147	113	300	372	Agressive, chronic
	Control	156	37,0 ± 8,2	29	78	49	136	176	
ZHANG et al., 2014	Case	400	50,5 ± 9,1	71	177	152	318	482	Chronic
	Control	750	50,3 ± 8,3	140	387	223	668	833	

Table 2 – Characteristics of studies focusing on IL-8 (-251 A/T) polymorphism and periodontitis

Form of periodontitis	A vs T			
	OR (95% CI)	P	I ² (%)	P [†]
Aggressive	1.28 (0.95, 1.72)	0.11	0	0.42
Chronic	1.24 (0.84, 1.83)	0.28	68	0.28

DISCUSSION

Our results showed higher frequency in A allele evidencing the relation between this polymorphism with change in development of periodontitis. Scarel-Caminaga et al.²² found in their study significant association of rs4073 polymorphism and chronic periodontitis susceptibility in Brazilians but when associated to others polymorphisms as haplotype, although others data bring different results¹⁵.

Studies with small simple size, as genetic studies, give what some authors call small effects or limited coverage of genetic variability, so the use of meta-analysis has been increased by larger capacity of detect association between studies²³. The meta-analysis composed by four studies owing to elevated heterogeneity revealed in Funnel plots. Heterogeneity proves how these studies are inconsistent, important fact to meta-analysis because the presence or absence

of true heterogeneity can affect the statistical model applied on data^{24,25}.

With use of Fixed-effects A allele was significantly associated with increased risk of periodontitis by production of interleukin 8. The IL-8 is a cytokine implicated in the recruitment of PMN and a key inflammatory mediator^{26,27}.

The IL-8 -251 A allele is involved in prostate cancer, colorectal cancer and enteroaggregative *Escherichia coli* diarrhea by increased IL-8 production when stimulated by lipopolysaccharide in blood²⁸ and transcriptional activity *in vitro* as response to IL-1-1 β and necrosis factor- α stimulation²⁹, it can explain its association with periodontitis group as demonstrated by Andia et al¹³. Vairaktaris et al.³⁰ demonstrated A allele in rs4073 polymorphism is involved in oral squamous cell carcinoma by elevated levels of interleukin 8 and its ability to induce cell proliferation and promote DNA damage.

Being an important inflammatory mediator, higher IL-8 levels may influence in progression of periodontitis as well as occurrence of lymphoid infiltrates in brain tumors or peritumoral cells infiltrates in gastric cancer³¹. The changes in IL-8 levels are also reported in human gingival sulcus during orthodontic treatment by forces that provide modifications in periodontium³², such changes also occur in periodontal disease evidencing its role in periodontitis.

Our results about AA genotype and its associated to development of periodontitis corroborate the study about increased risk for gastric cancer in Chinese population as well as Kaposi's sarcoma³³.

The association with T allele and control group may suggest a protection factor against the disease¹³ and this allele holds high prevalence in Caucasian (T/T genotypes 47-57%) and Japanese populations (T/T genotypes 59-61%)³⁴.

The pathogenesis of periodontitis is multifactorial, this polymorphism, alternatively, may also be in linkage disequilibrium with functional variants of neighboring genes in IL-8 locus³⁵ so our results should be evaluated with caution.

The study of Chen et al.³⁶ also performed a meta-analysis to evaluate the association between rs4073 polymorphism in interleukin 8 and the risk of development of periodontitis. However, the authors did not exclude the study of Sippert et al.²⁰ which has been an analysis associating patients Duffy-blood positive with presence of -251 A/T polymorphism in IL-8 gene and risk of periodontitis. This fact causes a bias of selection in which patients was submitted to previous selection, so that study must be treated with caution.

Other point to be evaluated is the different types of periodontitis. Chronic periodontitis and aggressive periodontitis have distinct pathophysiological aspects with different genetic factors³⁷, therefore separate analysis for two forms of periodontitis was performed where A allele frequency was not significant associated to aggressive periodontitis corroborating the finding of Andia et al¹⁴. This form of periodontitis has stronger genetic component and it is the most prevalent within families³⁸.

Even Odds Ratio has been calculated by Random-effects, A allele was no associated to chronic periodontitis, however this found must be interpreted carefully because in overall analysis this allele showed association statistically significant with periodontitis.

Smokers and non-smokers patients also exhibit difference in response pattern across this polymorphism, thus representing a potential limitation which may induce to false positive results, for example, observed association with rs4073 polymorphism and non-smokers Brazilian with chronic periodontitis and higher frequency of A allele in this group³⁹ while in other study this relation is not valid¹⁷.

This current meta-analysis had limitations. First some studies included in analysis did not bring total data about the patients (description of smokers and non-smokers, the exact ethnicity of patients). Many studies demonstrated variation in alleles influence when associated with ethnicity in periodontitis or others diseases^{16,29}, this variation is a potential

limitation of current meta-analysis, studies that bring data most specific are required to assure the results.

Second, the use of both statistical methods to Odds Ratio calculation may be a limitation in interpretation of results. The authors preferred use the Random-effects because the errors created by Fixed-effects, it is remarkable that statistical method causes an evidenced Type I bias with serious distortions in conclusions about cumulative knowledge in the research literature⁴⁰. So, it is therefore recommended the Random-effects than Fixed-effects⁴¹. Lastly, to ensuring the meta-analysis Funnel plots revealed no bias of publication validating our data⁴².

CONCLUSION

This meta-analysis, with 1,780 patients with periodontitis and 2,170 controls, indicated that the rs4073 polymorphism in IL-8 gene (-251 A/T) is not associated with risk of developing periodontitis using Random-effects as statistical model to Odds Ratio calculation.

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