

MICROBIOLOGICAL EFFECTS OF PERIODONTAL THERAPY PLUS AZITHROMYCIN IN PATIENTS WITH DIABETES: RESULTS FROM A RANDOMIZED CLINICAL TRIAL

Juan P. Hincapié¹, Cesar A. Castrillón¹, Fanny L. Yepes¹, Natalia Roldan¹, María A. Becerra¹, Sandra M. Moreno², Jessika Consuegra², Adolfo Contreras², Javier E. Botero¹

¹ Faculty of Dentistry, University of Antioquia, Medellín, Colombia.

² School of Dentistry, University del Valle, Cali, Colombia

ABSTRACT

Current evidence suggests that periodontal infection may aggravate diabetes control. The aim of this study was to determine the changes in the frequency with which *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola* and *Aggregatibacter actinomycetemcomitans* were detected in patients with diabetes with the use of non-surgical therapy plus azithromycin in a randomized clinical trial. One hundred and five (105) patients with diabetes and chronic periodontitis were randomly assigned to three treatment groups: subgingival mechanical therapy with azithromycin, subgingival mechanical therapy with placebo and supragingival prophylaxis with azithromycin. Complete periodontal clinical examinations and detection of periodontal pathogens

using polymerase chain reaction were carried out at baseline, 3, 6 and 9 months after periodontal therapy. The frequency with which *Porphyromonas gingivalis*, *Treponema denticola* and *Aggregatibacter actinomycetemcomitans* were detected decreased at 3 months in all groups. *Tannerella forsythia* increased after 3 months in all groups. All organisms had similar frequencies at 9 months in all groups. Subgingival mechanical therapy with adjunctive azithromycin had no additional effect on the frequency with which the periodontal pathogens investigated were detected in patients with diabetes.

Key words: diabetes mellitus; chronic periodontitis; microbiota, azithromycin

EFFECTOS MICROBIOLÓGICOS DE LA TERAPIA PERIODONTAL MÁS AZITROMICINA EN PACIENTES DIABÉTICOS: RESULTADOS DE UN ESTUDIO CLÍNICO CONTROLADO ALEATORIZADO

RESUMEN

Evidencia actual sugiere que la infección periodontal puede agravar el control de la diabetes. El objetivo de este estudio fue determinar los cambios en la frecuencia de detección de *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola* y *Aggregatibacter actinomycetemcomitans* en pacientes con diabetes con el uso de terapia mecánica no quirúrgica más azitromicina en un estudio clínico controlado aleatorizado. Ciento cinco pacientes (105) con diabetes y periodontitis fueron asignados aleatoriamente en tres grupos: terapia mecánica más azitromicina, terapia mecánica más placebo y profilaxis supragingival más azitromicina. Un análisis periodontal completo y detección de patógenos periodontales por medio de reacción en cadena de la polimerasa (PCR) se

realizaron al inicio, 3, 6 y 9 meses después de la terapia periodontal. La frecuencia de detección de *Porphyromonas gingivalis*, *Treponema denticola* y *Aggregatibacter actinomycetemcomitans* disminuyó a los 3 meses en todos los grupos. La frecuencia de detección de *Tannerella forsythia* se incrementó a los 3 meses en todos los grupos. Todos los patógenos periodontales mostraron una frecuencia de detección similar a los 9 meses en todos los grupos. La terapia periodontal más azitromicina no tuvo efectos adicionales sobre la frecuencia de detección de los patógenos periodontales investigados en pacientes diabéticos.

Palabras claves: diabetes mellitus; periodontitis crónica; microbiota; azitromicina

INTRODUCTION

Patients with poor glycemic control present increased loss in the levels of periodontal attachment (CAL) and in consequence higher tooth loss¹⁻³. Furthermore, type 1 and 2 diabetes patients are equally susceptible to further periodontal attachment and tooth loss

related to the degree of glucose control rather than to diabetes etiology^{2,3}. On the other hand, periodontitis may impair glucose control in patients with diabetes by promoting systemic inflammation⁴⁻⁶.

It has been observed that periodontal treatment has beneficial effects on patients with diabetes⁷. Non-

surgical periodontal treatment with or without the use of systemic antibiotics showed not only an improvement in periodontal parameters but also in the levels of glycated hemoglobin (HbA1C) in poorly controlled diabetes patients compared to placebo groups in recent randomized clinical trials^{8,9}. A reduction of 0.40% in HbA1C has been reported, but further studies are necessary to confirm these results¹⁰.

Studies have found that the presence of *P. gingivalis* after periodontal treatment was associated with poor glucose control¹¹. In a previous study, we found that the frequency of *P. gingivalis*, *Tannerella forsythia* and *Treponema denticola* (red complex microorganisms) was lower in patients with diabetes while the frequency of *A. actinomycetemcomitans* was higher than in non-diabetic patients¹². However, Yuan et al.¹³ found no difference in the subgingival microbiota between patients with and without diabetes. Further analysis of the subgingival microbiota in patients with diabetes could help improve periodontal treatment and further the understanding of periodontal-systemic relationships.

Results of studies have shown improvement in periodontal parameters as well as in the counts of red and orange complex microorganisms with the combined use of non-surgical therapy and azithromycin¹⁴. Azithromycin is a macrolide that has anti-inflammatory effects, accumulation at inflamed sites and slow release through periodontal pockets that could add beneficial effects to non-surgical therapy^{15,16}. To the extent of our knowledge there is no study addressing the use of azithromycin with periodontal therapy in subjects with diabetes.

The aim of this study was to determine changes in the frequency with which *P. gingivalis*, *T. forsythia*, *T. denticola* and *A. actinomycetemcomitans* were detected in patients with diabetes who were treated with non-surgical therapy plus azithromycin in a randomized clinical trial.

MATERIALS AND METHODS

Study Design

The microbiological results presented here are from a secondary data analysis of a randomized clinical trial (Clinical Trials NCT01271231) that was previously published⁹. The impact of periodontal intervention on periodontal parameters (probing depth, clinical attachment loss) and glycemic control (reduction of glycated hemoglobin and glycemia)

over a 9-month period have been analyzed elsewhere⁹. Briefly, the study was designed in accordance with the Declaration of Helsinki and approved by the Ethics Committee in Research of the Faculty of Dentistry (University of Antioquia 010-2011) and Hospital San Vicente Foundation (Medellín, Colombia). Potential candidates from the Hospital San Vicente Foundation were invited to participate in the study between January 2011 and July 2012. Volunteers were informed of the purpose of the study and screened for clinical parameters. Inclusion criteria included: ≥ 18 years of age, confirmed diagnosis of diabetes type I or II with duration of at least 2 years, ≥ 10 teeth present and chronic moderate periodontitis (ChP). ChP was defined as the presence of 2 or more interproximal sites with clinical attachment loss (CAL) ≥ 4 mm in different teeth, or, 2 or more interproximal sites with probing depth (PD) ≥ 5 mm in different teeth¹⁷. Exclusion criteria were administration of systemic antibiotics in the previous 3 months, any use of immunosuppressants or bisphosphonates, pregnancy, HIV, rheumatoid arthritis, cardiovascular disease or other systemic condition that contraindicated periodontal treatment. Patients were under treatment and control for diabetes at the hospital and received medications for diabetes (Insulin, Glibenclamide, Metformin) and other medications for hypertension (Enalapril, Captopril, Losartan) and cholesterol (Statins). The mean duration of diabetes in the volunteers was 13 years. Participants signed a written informed consent prior to the beginning of the study.

Sample, Allocation and Clinical Procedures

The original sample of 90 patients was calculated to detect changes of 0.7% in the values of glycated hemoglobin (HbA1C), with 0.05 significance and 85% power. To compensate for dropouts, the sample was increased by 10%. Patients were allocated to one of three groups by a block randomization process performed by one of the investigators (JEB) independent of the clinical intervention. The following groups were formed:

AZSRP group: azithromycin (MK, Cali, Colombia) 500mg/day x 3 days plus subgingival scaling using an ultrasonic device (Cavitron, Dentsply, York, PA, USA) at one session at medium intensity until the root surface was smooth. Patients were instructed to take one tablet (500 mg) per day during a three-day period.

PBSRP group: Placebo 500mg /day x 3 days plus subgingival scaling using an ultrasonic device at one session at medium intensity until the root surface was smooth. Patients were instructed to take one tablet (500 mg) per day during a three-day period.

AZPRO group: azithromycin 500mg/day x 3 days plus supragingival prophylaxis with polishing cups. Supragingival prophylaxis was repeated one week later. Patients were instructed to take one tablet (500 mg) per day during a three-day period. All patients assigned to AZPRO group received subgingival scaling at the end of study.

Azithromycin and placebo tablets were identical in shape and color and were given to patients in closed opaque coded envelopes with the corresponding instructions and contact number in the case of any adverse effects. Placebo tablets were manufactured by the Faculty of Pharmaceutical Chemistry (University of Antioquia, Medellin, Colombia). The type of antibiotic / placebo assigned was unknown by the patient and the clinicians who performed the interventions and clinical examinations.

A full periodontal examination was performed by 2 clinicians (JPH, CAC), who had been previously calibrated when clinical parameters were collected ($\kappa > 0.80$). PD, CAL and bleeding on probing (BOP) were measured at six sites around all teeth excluding third molars using a periodontal probe (UNC-15 USA Delta, Chicago, USA). Plaque index (PI) was measured with disclosing agent and establishing the percentage (%) of surfaces with plaque. HbA1C values (%) were also recorded. All parameters were measured at baseline 3, 6 and 9 months after periodontal interventions. Periodontal interventions were performed by 3 clinicians (FLY, NR, MAB). Any patient that presented worsening of periodontal or diabetes condition was removed from the study and treatment was initiated. Patients were contacted during the first week to ensure compliance with taking tablets and to check for any side effects: e.g. gastrointestinal problems, dizziness, etc.

Microbiological Analysis

Subgingival plaque samples were obtained from the 3 deepest sites by inserting sterile paper points to the bottom of the pocket for 30 seconds. The paper points were then transferred to Eppendorf vials and stored at -70°C until they were analyzed. DNA was extracted by the methods described by Botero et al.¹⁸. *P. gingivalis*, *T. forsythia*, *T. denticola* and *A. acti-*

nomycetemcomitans were detected by polymerase chain reaction (PCR) using primers and conditions described elsewhere¹⁹. Data were expressed as positive or negative and the frequency (%) of each microorganism was determined. Microbial samples were obtained at baseline and then at 3, 6 and 9 months after periodontal interventions.

Statistical Analysis

Demographic and clinical data are presented as mean \pm SD unless otherwise indicated. To test for differences in clinical parameters, the paired and unpaired *t* test and Mann-Whitney test were used when appropriate. The primary outcome was the change in the detection of each microorganism and this was assessed with ANOVA and Chi² where indicated. Statistical software (GraphPad Prism 5, GraphPad Software, San Diego, CA, USA) was used and statistical significance was assumed when $P \leq 0.05$.

RESULTS

Figure 1 shows the flowchart of patient inclusion in the study. Periodontal clinical parameters and HbA1C values were similar in all the allocated groups. There were more female patients participating in the study but they were balanced in all groups (Table 1). No side effects were reported in this study. Changes in the frequency of detection of red complex microorganisms and *A. actinomycetemcomitans* are presented in Table 2. The frequency of detection of *T. forsythia* tended to increase at 3

Table 1: Demographic and clinical description of the study sample.

	AZSRP	AZPRO	PBSRP
Number of patients	33	35	37
Gender (M/F)	11/22	10/25	10/27
Age (years \pm SD)	55.9 \pm 12.6	56.1 \pm 11.3	58.2 \pm 11.1
Number of teeth present (mean \pm SD)	20.9 \pm 5.0	19.7 \pm 6.6	21.1 \pm 4.7
BOP (% \pm SD)	52.4 \pm 26.0	43.8 \pm 24.1	45.7 \pm 27.2
Mean PD (mm \pm SD)	2.7 \pm 0.6	2.4 \pm 0.6	2.6 \pm 0.7
Mean CAL (mm \pm SD)	2.8 \pm 0.8	2.9 \pm 1.1	3.1 \pm 1.1
Mean HbA1C (% \pm SD)	7.5 \pm 1.9	7.9 \pm 2.4	7.7 \pm 2.0

M: male; F: female; SD: standard deviation; BOP: bleeding of probing. PD: probing depth, CAL: clinical attachment level, HbA1C: glycated hemoglobin. AZSRP: SRP plus azithromycin group; AZPRO: supragingival prophylaxis plus azithromycin; PBSRP: SRP plus placebo.

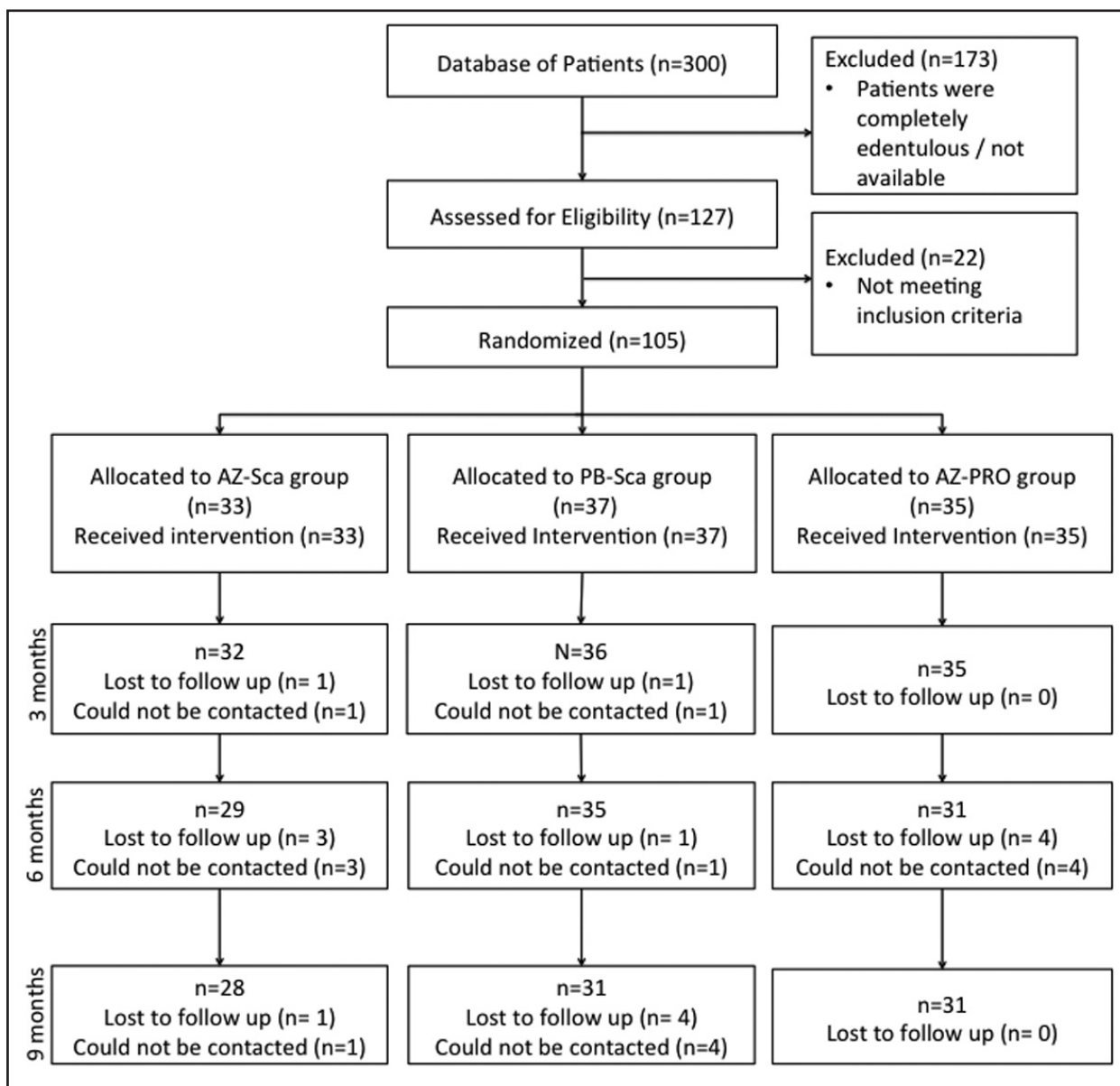


Fig.1: Flowchart of patient inclusion in the study.

Table 2: Changes in the detection frequency (%) of periodontal pathogens before and after interventions.

Microorganisms	AZSRP				AZPRO				PBSRP			
	Baseline	3mo	6mo	9mo	Baseline	3mo	6mo	9mo	Baseline	3mo	6mo	9mo
<i>T. forsythia</i> (%)	8/33 (24.2)	13/32 (40.6)	9/29 (31)	2/28 (7.1)	7/35 (20)	8/35 (22.9)	8/31 (25.8)	1/31 (3.2)	12/37 (32.4)	15/37 (40.5)	16/35 (45.7)	9/31 (19.4)
<i>P. gingivalis</i> (%)	15/33 (45.5)	4/32 (12.5)*	6/29 (20.7)	7/28 (25)	5/35 (14.3)	0/35 (0)	6/31 (19.4)	10/31 (32.3)	10/37 (27)	0/37 (0)	8/35 (22.9)	11/31 (35.5)
<i>T. denticola</i> (%)	9/33 (27.3)	3/32 (9.4)	4/29 (13.8)	8/28 (28.6)	3/35 (8.6)	0/35 (0)	1/31 (3.2)	7/31 (22.6)	13/37 (35.1)	5/37 (13.5)	6/35 (17.1)	13/31 (41.9)
<i>A. actinomycetemcomitans</i> (%)	5/33 (15.2)	1/32 (3.1)	0/29 (0)	0/28 (0)	11/35 (31.4)	1/35 (2.9)	1/31 (3.2)	7/31 (22.6)	6/37 (16.2)	1/37 (2.7)	0/35 (0)	2/31 (6.5)

AZSRP: SRP plus azithromycin group; AZPRO: supragingival prophylaxis plus azithromycin; PBSRP: SRP plus placebo. * P<0.05 Chi².

Table 3: Changes in clinical attachment levels before and after interventions.

Groups		Clinical Attachment Level (CAL)					
		Number of sites CAL 1-3mm		Number of sites CAL 4-5mm		Number of sites CAL ≥6mm	
		Mean	SD	Mean	SD	Mean	SD
AZSRP	Baseline	97.5	41.3	21.1	13.0	21.1	13.0
	3mo	101.5	42.7	19.7	14.8	19.7	14.8
	6mo	101.6	40.7	18.9	15.1	18.9	15.1
	9mo	101.3	41.2	20.1	12.8	20.1	12.8
AZPRO	Baseline	90.3	47.5	21.9	15.4	21.9	15.4
	3mo	91.1	46.9	21.7	14.7	21.7	14.7
	6mo	92.2	46.4	23.1	21.5	23.1	21.5
	9mo	96.4	48.6	20.1	14.9	20.1	14.9
PBSRP	Baseline	90.2	43.9	25.0	17.8	25.0	17.8
	3mo	91.2	43.3	25.2	17.6	25.2	17.6
	6mo	90.3	44.3	23.6	16.5	23.6	16.5
	9mo	94.0	42.0	23.9	16.8	23.9	16.8

AZSRP: SRP plus azithromycin group; AZPRO: supragingival prophylaxis plus azithromycin; PBSRP: SRP plus placebo.

months in all groups. Nonetheless, the placebo group presented higher frequencies of *T. forsythia* at 9 months than the other groups. In contrast, *P. gingivalis*, *T. denticola* and *A. actinomycetemcomitans* were detected less frequently at 3 months and then increased progressively over the 9-month period. The frequency of detection of all periodontal pathogens studied was similar at 9 months.

Table 3 depicts the changes in clinical attachment levels. An improvement in CAL was observed in the AZSRP and PBSRP groups, while no improvement was observed in the AZPRO group (Table 3). Changes in glycated hemoglobin values are presented in Table 4. A reduction in HbA1C was observed in the AZSRP and PBSRP groups over the 9-month follow-up. However, the reduction was greater in the AZSRP group. No improvement was observed in the AZPRO group.

Discussion

The subgingival microbiota associated to the development of periodontitis is frequently composed of microorganisms of the red complex (*T. forsythia*, *P. gingivalis* and *T. denticola*) and *A. actinomycetemcomitans*¹⁹. While it is accepted that diabetes affects the progression of periodontitis, there is no consensus on how diabetes affects the subgingival microbiota. In previous studies, these microorganisms were detected in similar proportions as in systemically healthy patients with periodontitis¹¹⁻¹⁴. The establishment of chronic subgingival infection in

Table 4: Changes in HbA1C values (%) before and after interventions.

Groups		HbA1C (%)	
		Mean	SD
AZSRP	Baseline	8.1	2.3
	3mo	7.3	1.7
	6mo	7.7	1.9
	9mo	7.3	1.7
AZPRO	Baseline	7.9	2.0
	3mo	8.1	2.6
	6mo	8.1	2.4
	9mo	7.8	2.0
PBSRP	Baseline	8.0	2.5
	3mo	7.4	1.7
	6mo	7.9	1.7
	9mo	7.8	2.4

AZSRP: SRP plus azithromycin group; AZPRO: supragingival prophylaxis plus azithromycin; PBSRP: SRP plus placebo.

patients with diabetes and periodontitis may preclude the appropriate control of glycemia¹¹. Hence, treatment of periodontitis and infection is an important objective in patients with diabetes.

Azithromycin has been suggested for the treatment of periodontitis due to its antimicrobial activity, high compliance protocol, anti-inflammatory activity and persistence at low levels in macrophages and fibroblasts with good clinical results^{20,21}. To the extent of our knowledge, this is the first RCT to assess the microbiological effects of azithromycin as adjunct to periodontal mechanical therapy in

patients with diabetes. All microorganisms analyzed in this study showed a marked reduction at 3 months except for *T. forsythia*. Overall, the reduction in the frequency detection of red complex microorganisms and *A. actinomycetemcomitans* was slightly better with the adjunctive use of azithromycin at 3 and 6 months. The frequency of detection of these microorganisms was similar at 9 months. Due to limited information regarding the use of azithromycin in patients with diabetes and periodontitis, the results observed in this study are comparable to other studies with chronic and aggressive periodontitis²²⁻²⁴. However, this study is limited since a non-quantitative PCR technique was used to detect the microorganisms and hence it is not possible to determine changes in the bacterial counts. It would be relevant to test whether small changes in the microbiota are related to changes in clinical parameters and in this case, glycemic control in patients with diabetes and periodontitis. The studies by Han et al.²³ and Emingil et al.²⁴ showed that there is no significant difference in the counts of *A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythia* and clinical parameters when compared to SRP and placebo in otherwise healthy subjects. It is not known whether the systemic condition of our patients may have influenced the results. In contrast, the use of the combination of amoxicillin and metronidazole appears to provide the best clinical and microbiological results available²⁵. Differences in the pharmacological properties of different antibiotics may account for these differences. The microbiological adjunctive effects of azithromycin

for the treatment of periodontitis remain to be elucidated.

Although the detection of red complex and *A. actinomycetemcomitans* was similar in the AZSRP and PBSRP groups, clinical parameters and glycemic control showed an improvement when azithromycin was used as adjunct to mechanical therapy in patients with diabetes and this is analyzed and reported in a previous article⁹. Despite the moderate beneficial effects, it is possible that the anti-inflammatory effects of azithromycin helped to reduce periodontal inflammation independently of high microbial suppression and consequently improved the values in HbA1C²¹. Further studies are needed to understand the immuno-modulatory effects of azithromycin and its consequences on periodontal parameters.

One shortcoming of this study is attrition of the study sample as results of patients lost to follow-up at 9 months. However, most beneficial effects are believed to occur within the first 6 months after therapy and results may have been maintained. In this study, variables other than the antimicrobial effects of azithromycin such as diabetes and medical control and periodontal maintenance may have an influence on the results. Long-term studies on larger samples are necessary to comprehend the results of periodontal therapy in patients with diabetes.

The results from the present RCT indicates that SRP therapy with adjunctive azithromycin had no additional effect compared to SRP and placebo alone on the frequency of detection of the periodontal pathogens investigated in patients with diabetes.

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REFERENCES

1. Botero JE, Yepes FL, Roldán N, Castrillón CA, Hincapié JP, Ochoa SP, Ospina CA, Becerra MA, et al. Tooth and periodontal clinical attachment loss are associated with hyperglycemia in patients with Hyperglycemia in Diabetic Patients. *J Periodontol* 2012;83:1245-1250.
2. Demmer RT, Holtfreter B, Desvarieux M, Jacobs DR Jr, Kerner W, Nauck M, Völzke H, Kocher T. The influence of type 1 and type 2 diabetes on periodontal disease progres-

CORRESPONDENCE

Dr. Javier Enrique Botero,
Facultad de Odontología, Universidad de Antioquia,
carrera 70 No. 52-21, Medellín, Colombia.
e-mail: drjavo@yahoo.com

- sion: prospective results from the Study of Health in Pomerania (SHIP). *Diabetes Care* 2012;35:2036-2042.
3. Ochoa SP, Ospina CA, Colorado KJ, Montoya YP, Saldarriaga AF, Miranda Galvis M, Muñoz Pino N, Gómez ME, Yepes FL, Botero JE. Periodontal condition and tooth loss in diabetic patients. *Biomedica* 2012;32:52-59.
4. Zadik Y, Bechor R, Galor S, Levin L. Periodontal disease might be associated even with impaired fasting glucose. *Br Dent J* 2010; 208: E20.

5. Choi YH, McKeown RE, Mayer-Davis EJ, Liese AD, Song KB, Merchant AT. Association between periodontitis and impaired fasting glucose and diabetes. *Diabetes Care* 2011; 34:381-386.
6. Gurav AN. Periodontitis and insulin resistance: casual or causal relationship?. *Diabetes Metab J* 2012; 36: 404-411.
7. Sgolastra F, Severino M, Pietropaoli D, Gatto R, Monaco A. Effectiveness of periodontal treatment to improve metabolic control in patients with chronic periodontitis and type 2 diabetes: a meta-analysis of randomized clinical trials. *J Periodontol* 2013;84:958-973.
8. Moeintaghavi A, Arab HR, Bozorgnia Y, Kianoush K, Alizadeh M. Non-surgical periodontal therapy affects metabolic control in diabetics: a randomized controlled clinical trial. *Aust Dent J* 2012;57:31-37.
9. Botero JE, Yepes FL, Ochoa SP, Hincapie JP, Roldan N, Ospina CA, Castrillon CA, Becerra MA. Effects of periodontal non-surgical therapy plus azithromycin on glycemic control in patients with diabetes: a randomized clinical trial. *J Periodontal Res* 2013;48:706-712.
10. Teeuw WJ, Gerdes VE, Loos BG. Effect of periodontal treatment on glycemic control of diabetic patients: a systematic review and meta-analysis. *Diabetes Care* 2010;33:421-427.
11. Makiura N, Ojima M, Kou Y, Furuta N, Okahashi N, Shizukuishi S, Amano A. Relationship of Porphyromonas gingivalis with glycemic level in patients with type 2 diabetes following periodontal treatment. *Oral Microbiol Immunol* 2008;23:348-351.
12. Castrillon CA, Hincapie JP, Yepes FL, Roldan N, Moreno SM, Contreras A, Botero JE. Occurrence of red complex microorganisms and Aggregatibacter actinomycetemcomitans in patients with diabetes. *J Invest Clin Dent* 2013 Jul 16. doi: 10.1111/jicd.12051. [Epub ahead of print].
13. Yuan K, Chang CJ, Hsu PC, Sun HS, Tseng CC, Wang JR. Detection of putative periodontal pathogens in non-insulin-dependent diabetes mellitus and non-diabetes mellitus by polymerase chain reaction. *J Periodontal Res* 2001;36:18-24.
14. Smith SR, Foyle DM, Daniels J, Joyston-Bechal S, Smales FC, Sefton A, Williams A. A double-blind placebo-controlled trial of azithromycin as an adjunct to nonsurgical treatment of periodontitis in adults: clinical results. *J Clin Periodontol* 2002;29:54-61.
15. Ernst EJ, Klepser ME, Klepser TB, Nightingale CH, Hunsicker LG. Comparison of the serum and intracellular pharmacokinetics of azithromycin in healthy and diabetic volunteers. *Pharmacotherapy* 2000;20:657-661.
16. Gomi K, Yashima A, Lino F, Kanazashi M, Nagano T, Shibukawa N, Ohshima T, Maeda N, Arai T. Drug concentration in inflamed periodontal tissues after systemically administered azithromycin. *J Periodontol* 2007;78:918-923.
17. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. *J Periodontol* 2007;78 Suppl 7:1387-1399.
18. Botero JE, Vidal C, Contreras A, Parra B. Comparison of nested polymerase chain reaction (PCR), real-time PCR and viral culture for the detection of cytomegalovirus in subgingival samples. *Oral Microbiol Immunol* 2008;23: 239-244.
19. Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol* 1996;11:266-273.
20. Heitz-Mayfield LJ. Systemic antibiotics in periodontal therapy. *Aust Dent J* 2009; 54 Suppl 1:S96-101.
21. Hirsch R, Deng H, Laohachai MN. Azithromycin in periodontal treatment: more than an antibiotic. *J Periodontal Res* 2012;47:137-148.
22. Oteo A, Herrera D, Figuero E, O'Connor A, González I, Sanz M. Azithromycin as an adjunct to scaling and root planing in the treatment of Porphyromonas gingivalis-associated periodontitis: a pilot study. *J Clin Periodontol* 2010; 37:1005-1015.
23. Han B, Emingil G, Özdemir G, Tervahartiala T, Vural C, Atilla G, Baylas H, Sorsa T. Azithromycin as an adjunctive treatment of generalized severe chronic periodontitis: clinical, microbiologic, and biochemical parameters. *J Periodontol* 2012; 83:1480-1441.
24. Emingil G, Han B, Ozdemir G, Tervahartiala T, Vural C, Atilla G, Baylas H, Sorsa T. Effect of azithromycin, as an adjunct to nonsurgical periodontal treatment, on microbiological parameters and gingival crevicular fluid biomarkers in generalized aggressive periodontitis. *J Periodontal Res* 2012;47:729-739.
25. Powell LV. Amoxicillin/metronidazole therapy may improve the effectiveness of scaling and root planing in patients with periodontitis. *J Am Dent Assoc* 2013;144:640-642.