VERSITA
DOI: 10.2478/rrlm-2013-0020

Original article

Study regarding the use of salivary 8-hydroxideoxyguanosine and Interleukin-1 gene polymorphism - as potential biomarkers in the diagnosis of aggressive periodontitis

Studiu privind utilizarea 8-hidroxideoxiguanozinei şi a polimorfismului genei interleukinei-1 ca potenţiali biomarkeri în diagnosticul parodontitei agresive

Victoria Badea[1], Mircea Grigorian[1*], Cristina Nucă[1], Corneliu Amariei[1], Alina Martinescu[2], Lavinia Voineagu[2]

1. "Ovidius" University of Constanta, Faculty of Dentistry Medicine
2 ."Ovidius" University of Constanta, Faculty of Medicine

Abstract

The aim of the present study is to explore the use of salivary 8-hydroxideoxyguanosine (8-OHdG) and Interleukin-1 (IL-1) gene polymorphism in the diagnosis of the patients with aggressive periodontitis. The correlation between salivary 8-OHdG level and clinical parameters was analyzed, at the same time as the use of 8-OHdG level and IL-1gene polymorphism in patients with aggressive periodontitis. Eighteen patients suffering from aggressive periodontitis and 18 healthy subjects without any sign of periodontitis were enrolled into the study after clinical examination. The analysis of genetic polymorphism of IL-1 gene was carried out from oral swabs by using the GenoType IL-1 test; the 8-OHdG biomarker was quantified from saliva samples by using an ELISA competition test. The salivary level of 8-OHdG in the control group was 0.70±0.54 ng/mL and in aggressive periodontitis, 6.93±2.90 ng/mL ($p<0.001$). A positive genotype consisting of allele 2 (Thymine/Thymine) was found with lower prevalence in healthy subjects - 5.56% - when compared to aggressive periodontitis, respectively 72.22 % ($p<0.001$). Our study demonstrated that the salivary level of the 8-OHdG biomarker and IL-1 gene polymorphism can be used in the evaluation of the oro-dental status at patients with aggressive periodontitis.

***Keywords**: 8-OHdG, IL-1, saliva, periodontitis.*

Rezumat

Scopul acestui studiu este de a explora posibilitatea de a folosi 8-hidroxideoxiguanozina salivară (8-OHdG) şi polimorfismul genei interleukinei-1 (IL-1) salivare în diagnosticul pacienţilor cu boală parodontală agresivă. Au fost analizate corelaţii dintre nivelul 8-OHdG salivar şi parametrii clinici, în acelaşi timp cu cele dintre nivelul 8-OHdG şi polimorfismul genei IL-1 la pacienţii cu parodontită agresivă. Pe baza examenului clinic, în studiu au fost incluşi 18 pacienţi diagnosticaţi cu parodontită agresivă şi 18 subiecţi sănătoşi, fără nici un semn de parodontită. Analiza

[*]**Corresponding author:** Grigorian Mircea – Faculty of Dental Medicine, "Ovidius" University Constanta, 7 Ilarie Voronca Street, Constanta 900684. Phone: +4 0726310090, Email: mirceagrigorian@yahoo.com

polimorfismului genetic al genei IL-1 a fost determinat din eşantioane de salivă folosind testul GenoType IL-1; biomarkerul 8-OHdG a fost cuantificat din probele de salivă folosind testul ELISA, metoda prin competiţie. Nivelul 8-OHdG salivar în grupul martor a fost de 0,70 ±0,54 ng/mL, iar în grupul pacienţilor cu parodontită agresivă a fost de 6,93±2,90 ng/mL (p<0,001). Genotipul pozitiv reprezentat de alela 2 (Timină/Timină) a fost identificat ca având o prevalenţă mai mică la subiecţii sănătoşi - 5,56% - comparativ cu pacienţii cu parodontită agresivă, respectiv 72,22% (p<0,001). Studiul nostru demostrează că nivelul salivar al biomarkerului 8-OHdG şi polimorfismul genei IL-1 pot fi folosite în evaluarea statusului oro-dentar la pacienţii cu parodontită agresivă.

Keywords: *8-OHdG, IL-1, salivă, parodontită.*

Received: *1st November 2011;* ***Accepted:*** *30th November 2012;* ***Published:*** *27th February 2013.*

Introduction

Dental caries and periodontal diseases are the most common diseases in the oral cavity.

The Global Oral Data Bank of the World Health Organization shows that by the age of 12 only 15% to 30% of the population is caries-free; the same source also shows that periodontal disease affects between 10% and 15% of the world's population (1,2).

Periodontal disease can be defined as disorders of supporting structures of the teeth, including the gingivae, periodontal ligament and supporting alveolar bone, broadly characterized into gingivitis and periodontitis (3, 4). It should be noted that there is no universally acknowledged classification of periodontal disease, but the clinical descriptors used relate to: the rate of disease progress (chronic or aggressive); lesion distribution (localized or generalized) and age group of the person (prepubertal, juvenile or adult) (4).

Salivary analysis has become an important resource for monitoring general health and the state of disease due to its origin, composition equivalent to serum, and interactions with other organs (5); so, the use of saliva has many advantages, including the simple and non-invasive method of collection and low-cost storage (6).

In the present, medical researchers are finding that saliva contains molecular biomarkers, which may be useful in the diagnosis, prognosis, and monitoring of periodontal disease (6).

Therefore, periodontitis that manifests itself as a multifactor phenomenon, includes generation of reactive oxygen species (ROS) which are capable of crossing the DNA nuclear membrane and also producing oxidative damage of the nitrogenous bases (7, 8).

An important biomarker for the DNA damage present in saliva is 8-hydroxideoxyguanosine (8-OHdG) - as the result of guanosine oxidation by ROS (7, 8).

Many researchers have analyzed the salivary 8-OHdG molecule in connection with the periodontal tissue damage, before and after treatment and their results shows that this biomarker can be used in assessing the periodontal status of the patients (9, 10).

Proinflammatory cytokines (interleukins) are a family of mediators closely associated with the pathogenesis of periodontitis; the levels of several interleukins, including interleukin-1 family, were increased in saliva of the patients with chronic periodontitis (11).

The interleukin-1 (IL-1) consists of three proteins: interleukin-1alfa and interleukin-1beta, which are proinflammatory proteins and an antagonist protein, namely interleukin-1ra (11).

The risk of periodontitis is not equally distributed in the population; the source of this susceptibility in periodontitis is represented by the IL-1 Alfa (IL-1A) and IL-1 Beta (IL-1B) gene polymorphism, gene that are present in chromosome 2q13 (11).

The most common form of polymorphism is the single nucleotide polymorphism (SNP); in fact it is represented by the change of a single base pair in the DNA (11).

Genetic predisposition for aggressive periodontitis appear to have a strongly linked with IL-1 gene polymorphism (*Figure 1*).

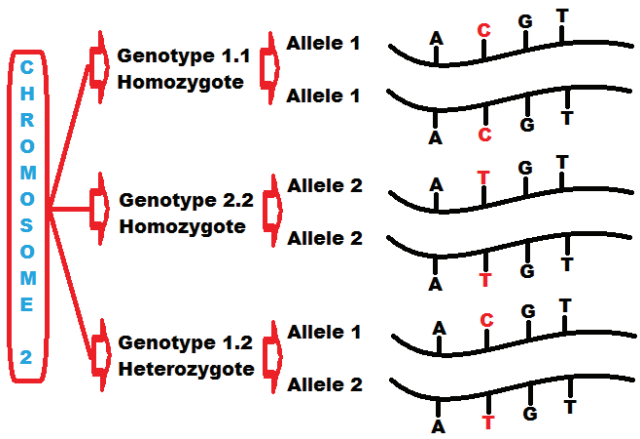


Figure 1. Relationship between Polymorphism and Genotype (after Herbert F. Wolf and Thomas M. Hassell – Periodontology; Georg Thieme Verlag, Stuttgart, Germany, 2006)

In the present, researchers have been focusing their studies on developing new biomarkers in periodontitis (12) and on discovering a possible relationship between genetic polymorphism of the IL-1A gene and its use in periodontitis diagnosis, prognosis and appropriate disease therapy (13).

We conducted this study starting from the hypothesis that both salivary levels of 8-OHdG, a specific biomarker for oxidative stress in periodontitis, and the positive genotype for IL-1 gene can be associated for the early diagnosis of localized aggressive periodontitis. This study is an absolute novelty for Romania and is one of the few among the countries of Eastern Europe.

Materials and methods

Study group

All subjects were from Romania, part of the Eastern European Caucasian ethnic group.

The study group comprised:

- Eighteen subjects (8 males and 10 females) with localized aggressive periodontitis (LAgP); clinical periodontal profile was evaluated by two trained and calibrated examiners who measured the following parameters: probing depth (PD), bleeding on probing (BOP), gingival index (GI) which together have allowed the classification of patients using the clinical index used for the evaluation of periodontal disease according to 1997 criteria of World Health Organization, namely Community Periodontal Index (CPI) (14).
- Eighteen individuals (9 males and 9 females) with clinically healthy periodontium served as control group.

Both groups included subjects with ages between 35 and 44 years, non-smokers, without systemic diseases and who did not use antibiotics for the last 3 months prior to the study. The individuals were randomly selected for this study and they are unrelated. We used adult patients because the LAgP generally affect this age. During the oro-dental examination, saliva samples were collected for 8-OHdG quantification and IL-1 evaluation.

Ethical permission

Ethical permission was given by the Professional Ethical Committee of "Ovidius" University, Constanta, in order to respect the ethical principles for medical research involving human subjects, given by the World Medical Association Declaration in Helsinki. Subjects were verbally informed about the purpose of the investigation. Subjects participated in the study on the basis of informed consent (Free-Consent form – signed by the researcher and the subjects).

Saliva sampling

Saliva samples were collected in sterile containers after five minutes of paraffin stimulation. For 8-OHdG quantification, the samples were centrifuged at 8000 rpm/10min and then stored at -80°C until they were analyzed. Saliva sampling for IL-1 genotype analysis was done using oral swabs.

8-OHdG quantification

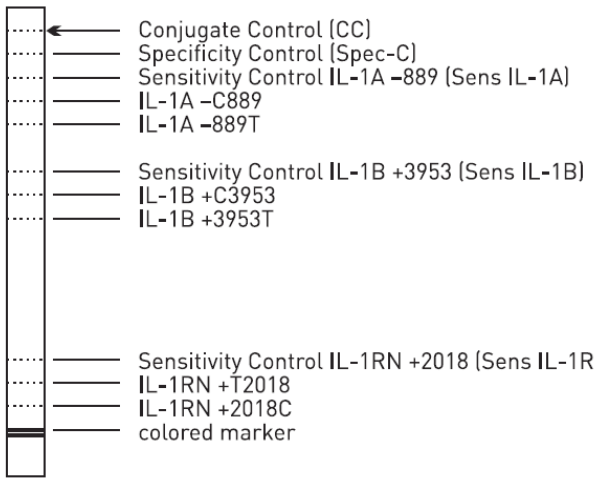
Determination of salivary 8-OHdG by ELISA was performed using a competition method (Cayman Chemical, USA).

IL-1 genotype analysis

The detection of IL-1 genes was done using the GenoType IL-1 test (HAIN Lifesci-

ence, Germany). This test is based on the DNA STRIP technology and permits the combined characterization of polymorphisms in position -889 of the human interleukin (IL)-1A gene, in position +3953 of the human IL-1B gene, and at position +2018 of the human interleukin-1 receptor antagonist gene. The whole procedure is divided into three steps: DNA extraction from the patient sample, a multiplex amplification and a reverse hybridization.

In the first step we extracted the DNA according to the protocol for purification of total DNA from buccal swabs using a microcentrifuge.



strip. (GenoType IL-1; ver 1.0; Hain Lifescience GmbH; www.hain-lifescience.de)

The multiplex amplification was made using the HotStarTaq DNA Polymerase from Quiagen and the thermocycler PTC200.

The hybridization was done with specific oligonucleotide probes, which were immobilized on nitrocellulose strips. During the hybridization, the denatured and amplified DNA was bound to the gene probes attached to the strips. This complex was then detected by a color reaction with alkaline phosphate and band pattern was analyzed using the template supplied.

Each strip has a total of 11 reaction zones and we used a key (*Figure 2*) offered by the producer for identifying our results (*Figure 3*).

Statistical analysis

Student's t-test and SPSS 17 test (p value<0.05) were used to evaluate significant difference between the groups of subjects; analysis of variance (ANOVA) and Chi-square statistics were used for testing intra-group variation. The correlation between clinical signs, genetic profile and the 8-OHdG biomarker levels were analyzed using Pearson coefficient.

All statistical analyses were done using statistical software; statistical significance of the results was defined by p<0.05 (two tail).

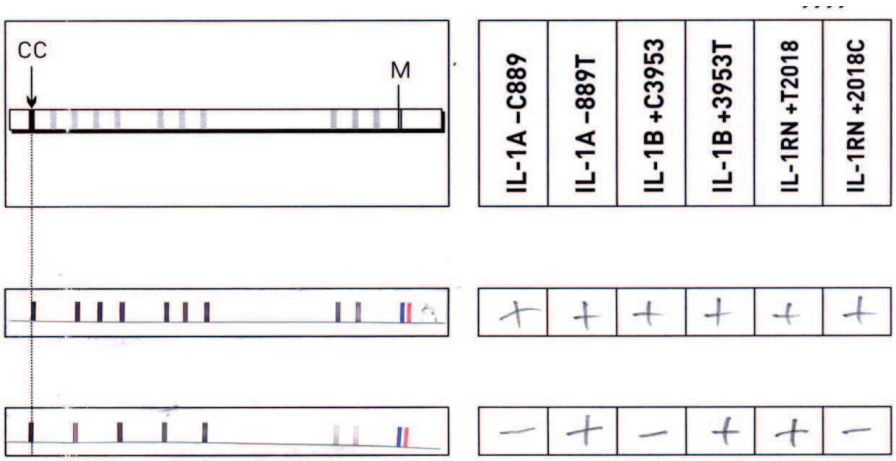


Figure 3. Sample of our results.

Table 1. Salivary 8-OHdG level of control and localized aggressive periodontitis patients

Group	8OHdG ng/mL		
	Mean	SD	p value (*t* test)
Control	0.70	0.54	0.000
Localized aggressive periodontitis	6.93	2.90	

Table 2. Correlation between salivary 8-OHdG level and clinical parameters in localized aggressive periodontitis patients

	Localized aggressive periodontitis			
8-OHdG (ng/mL)	PD(mm)	BOP(%)	GI(%)	CPI
Pearson Correlation /Sig.(2-tailed)	0.496*	0.456*	0.388*	0.984**

* Pearson Correlation is significant at the 0.05 level (2-tailed),
** Correlation is significant at the 0.001 level (2-tailed).
Abbreviations: PD – probing depth, BOP-bleeding on probing, GI – gingival index, CPI-Community Periodontal *Index*.

Table 3. Prevalence of the IL-1 A genotype and gene frequency in the study groups

Genotype	Control (n=18)		LAgP (n=18)		Total (n=36)		p-value
	n	(%)	n	(%)	n	(%)	
CC	11	61.11%	4	22.22%	15	41.67%	**0.018**
CT	6	33.33%	1	5.56%	23	63.89%	**0.000***
TT	1	5.56%	13	72.22%	14	38.89%	**0.000***

* p< 0.005, Chi-square test

Results

The salivary level of 8-OHdG in the control group was 0.70 ± 0.54 ng/mL (the normal mean of salivary 8-OHdG is 1.56 ± 0.1 ng/mL (9,10)) and in LAgP, 6.93 ± 2.90 ng/mL.

Statistically processed data showed significant differences (p< 0.001) in the mean values of the 8-OHdG level in saliva of the patients with LAgP as compared with healthy subjects (*Table 1*).

The analysis of the correlation between the clinical parameters and the salivary 8-OHdG levels in LAgP patients shows that there is a positive relationship between these variables (p< 0.05).

In the same time, analysis of the correlation between the 8-OHdG salivary level and CPI (Pearson coefficient) shows that there is a significant relationship (p<0.001) between these two variables (*Table 2*).

Regarding the Interleukin-1 gene polymorphism, our study demonstrated that there is a positive genotype consisting of allele 2 Thymine/Thymine (T/T) carried by 5.56 % of the healthy subjects and 72.22 % of subjects with LAgP.

The heterozygote variant for IL-1A Cytosine/Thymine (C/T) was present in only one subject with LAgP and in 6 healthy controls (33.33%); the heterozygote variant for IL-1B (C/T) was not found in our groups.

There were significant differences (Chi-square) in the genotype frequencies Cytosine/Cytosine (C/C) (p<0.05), C/T and T/T (p<0.001) in healthy controls subjects as compared with the patients with LAgP (*Table 3*).

Discussions

Any new diagnosis strategy in periodontitis should have a double effect: on oral health and

on systemic health. This expected effects are based on the correlation between periodontitis and other systemic diseases, relationship which is already demonstrated by many authors (8,13).

During the past 10 years, many studies have showed that the severity of periodontitis is closely linked with IL-1A and IL-1B gene polymorphism and local inflammatory mediators, among which IL-1 plays an important role by increasing the susceptibility of aggressive periodontitis (15).

Regarding the relationship between the IL-1 gene polymorphism and aggressive periodontitis susceptibility, conflicting results have been presented in different ethnic populations, including Caucasian-American, African-American, European-Caucasian and Asian Population (15,16).

In this order, Scapoli et al. (17) have failed to support the existence of a causative variant for aggressive periodontitis with IL-1 gene polymorphism in a representative Italian population; similar results in Indian population were published by G. Kaarthikeyan et al. (18), by Drozdik A. et al. (19) in Polish population and Fiebig et al. (20) in Nord-European Caucasians.

In contrast to these results, Kormman et al. (21) provided a highly significant association between IL-1 gene polymorphism and periodontitis; the frequency of positive patients reported by these authors for allele 2 was 36%.

According to Kratka et al. (22), in Czech population, IL-1 gene polymorphism was found in 60% patients with early onset periodontitis. Similar results were reported by Prakash (23) who studied the association between aggressive periodontitis and IL-1 gene polymorphism and reported predominance of subject characterized by positive genotype among patients with aggressive periodontitis. On the other hand, Parkhill (24) found significantly increased IL-1B gene polymorphism at patients with early onset periodontitis. Guzeldemir et al. (25), who studied the Turkish Caucasian population, also identified that IL-1A and IL-1B gene polymorphism are associated with LAgP.

The results of the present study are very similar to the last authors - a difference due to the presence of allele 2 in the healthy and LAgP subjects was present; so, positive genotype consisting of allele 2 (-889 IL-1A and +3954 IL-1B) was carried by 72.22 % of subjects with LAgP and only by 5.56 % of the healthy subjects.

However, it should be noted that there is statistical significance between these groups involving allele 2 (*Figure 1*) – positive genotype ($p<0.001$).

A difference due to allele 1 (normal genotype - *Figure 1*) in the study groups was found; 61.11% in the healthy control group and 22.22% in the LAgP group. These results are in accordance with other studies (22-24).

In a recent study (26) we have demonstrated the high sensibility of the salivary 8-OHdG biomarker for the periodontal disease; this biomarker is increased before the appearance of the clinical sign of LAgP and so, it can be used in the early diagnosis of LAgP. We have continued the study and now we discovered a strong link between the presence and level of 8-OHdG biomarker and the aggressiveness of periodontal disease.

Based on the present data and within the limit of this study, our results showed that salivary levels of 8-OHdG and IL-1 polymorphism are instruments which can be used for appreciating the oro-dental status and for establishing the periodontitis diagnosis. This is based on the fact that in our study we detected higher values of salivary levels of 8-OHdG biomarker and IL-1 gene polymorphism at patients with LagP.

Considering that our study's results have showed an association between periodontal disease and the IL-1 genetic polymorphism, we can affirm retrospectively that the susceptibility to the progression of the disease in patients with LAgP is linked with the genetic profile; this means that subjects from the healthy group without signs of periodontitis but with positive genotype must be monitored because they present IL-1 genetic polymorphism as a risk factor for periodontal disease, in the absence of the other risk factors tested.

Identification of salivary biomarkers and genotype will establish a more correct dia-

gnostic and make possible a more efficient prognosis. Our results indicate that the salivary level of 8-OHdG can be used to appreciate the oro-dental status. At present, the functional significance of polymorphisms is not completely understood and that is why we propose for our future studies to evaluate the association between the salivary level of 8-OHdG biomarker, IL-1 genes polymorphism and their phenotypic expression, respectively the quantitative determination of salivary IL-1, in periodontitis.

Conclusion

Early detection of disease plays a crucial role in successful therapy; and for this, we think that in the future screening by salivary level of 8-OHdG and IL-1 genotypes will be a valuable and necessary tools in early diagnosis and monitoring the patients with periodontitis.

Acknowledgments

This work was supported by CNCSIS-UEFISCSU, project PN II- IDEAS, 1131/ 16.01.2009 - "The study for the determination of salivary 8-hidroxydeoxyguanosine-oxidative stress biomarker, as a screening population method and monitoring of the periodontal disease".

Abbreviations

8-OHdG - 8-hydroxideoxyguanosine
IL-1 - Interleukine-1; IL-1A – Interleukine-1 Alfa; IL-1B – Interleukine-1 Beta
ELISA - Enzyme-Linked ImmunoSorbent Assay
ROS - reactive oxygen species
SNP - single nucleotide polymorphism
LAgP - localized aggressive periodontitis
PD - probing depth
BOP - bleeding on probing
GI - gingival index
CPI - Community Periodontal *Index*
C/C – Cytosine/ Cytosine
C/T – Cytosine/ Thymine
T/T – Thymine/ Thymine

References

1. W.H.O. Strategy for prevention and control of chronic diseases; Oral Health Surveys: Basic Method - 4th Ed. W.H.O. Library Cataloguing in Publication Data, Geneva, Switzerland, 1997.
2. Baehni PC. Potential of diagnostic microbiology for treatment and prognostic of dental caries and periodontal disease. Oral Biol Med. 1996,7(3):259-277.
3. Barsotti O, Poulet PP. Les infections du parodonte. Valerie Laruelle-Bancel. Microbiologie en odonto-stomatologie. Maloine, Paris, 2006,256-257.
4. Samaranayake LP. Microbiology of periodontal disease. Michael Parkinson. Essential Microbiology for Dentistry - 2nd Ed. Churchill Livingstone, London, 2002, 224-225.
5. Limaa DP, Diniza DG, Moimaza SAS, Sumidab DH, Okamotoc AC. Saliva: reflection of the body. International Journal of Infectious Diseases. 2010,14(3):184-188.
6. Yu-Hsiang L. Saliva-An emerging biofluid for early detection of diseases. Am J Dent. 2009,22(4):241-248.
7. Borges IJr, Moreira EA, Filho DW, de Oliveira TB, da Silva MB, Fröde TS. Proinflamatory and oxidative stress biomarkers in patients with periodontal disease. Mediators Inflamm. Volume 2007,2007:45794.
8. Zhang L, Xiao H, Wong DT. Salivary Biomarkers for Clinical Applications. Molecular Diagnosis & Therapy. 2009,13(4):245-259.
9. Takane M, Sugano N, Ezawa T, Uchiyama T. A marker of oxidative stress in saliva-association with periodontaly-involved teeth of a hopeless prognosis. Journal of Oral Science. 2005,47(1):53-57.
10. Cenk FC, Yasin C, Abdulkadir Y. Increased levels of 8-hidroxydeoxyguanosine and malondialdehyde and its relationship with antioxidant enzymes in saliva of periodontitis patients. European Journal of Dentistry. 2009,3(2):100-106.
11. Dumitrescu AL, Dogaru BC, Dogaru CD. The role of interleukin-1 gene polymorphism in periodontal disease. InfoMedica, 2007, 142(4):23-29.
12. Nabors TW, McGlenen RC. Salivary testing for disease diagnosis and treatment. Dentistry Today. Course nr.126, June 1 2009 to May 31 2011.
13. Brinkmann O, Zhang L, Giannobile W, Wong DT. Salivary biomarkers for periodontal diagnostics. Expert Opinion on Medical Diagnostics. 2011,5(1):25-35.
14. W.H.O. Oral Health Surveys: Basic Method - 4th Ed. W.H.O. Library Cataloguing in Publication Data. Geneva, Switzerland,1997.
15. Meng H, Xu L, Li Q. Determination of host susceptibility in aggressive periodontitis. Periodontology 2000. 2007,43:133-159.
16. Kabayasit YH, Tai H, Galicia JC. The role of genetic polymorphism in periodontitis. Periodontology 2000. 2007,43:102-132.

17. Scapoli C, Borzani I, Guarnelli ME, Mamolini E. IL-1 gene cluster is not linked to aggressive periodontitis. J Dent Research. 2010,89(5):457-461.
18. Kaarthikeyan G, Jayakumar ND, Padmalatha O, Sheeja V, Sankari M, Anandan B. Analysis of the association between IL-1(+3954) gene polymorphism and chronic periodontits in a sample of the soth Indian population. Indian Journal of Dental Research. 2009,20:37-40.
19. Kurzawski DA. Polymorphism in interleukin-1 gene and the risk of periodontitis in a polish population. Adv Med Sci. 2006,51:13-20.
20. Fiebig A, Jepsen S, Loos BG et al. Polymorphisms in the interleukin-1 (IL1) gene cluster are not associated with aggressive periodontitis in a large Caucasian population. Genomics, 2008,92(5):309–315.
21. Kornman KS, Crane A, Wang HY. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol. 2009,24:72-77.
22. Kratka Z, Bartowa J, Kresja O, Janatova T, Duskova J. Interleukin-1 gene polymorphism as assessed in a 10 year study of patients with early-onset periodontitis. Foliea Microbiol. 2007,52(2):183-187.
23. Prakash PSG. Interleukin 1b gene polymorphism and its association with chronic periodontitis in South Indian population. International Journal of Genetics and Molecular Biology. 2010,2:178-183.
24. Parkhill JM. Association of IL-1 gene polymorphism with early-onset periodontitis. Journal of Clinical Periodontology. 2011, 27(9):682-689.
25. Guzeldemir E, Gunhan M, Ozcelik O, Tastan H. Interleukin-1 and tumor necrosis factor-alpha gene polymorphisms in Turkish patients with localized aggressive periodontitis. Journal of Oral Science. 2008, 50(2):151–159.
26. Badea V, Balaban DP, Amariei C, Nuca C, Bucur L. Salivary 8- hidroxy-2deoxy guanosine as oxidative stress biomarker for the diagnosis of periodontal disease. Farmacia. 2010, 58:660-670.