Optimization of periodontal disease diagnosis by the results of clinical laboratory tests

M.A.M. AL-Qufaish¹, I.N. Usmanova², M.M. Tuigunov², R.F. Khusnarizanova², M.I. Gumerova², ³, A.I. Shangareeva², ⁴University of Aden, School of Dentistry, Aden, Yemen ²Bashkir State Medical University, Ufa, Russian Federation ³«My dentist» Dental clinic, Ufa, Russian Federation ⁴«Kremlin-Denta» Dental clinic, Ufa, Russian Federation

Abstract

Relevance. The authors have established that the microbiological and local risk factors prevail in changing the clinical condition of the periodontium. Aim – clinical and diagnosis argumentation of the gingival tissue condition according to the criteria of the New International Classification of Periodontal and Peri-implant Diseases and Conditions and Proceedings of the 2017 World Workshop jointly held together by the American Association of Periodontology (AAP) and the European Federation of Periodontology (EFP).

Materials and methods. Clinical and laboratory assessment of 105 young patients was conducted. Three patient groups were formed according to the detected risk factor and according to the data of the New International Classification of Periodontal and Peri-implant Diseases and Conditions. The main group consisted of 70 (66.6%) patients with diagnosed chronic plaque-induced gingivitis (33.3%) and initial periodontitis (33.3%) (mild chronic periodontitis). The control group comprised 35 patients with clinically healthy gingiva on an intact periodontium (71.1%) and reduced periodontium (22.9%). Periodontal pathogens as a risk factor were assessed by PCR using DNA-express commercial sets (Liteh, LLC, scientific manufacturing company, Russia). Cytology of the gingival crevicular fluid impression smears stained by Romanovsky-Giemsa method was performed.

Results. Changes in the hygiene and periodontal indices were revealed on full dental examination. PCR detected low or critical number of periodontal pathogens in the studied samples. Neutrophilic leukocytes, histiocytes and epithelial cells were present in the impression smears, polymorphonyclear neutrophils significantly increased and macrophages, histiocytes, epithelial cells appeared; macrophages decreased.

Conclusion. Full dental examination and laboratory tests revealed the following clinical conditions: clinically healthy gingiva on an intact periodontium, clinically healthy gingiva on a reduced periodontium, plaque-induced gingivitis, stage I periodontitis – initial periodontitis, which corresponded to the New International Classification of Periodontal and Peri-Implant Diseases and Conditions and Proceedings of the 2017 World Workshop held by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP).

Key words: clinically healthy gingiva, intact periodontium, gingivitis, initial periodontitis, microbial biofilm, periodontal pathogens

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INTRODUCTION

Periodontal diseases remain one of the most common oral pathologies worldwide irrespective of the sex and age as well as causative risk factors [1-4].

In modern international and Russian scientific publications, authors consider different risk factors for developing periodontal diseases, thereby, the key role belongs to microbial [1, 3, 5], local and environmental risk factors [6]. Periodontal pathology progresses only in the presence of the supragingival and subgingival biofilm which most frequently consists of the aggressive periodontal pathogens - representatives of especially aggressive "red" and "orange" complexes: Porphyromonas gingivalis, Prevotella intermedia Tannerella forsythia, Treponema denticola [7]. Complex representatives in association with other oral microbiome representatives, are able to invade gingival epithelial cells [7, 8]. Healthy gingival condition is stated if there are no histological or structural signs of inflammation and good oral hygiene. Stable periodontally diseased tissues can also be considered healthy [9]. According to the New International Classification of Periodontal and Peri-Implant Diseases and Conditions and Proceedings of the 2017 World Workshop held by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP), healthy periodontal condition is defined by certain criteria which explain the relevance of our research [2, 7].

Aim – clinical and diagnostic rationale for the gingival tissue condition based on the criteria of the New International Classification of Periodontal and Peri-Implant Diseases and Conditions and Proceedings of the 2017 World Workshop held by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP).

MATERIAL AND METHODS

Full clinical and diagnostic examination of 105 young subjects aged 20 to 35 years allowed assessing periodontal tissue condition and form three groups. The control group consisted of 35 patients (33.3%), in which 77.1% had clinically healthy gingiva on an intact periodontium and 22.9% had clinically healthy gingiva on a reduced periodontium. Group 1 consisted of 35 patients (33.3%) with plaque-induced gingivitis (K05.1), group 2 was composed of 35 patients (33.3%) with initial periodontitis (K05.31). The groups were formed according to the criteria of the New International Classification of Periodontal and Peri-Implant Diseases and Conditions and Proceedings of the 2017 World Workshop held



by the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP) [10].

On full oral examination, the periodontal condition was evaluated by the following indices: PMA, OHI-S, PI, SBI.

The periodontal condition was assessed by CBCT (Planmeca), bone tissue density was evaluated by densitometry in Hounsfield units (HU).

The impression smears of the gingival crevicular fluid stained by Romanowsky-Giemsa method were cytologically studied. The number of cellular elements was detected, epithelial cell maturity was assessed, leukocytes and lymphocytes were revealed.

Periodontal pathogen DNA, namely Porphyromonas endodontalis, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Treponema denticola, Fusobacterium nucleatum, Prevotella intermedia, Tannerella forsythia, was detected and quantified in the biological material (periodontal pocket content, dental plaque, gingival crevicular fluid) using "Dentoscreen" reagent set (Liteh, LLC, scientific manufacturing company, Russia).

The obtained data were statistically analyzed using Statistica 7.0 and Excel 2007 software. Difference probability (p) was found in the T-distribution table by the Student t-test value and degrees of freedom (n). Data, where error probability (p) was less than 0.05 (p < 0.05) were statistically significant. Biostat software package, including X2 criterion, was used for non-parametric data processing.

RESULTS AND DISCUSSION

The results of the full dental examination demonstrated that 77.1% of patients with clinically healthy gingiva on an intact periodontium did not have any complaints, hygiene and periodontal indices were within normal limits. 22.9% of patients with clinically healthy gingiva on an intact periodontium complained of itching, spontaneous bleeding on brushing and eating hard food; there were the signs of the slight gingival inflammation: swelling, hyperemia or engorgement. The hygiene and periodontal indices (PMA, GI, OHI-S, PI) were not within normal limits: PMA - 10.70 \pm

0.03, GI - 0.98 \pm 0.01, OHI-S - 1.57 \pm 0.03, PI - 0.57 \pm 0.03. That's why a CT scan was performed.

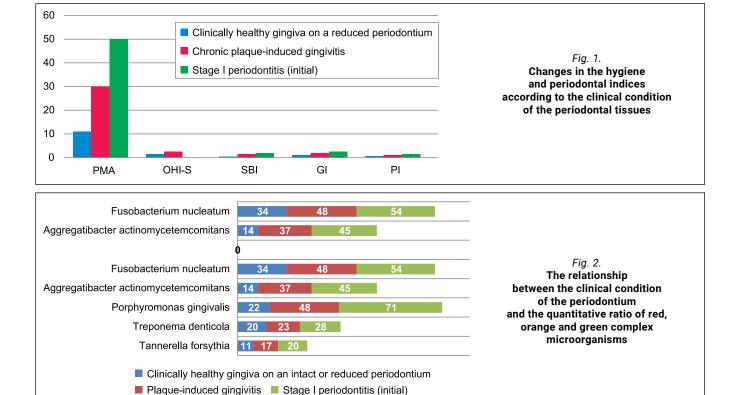
In 22.9% of cases, the CT scans demonstrated changes in the external and internal cortical plates – thinning around single teeth, small foci of osteoporosis at the alveolar bone crest. The densitometry revealed that the bone density was 1398.00 \pm 53.42 HU in the middle of the interproximal bone crest at the mandibular anterior teeth, 1567.00 \pm 49.64 HU – at the mandibular posterior teeth, 1166.00 \pm 46.58 HU – maxillary anterior teeth, 1585.00 \pm 51.31 HU – maxillary posterior teeth. The obtained data defined suballocation of patients with clinically healthy gingiva on a reduced periodontium.

Patients with plaque-induced gingivitis (42.85%) and 65.71% of patients with stage I periodontitis (initial) complained of bleeding on brushing and eating hard food, occasional dull ache and discomfort in the gum. The gingival papillae were swollen, enlarged, spongy, hyperemic, cyanotic, rolled.

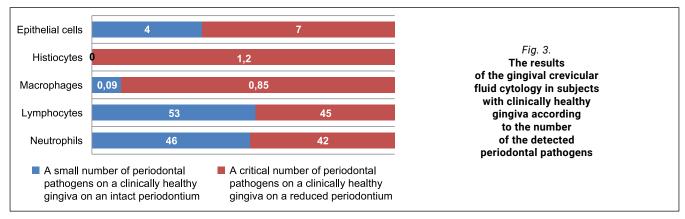
Mean values of the hygiene and periodontal indices in patients with plaque-induced gingivitis were higher than those in healthy subjects: PMA - 15.30 \pm 0.03 to 29.80 \pm 0.03, OHI-S - 1.75 \pm 0.05 to 2.57 \pm 0.05, SBI - 1.08 \pm 0.05 to 1.48 \pm 0.05, GI - 1.43 \pm 0.04 to 1.99 \pm 0.01, PI - 0.85 \pm 0.03 to 1.02 \pm 0.03. The values in stage I periodontitis (initial) patients were as follows: PMA - 39.30 \pm 0.08 to 49.30 \pm 0.08, OHI-S - 2.72 \pm 0.01 to 2.92 \pm 0.01, SBI - 1.75 \pm 0.75 to 1.85 \pm 0.75, GI - 2.10 \pm 0.04 to 2.60 \pm 0.01, PI - 1.32 \pm 0.05 to 1.52 \pm 0.05 (Fig. 1).

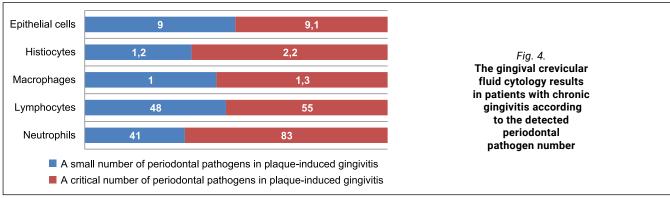
CT scans demonstrated widening of the periodontal gap in the area of single teeth in 42.85% of patients with plaque-induced gingivitis and in 65.71% of patients with stage I periodontitis (initial). The densitometry showed that the mean values of bone density were 1458.00 \pm 46.35 HU in the middle of the mandibular anterior interproximal bone, 1597.00 \pm 51.22 HU at the mandibular posterior teeth, 1256.00 \pm 33.54 HU at the maxillary anterior teeth and 1599.00 \pm 47.34 HU at the maxillary posterior teeth.

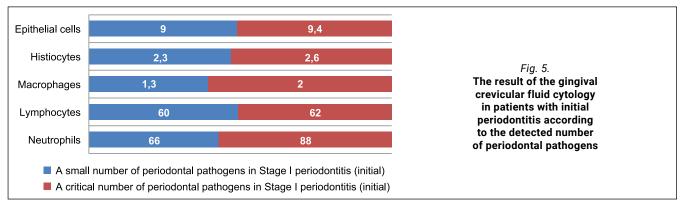
The hygiene and periodontal indices were statistically significantly different in the groups of patients with clinically healthy gingiva on a reduced periodontium, with



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plaque-induced gingivitis and stage 1 periodontitis (initial), compared to the subjects with clinically healthy gingiva on an intact periodontium (p < 0.05).

Real-time qualitative PCR revealed specific DNA fragments of red and orange complex microorganisms: Treponema denticola, Tannerella forsythia, P. gingivalis, Fusobacterium nucleatum, Aggregatibacter actinomy-cetemcomitans, in the samples (periodontal pocket content, dental plaque, crevicular fluid) of subjects with diagnosed healthy periodontium, plaque-induced gingivitis as well as stage I periodontitis.

The mean detection rate of the red complex microorganisms was 22.9% - 71.4% for P. gingivalis, 20.0% - 28.6% for Treponema denticola, 11.4% - 20.0% for Tannerella forsythia (Fig. 2).

The orange complex was formed by the representatives of anaerobic gram-negative microorganisms – Fusobacterium nucleatum detected in 34.3% to 54.3% of the studied cases. Aggregatibacter actinomycetemcomitans was the most frequently detected green complex representative, 14% to 45% of the studied cases (Fig. 2).

Red complex bacteria were revealed in the samples of patients with clinically healthy gingiva on a reduced periodontium. Fusobacterium nucleatum was detected in 34.28% of cases, Porphyromonas gingivalis – 22.85%, Aggregatibacter

actinomycetemcomitans – 14.29%. In plaque-induced gingivitis and initial periodontitis, their prevalence was significantly higher: Porphyromonas gingivalis was encountered twice as often, Fusobacterium nucleatum – 1.5 times, Aggregatibacter actinomycetemcomitans – 2.6 and 3.2 times respectively.

The dependence of the clinical manifestations from the risk factors in the form of critical number of periodontal pathogens was revealed by the clinical examination of periodontal tissues, index assessment and CT scan.

In the large majority of patients with Stage I periodontitis (initial) (K05.31), a high number of Fusobacterium nucleatum, P. gingivalis, Aggregatibacter actinomycetemcomitans, Porphyromonas endodentalis and T. Denticola was reveled in the studied samples – periodontal pocket content. The mean detection rate was 54.3 to 34.3%.

The studied microorganisms were encountered 1.4 to 3.1 times less frequently (p < 0.05) in the gingival crevicular fluid samples of patients with plaque-induced gingivitis and clinically healthy gingiva on a reduced periodontium.

In the studied samples (dental plaque) of the majority of patients with clinically healthy gingiva on a reduced periodontium, orange, red and green complex microorganisms prevailed: Fusobacterium nucleatum – 34.28% of cases, Porphyromonas gingivalis – 22.85%, Aggregatibacter actinomycetemcomi-

tans – 14.29%, which became the criterium for predicting the further development of the periodontal inflammatory diseases.

Low and critical number of the studied periodontal pathogens was revealed respectively in the clinical subgroups of patients with clinically healthy gingiva on an intact periodontium (77.1% of cases) and on a reduced periodontium (22.9%). The clinical group of subjects with plaque-induced gingivitis was divided into 2 subgroups according to the detected low (57.1%) or critical (14.9%) number of the periodontal pathogens.

The clinical group of patients with Stage I periodontitis (initial) was also subdivided into 2 groups according to the detected low (34.3%) or critical (65.7%) number of the periodontal pathogens.

The performed Student t-test under the null hypothesis, which proposed there was no difference between mean values in two samples, showed that the samples of the clinical subgroups with clinically healthy gingiva on a reduced periodontium, plaque-induced gingivitis and Stage I periodontitis (initial) were statistically significantly different from the similar subgroups with low number of periodontal pathogens, p < 0.001.

The cytology of the gingival crevicular fluid impression smears revealed quantitative and qualitative changes regardless the clinical condition of the periodontium and the detected number of periodontal pathogens. Neutrophil leukocytes, histiocytes and epithelial cells were encountered in the impression smears of 22.9% cases in subjects with clinically healthy gingiva on a reduced periodontium (Fig. 3).

In plaque-induced gingivitis, polymorphonuclear neutrophils significantly increased, macrophages, histiocytes, epithelial cells appeared (Fig. 4).

The cytology of the gingival crevicular fluid impression smears, in initial periodontitis patients, stained by Romanowsky-Giemsa demonstrated the increase in epithelial cells of different maturity, decrease in macrophages which, in our opinion, testify the depression of the non-specific immune protection in response to the presence of periodontal pathogens (Fig. 5).

CONCLUSION

1. 22.9% of patients with clinically healthy gingiva complained of itching, spontaneous bleeding while brushing and

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eating hard food. Slight inflammation in the form of swelling, hyperemia or engorgement of the gingiva, changes in the hygiene and periodontal indices, X-ray changes, changes in the cellular composition of the gingival fluid and the critical number of periodontal pathogens in the studied sample were revealed which allowed verifying chronic inflammation and periodontal tissue loss in the clinically healthy gingiva.

2. In patients with plaque-induced gingivitis (42.85%) and initial periodontitis (65.71%), complaints of bleeding while brushing and eating hard food, occasional dull ache and gingival discomfort prevailed. The oral hygiene was bad, which was confirmed by Green-Vermillon index, which was over 2 points (ref. 0.0-0.6), and the prevalence of the following symptoms (according to the WHO): gingival bleeding, hard dental deposits, was low and moderate respectively. The depth of the periodontal pockets in initial periodontitis varied in the range of 3.3 ± 0.6 mm, the pockets were most frequently interproximal. In the samples of patients with plaque-induced gingivitis, PCR revealed a critical (14.9%) number of periodontal pathogens, an increase in polymorphonuclear neutrophils, macrophages, histiocytes, epithelial cells in the cellular composition of the gingival crevicular fluid. In the group of patients with initial periodontitis, PCR revealed a critical (65.7%) number of periodontal pathogens, a decrease in macrophages and an increase in epithelial cells of different maturity were detected in the cellular composition of the gingival crevicular fluid.

Thus, the comprehensive examination and laboratory result analysis of the studied subjects allowed diagnosing the clinical periodontal conditions – clinically healthy gingiva on a full periodontium, clinically healthy gingiva on a reduced periodontium, plaque-induced gingivitis, Stage I periodontitis (initial), which corresponded to the New International Classification of Periodontal and Peri-Implant Diseases and Conditions and Proceedings of the 2017 World Workshop of the American Association of Periodontology (AAP) and the European Federation of Periodontology (EFP).

The newly introduced criteria, used by the experts for the development of the New International Classification of Periodontal and Peri-Implant Diseases and Condition, are more informative and important for periodontists and other dental clinicians as well researchers, not only for determining the clinical periodontal condition but for early diagnosis.

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СВЕДЕНИЯ ОБ АВТОРАХ

Аль Кофиш Мохаммед Али Мохаммед, кандидат медицинских наук, заместитель декана стоматологического факультета Университета Адена, Аден, Йемен

E-mail: alqufaesh@gmail.com

ORCID: https://orcid.org/0000-0002-3065-6831

Автор, ответственный за связь с редакцией:

Усманова Ирина Николаевна, доктор медицинских наук, профессор кафедры терапевтической стоматологии Башкирского государственного медицинского университета, Уфа, Российская Федерация

Для переписки: irinausma@mail.ru

ORCID: https://orcid.org/0000-0002-1781-0291

Туйгунов Марсель Маратович, доктор медицинских наук, профессор, заведующий кафедрой микробиологии и вирусологии Башкирского государственного медицинского университета, Уфа, Российская Федерация

E-mail: tuygunov@mail.ru

ORCID: https://orcid.org/0000-0002-5473-2034

Хуснаризанова Рауза Фазыловна, кандидат медицинских наук, доцент кафедры микробиологии и вирусологии Башкирского государственного медицинского университета, Уфа, Российская Федерация

E-mail: roza.khusna@mail.ru

ORCID: https://orcid.org/0000-0002-1001-9587

Гумерова Марьям Ильдаровна, кандидат медицинских наук, доцент кафедры терапевтической стоматологии Башкирского государственного медицинского университета, врач стоматолог-терапевт стоматологической клиники «Мой стоматолог», Уфа, Российская Федерация

E-mail: maguil@mail.ru.

ORCID: https://orcid.org/0000-0001-5765-4273

Шангареева Алия Ирековна, ассистент кафедры терапевтической стоматологии Башкирского государственного медицинского университета. Врачстоматолог-пародонтолог клиники «Кремль-Дента», Уфа, Российская Федерация

E-mail: 180780_lena@mail.ru

ORCID: https://orcid.org/0000-0002-9673-1654

INFORMATION ABOUT THE AUTHORS

Mohammed A.M. Al-Qufaish, MD, PhD (Medicine), deputy dean of the dental school of University of Aden, Aden, Yemen

E-mail: alqufaesh@gmail.com

ORCID: https://orcid.org/0000-0002-3065-6831

Corresponding author:

Irina N. Usmanova, Dr. Sci. (Med.), Professor of the Department of therapeutic dentistry, Bashkir State Medical University, Ufa, Russian Federation

For correspondence: irinausma@mail.ru.

ORCID: https://orcid.org/0000-0002-1781-0291

Marcel M. Tuigunov, Dr. Sci. (Med.), Professor, Head of the Department of Microbiology and Virology, Bashkir State Medical University, Ufa, Russian Federation

E-mail: tuygunov@mail.ru

ORCID: https://orcid.org/0000-0002-5473-2034

Rausa F. Khusnarizanova, Ph.D. (Med.), Associate Professor of the Department of Microbiology and Virology, Bashkir State Medical University, Ufa, Russian Federation

E-mail: roza.khusna@mail.ru

ORCID: https://orcid.org/0000-0002-1001-9587

Maryam I. Gumerova, MD, PhD, Associate Professor, Bashkir State Medical University. Operative dentist of dental clinic «My dentist», Ufa, Russian Federation

E-mail: maguil@mail.ru

ORCID: https://orcid.org/0000-0001-5765-4273

Aliya I. Shangareeva, PhD, assistant professor Department of therapeutic dentistry, Bashkir State Medical University. Dentist-periodontist of dental clinic «Kremlin-Denta», Ufa, Russian Federation

E-mail: 180780_lena@mail.ru

ORCID: https://orcid.org/0000-0002-9673-1654

