J Oral Res
research in oral sciences

ARTICLE

EFFECT OF PERIODONTAL DEBRIDEMENT PLUS SYSTEMIC AZITHROMYCIN IN SUBJECTS WITH STAGE III PERIODONTITIS: A RANDOMIZED CONTROLLED CLINICAL TRIAL.

Efecto del desbridamiento periodontal más azitromicina sistémica en sujetos con periodontitis estadio III: Un ensayo clínico controlado aleatorio.

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CITE AS:
Navarrete M, Oñate H, Loyola K & Olivares P.
Effect of Periodontal Debridement plus Systemic Azithromycin in subjects with Stage III Periodontitis: A Randomized Controlled Clinical Trial.
J Oral Res.2022;11(5):1-16.
doi:10.17126/joralres.2022.051

ABSTRACT:

Aim: To evaluate the effect of the systemic administration of azi-thromycin (AZM) as an adjunct to non-surgical periodontal therapy (NSPT) on the clinical and microbiological variables of patients with periodontitis

Material and Methods: Eighteen volunteers received NSPT combined with placebo or AZM (500 mg/day) for 3 days (n=9/group). They were monitored clinically for probing pocket depth (PPD), clinical attachment level (CAL), O'Leary index (OI), bleeding on probing (BoP) at baseline and during the first, third and sixth month and microbiologically, at baseline and at 3 and 6 months after therapy, by conventional polymerase chain reaction tests.

Results: Fourteen patients completed the study (n=7/group). Differences statistically significant were observed among both groups. The experimental group presented: A PPD mean (p=0.04) significantly lower and PPD reduction (p=0.02), at 6-months post NSPT. Regarding changes (Δ), at the third month post NSPT, there was a significant increase in the number of shallow sites (p<0.001) and a decrease in the intermediate sites (p<0.001). In addition, a significant decrease in the mean number of deep sites (p= 0.04) was detected at 6 months post treatment. There was also a significant decrease in periodontal index BoP at 1 (p=0.01), 3 (p<0.001) and 6 (p=0.01) months and OI at 3- and 6-months (p<0.001), post treatment. Regarding the presence of periodontal pathogens, no significant differences were observed, intra and inter groups.

Conclusion: AZM as an adjuvant to NSPT provides additional beneficial effects for PPD and BoP compared to NSPT alone.

KEYWORDS:

Azithromycin; periodontal debridement; Root Planing; Periodontal index; Periodontitis; Periodontal diseases.

Received: October 31, 2021 | **Accepted:** June 29,2022 | **Published online:** 30 October 2022.

RESUMEN:

Objetivo: Evaluar el efecto de la administración sistémica de azitromicina (AZM) como coadyuvante de la terapia periodontal no quirúrgica (TPNQ) en las variables clínicas y microbiológicas de pacientes con periodontitis.

Material y Métodos: Dieciocho voluntarios recibieron TPNQ combinado con placebo o AZM (500 mg/día) durante 3 días (n=9/grupo). Fueron monitoreados clínicamente para determinar Profundidad de Sondaje del Saco (PSS), Nivel de Inserción Clínica (NIC), Indice de O'Leary (IO), Sangrado al sondaje (SS) al inicio y durante el primer, tercer y sexto mes y microbiológicamente, al inicio y a los 3 y 6 meses después de la terapia, mediante la reacción en cadena de la polimerasa convencional.

Resultados: Catorce pacientes completaron el estudio (n=7/grupo). Se observaron diferencias estadísticamente significativas entre ambos grupos. El grupo experimental presentó una media de PSS significativamente menor (p=0,04) y una reducción de PSS (p=0,02), a los 6 meses post TPNQ. En cuanto al delta (Δ) pre y post tratamiento, al tercer mes post TPNQ, hubo un aumento significativo en el número de sitios poco profundos (p<0.001) y una disminución en los sitios intermedios (p<0.001). Además, se detectó una disminución significativa en la media de los sitios profundos (p=0.04) a los 6 meses post tratamiento. También hubo una disminución significativa en el indice SS al primer (p=0.01), tercer (p<0.001) y sexto mes (p=0.01) post TPNQ y del IO al tercer y sexto mes (p<0.001), post tratamiento. En cuanto a la presencia de patógenos periodontales, no se observaron diferencias significativas tanto intra como ínter grupos.

Conclusión: AZM como adyuvante a TPNQ proporciona efectos benéficos adicionales en la PSS y SS en comparación a TPNQ solo.

PALABRAS CLAVE:

Azitromicina; Desbridamiento periodontal; Pulido radicular; Índice periodontal; Periodontitis; Enfermedades periodontales.

INTRODUCTION.

Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic dental biofilms.[1] Its primary features include clinical attachment loss (CAL), alveolar bone loss, the presence of probing pocket depth (PPD), and bleeding on probing (BoP). It is the most common chronic inflammatory non-communicable disease of humans and is a major public health problem due to its high global prevalence.[1]

Since it may lead to tooth loss and disability, it negatively affects chewing function and aesthetics, which generates social inequality and significantly impairs quality of life.[2]

The recently introduced 2017 World Workshop on the classification of periodontitis, incorporates stages (I, I, III, IV) and grades (A, B, C) of disease.[1] Stage III refers to severe Periodontitis with potential for additional tooth loss and is defined by complexity and severity factors. For each stage, extent as localized (< 30% of teeth involved), generalized or, molar/incisor pattern is described. Grade B indicates a moderate level of disease progression, defined by primary criteria and grade modifiers.[1] Periodontitis can in many cases be treated using non-surgical periodontal therapy (NSPT).[3]

However, it has been demonstrated that access to deeper pockets to provide adequate root surface debridement is difficult, and therefore the results of non-surgical therapy for pockets of ≥7mm are less predictable.[3] Moreover, it is recognized that there are differences in an individual's susceptibility to severe periodontal disease.[3,4] This situation could also explain why the results obtained with NSPT are sometimes not successful.[3]

It has been proposed that the use of systemic

antibiotics may enhance the results of NSPT.[5] The most studied are tetracycline, minocycline, metronidazole, and metronidazole and amoxicillin combinations.[5]

Past research has reported positive results with each of these adjuncts; however, other studies have indicated that there is no additional benefit.[3] Recently, it has been suggested that azithromycin (AZM) may be a useful adjunct to NSPT.

Is an antibiotic of the macrolide family, semi-synthetic analogue of erythromycin, used to treat infections of the upper respiratory tract and middle ear, and sexually transmitted infections, among others.[3] It effective against the putative periodontal pathogens, it presents few gastrointestinal tract complications, and a simplified dosage regimen (500mg once a day for three or five days).[6]

However, the versatility of AZM goes beyond its antibiotic properties, since it possesses immunomodulatory properties and anti-inflammatory effects both *in vivo* and *in vitro*.[7,8] Researchers have suggested that its efficacy is due to effects on the innate and adaptive immune response, modifying the production of cytokines and pro-inflammatory mediators.[7-9] In addition to having a long half-life, AZM achieves higher concentrations in periodontal tissues than in plasma. It accumulates at high concentrations in macrophages, fibroblasts[7] and neutrophils,[9] key cells in the pathogenesis of periodontitis. The use of azithromycin as a coadjuvant for the treatment of periodontitis is widely supported in the literature.[13]

However, due to the overuse of antibiotics, and the development of bacterial resistance, its routine administration as an adjunct to NSPT is not recommended,[1] and it could be indicated in specific situations.[14] According to all the above, the current evidence is not conclusive in relation to its efficacy as an adjunct of NSPT. Consequently, the aim of this study was to assess the effect of the systemic administration of AZM as an adjunct to NSPT on the clinical and microbiological variables of patients with periodontitis, stage III, generalized, grade B.

MATERIALS AND METHODS.

Study Design

This was a 6-month, double-blind, randomized controlled trial with two arms and triple masking (participant, care provider, and outcomes assessor). The study protocol was approved by the Dentistry Faculty's Scientific Ethics Committee of the Andres Bello University's (UNAB) (Decision No. 038) and was registered at *http://www.clinicaltrials.gov* as NCT03629288.

It followed the Consolidated Standards of Reporting Trials (CONSORT) guidelines as well as the Helsinki Declaration for human research, as revised in 2013. The study was conducted by the principal investigator (MN) and secondary investigators (KL and PO).

Subject population

Thirty-six patients with periodontitis, stage III, generalized, grade B, were screened from the Diagnostic Unit of the UNAB School of Dentistry, Viña del Mar, and a total of 18 subjects (eight men and ten women) who met the inclusion and exclusion criteria were recruited for the study.

The chosen participants were informed of the nature of the research, the potential risks and the compensation for participating in the study, and informed consent was obtained from each patient. The patients were chosen according to inclusion and exclusion criteria (Table 1).

The average age was 50.83 years and they did not present statistically significant differences in the baseline registered clinical variables (Table 2).

Sample Size Calculation

To calculate the minimum sample size necessary for the groups, the variance of PPD differences before and after the intervention were considered as fixed values and a standard deviation (SD) of 1.0 mm. The study by Sampaio *et al.*,[10] was used to obtain mean probing depth difference ± SD for the control (placebo) and experimental (AZM) group. Based on these calculations, it was defined that 7 subjects per group would be necessary to provide a level of significance of 0.05, a statistical power of 80% and an estimation error of 1mm.

ISSN Print 0719-2460 - ISSN Online 0719-2479.

Considering an attrition of approximately 15%, nine subjects were included in each group.

Randomization

All subjects were assigned via randomization using the Epidat 4.0 program, with age-matched control and intervention groups. Two groups were formed for investigation: an experimental group that received NSPT plus AZM and a control group that received NSPT plus placebo, under the same conditions as the intervention group.

The patients, the examiner, the operators who performed the periodontal treatment, and the statistician did not know which subject was assigned to each study group. Only the main investigator (MN) who assigned the randomization had knowledge of the group to which each patient belonged in the study and of the contents of the medication containers. He, therefore, was in charge of labeling the containers. To maintain a double-blind condition, the containers used for the azithromycin and placebo presented the same characteristics of size and color. The AZM tablets and placebo tablets were visually identical.

Examiner calibration

As only one investigator (KL) performed the periodontal examination, an intra-examiner reliability assessment was conducted. This examiner (intra-class correlation coefficient of 0.96 and 0.95 for PPD and CAL) recorded all stipulated variables in a clinical file designed especially for this study. Microbiological sampling and the NSPT were performed by the other investigator (PO).

For standardization, measurements were performed under the same conditions using the same type of instrument in order to reduce any associated bias. The instruments used for data collection were all the same design and brand and consisted of a Basic Examination Kit: Mirror, caries probe, tweezers and a manual periodontal probe (North Carolina Probe, Hu-Friedy® Manufacturing Inc., Chicago, IL, USA); plaque disclosing tablets (Curaprox © CURADEN AG, Switzerland) and number 40 sterile paper cones (Johnson & Johnson, Tokyo, Japan).

Periodontal examination

Before data collection, all subjects belonging to the study were asked for a panoramic radiograph. The following clinical variables were measured: PPD (distance in mm from the gingival margin to the bottom of the sulcus/pocket). "Risk for disease progression" was defined at the patient level according to Lang and Tonetti.[11] Low risk was defined as ≤ 4 sites with PPD ≥ 5 mm, moderate risk was defined as 5–8 sites with PD ≥ 5mm, and high risk was defined as ≥ 9 sites with PD ≥ 5mm.[11] BoP (presence of immediate bleeding or up to 30 seconds after inserting the manual periodontal probe into the periodontal pocket, during the PPD measurement). CAL (distance in mm from the cement-enamel junction to the bottom of the sulcus/pocket).

O'Leary index (presence of bacterial plaque on tooth surfaces that were related to the gingival margin). Bop, PPD and CAL were measured at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual/palatine, lingual/palatine and mesiolingual/palatine).

O'Leary index (OI) was measured at four surfaces per tooth (buccal, mesial, lingual/palatine, distal). All teeth were evaluated, excluding the third molars. The PPD and CAL measurements were recorded to the nearest millimeter using a periodontal manual probe.

Bop and OI were recorded as a percentage of sites that bled when probing and the percentage of dental surfaces with staining after using bacterial plaque finder tablets. Patients were clinically monitored at baseline, 1-, 3- and 6-months post NSPT, and periodontal maintenance was performed.

Microbiological monitoring

After the clinical parameters had been recorded, a sample of the subgingival biofilm was taken by a single trained operator (PO) from the site with the highest CAL, PPD ≥5 mm, BoP and suppuration. First, the chosen area was isolated with cotton rolls and gently air dried.

Then, the supragingival deposits were carefully removed with sterile gauze. Subsequently, the

samples were obtained by inserting two standardized No. 40 sterile paper cones into the deepest part of the periodontal pocket for 20 seconds to ensure the absorption of the crevicular fluid and subgingival biofilm. The microbiological variables were measured again in the third and sixth month after NSPT.

Each biological sample obtained was suspended in an Eppendorf tube with 1ml of distilled water and left in a 4°C container. Immediately, the samples were transported to the Laboratory of the Faculty of Life Sciences UNAB and were stored at −80°C, until the subsequent extraction of deoxyribonucleic acid (DNA). This procedure was performed following the kit manufacturer's protocol (Promega Corporation, Madison, WI, USA). The time between sampling and DNA extraction did not exceed 48 h to avoid any deterioration of the biological material.

After DNA extraction, the detection of Porphyromona gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td), and Fusobacterium nucleatum (Fn) was performed by amplifying a fragment of the 16S rDNA gene following the manufacturer's recommendations by polymerase chain reaction (PCR) (Promega Corporation, Madison, WI, USA). Specific primers for each bacterium were used. For the reaction mixes, 0.2ml Eppendorf microtubes were used in a 25µL final volume protocol. Each tube contained 12.5µL of the GoTaq® Green Master Mix (Promega Corporation, Madison, WI, USA), 0.5µL of forward primer, 0.5µL of reverse primer, 6µL of nuclease-free water and 5.5µL of DNA template.

PCR

The sample was briefly homogenized and given a quick spin to settle the contents. Then, the tubes were deposited in the thermocycler, which was programmed with a cycle of 94°C for 5 min, followed by 36 cycles of 94°C for 30 seconds, 57°C for 30 seconds and 72°C for 30 seconds, followed by an extension cycle of 72°C for 10 min, and finally maintained at 4°C, to obtain the amplified PCR product.

The PCR products were separated on a 1.5% agarose gel by electrophoresis for 60 min at 100 volts, and bands were visualized under ultraviolet light. Finally, the image was captured and documented for analysis.

Periodontal intervention

Once the microbiological sample was obtained, all subjects were instructed in oral hygiene methods using a soft, straight filament toothbrush with a small head, interdental brushes and dental floss. The brushing technique used was modified Bass. Subsequently, they received one-stage full-mouth scaling and root planing (FM-SRP) performed under local anesthesia in one or two appointments of approximately 2 h each, over a maximum period of 24 h. NSPT was performed by a single trained operator (KL), using an ultrasonic scaler (DTE®, Guilin Woodpecker Medical Instrument Co., Ltd., Guilin, Guangxi, P.R. China) and hand instruments (Gracey Curettes. Hu-Friedy® Manufacturing Inc., Chicago, IL, USA). At the end of the last treatment session, 500 mg of AZM (azithromycin, Laboratorio Chile) once a day for three days immediately after the NSPT or placebo (lactose, Galenica Pharmacy, Chile) under the same conditions were administered depending on the group to which the patient was assigned, intervention or control, respectively.

Compliance and adverse events monitoring

On the last day of medication, the subjects were asked to return to the clinic and bring the medication bottles, which were checked for any possible remaining pills. During this visit and at the following follow-up appointments, subjects answered a questionnaire about any self-perceived side-effects of the medication/placebo. One study investigator (KL) conducted this inquiry and was also responsible for calling the subjects every day to monitor compliance.

The intervention activities carried out are summarized in a flow chart (Figure 1).

Primary and secondary outcome variables

Primary outcome variable was the difference between groups of sites with PPD 1mm – 3mm, PPD 4–6 mm and PPD ≥ 7 mm.

ISSN Print 0719-2460 - ISSN Online 0719-2479.

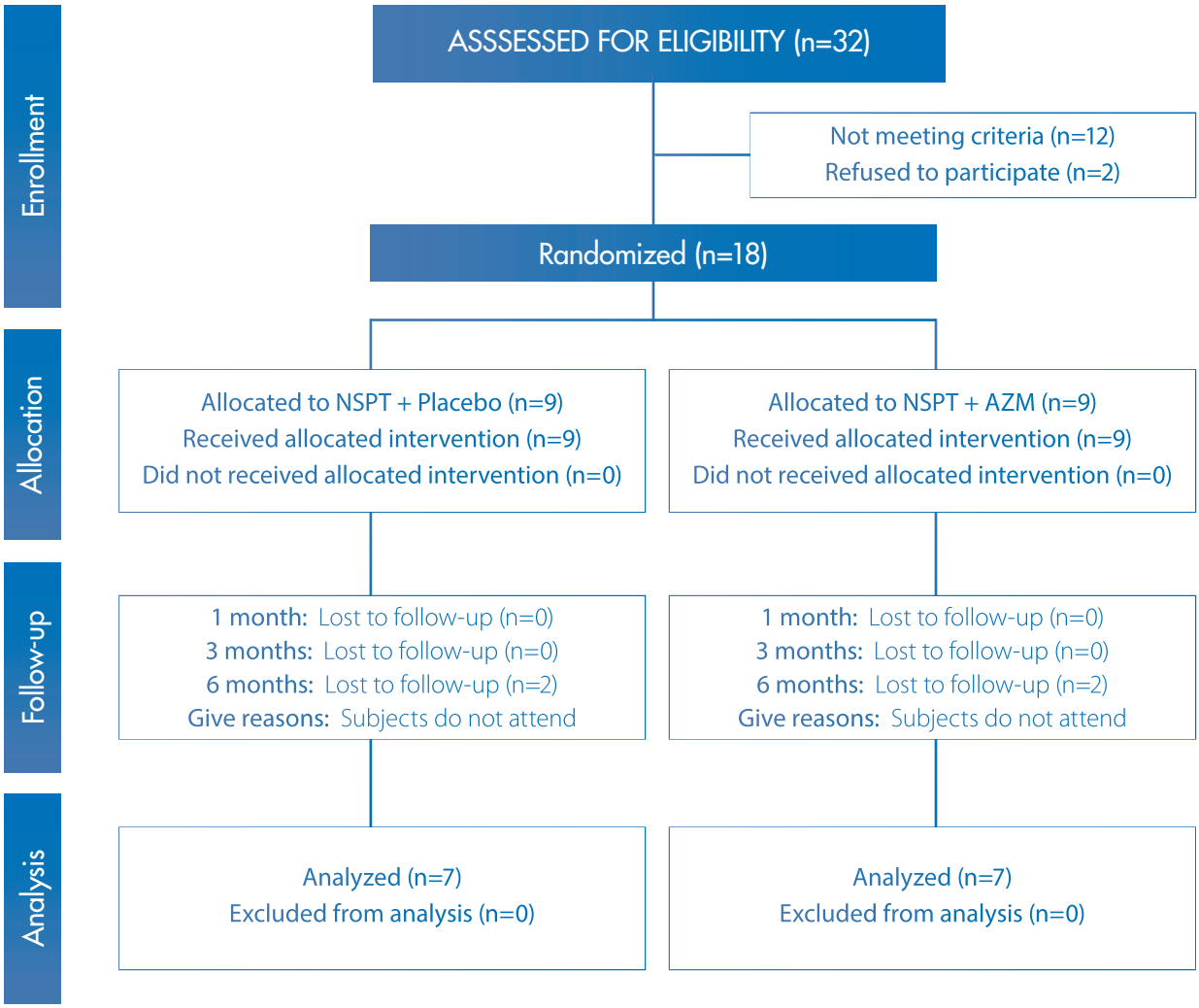
Secondary outcome variables were differences between groups for: number of subjects with low, moderate and high risk for disease progression according to Periodontal risk assessment.[11] CAL gain, BoP, PI and presence of periodontopathogens.

Statistical Analysis

A descriptive analysis was performed in which the qualitative variables were studied by frequency, while the quantitative ones were analyzed by averages. For each variable, a data normality was tested using the Shapiro-Wilk test. Afterwards, an inferential analysis was carried out for the quantitative variables. The level of statistical significance was measured by the unpaired t-test and Mann-Whitney U test, while for the qualitative variables, Fisher's exact test and chi-square tests were performed.

Significant *p*-values of <0.05 were considered, aiming to present a confidence level of 95%.

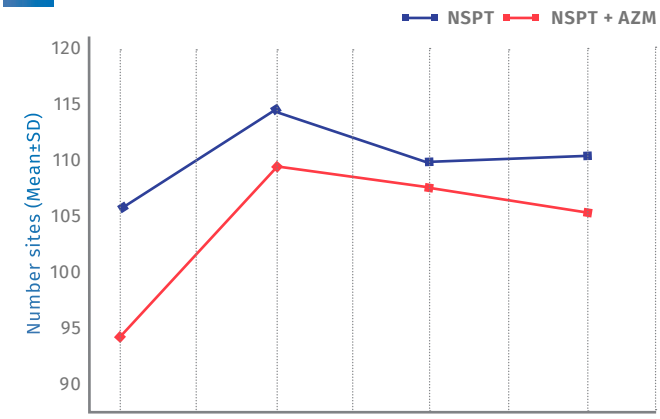
Figure 1. Flow chart of participation in the study.



AZM: Azithromycin. NSPT: Non-Surgical Periodontal Therapy.

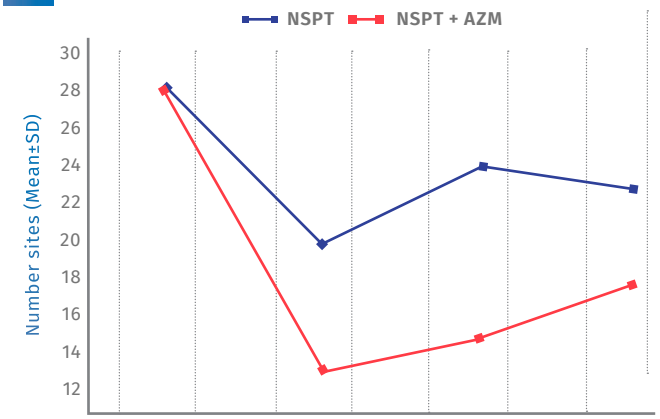
Figure 2. Mean (±SD) of the number of sites with different PPD (a, b, c) at 1,3- and 6-months post NSPT, and changes or delta (Δ) in the number of sites with: (d) PPD 1-3mm, (e) PPD 4-6 mm, (f) PPD ≥ 7mm, from at baseline to at follow-up visits.

A Initially shallow sites (PPD 1mm - 3mm)



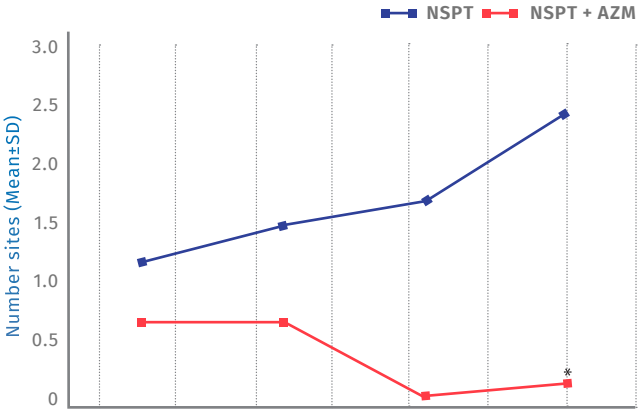
TIME POINT	BASELINE	1 MONTH	3 MONTHS	6 MONTHS
NSPT	10.6±26.3	114.4±25.7	109.8 ±25.1	110.4 ±22.6
NSPT + AZM	94±25.5	109.4±26.3	107.5±24	105.1±26.3
p-value	0.40	0.472	0.86	0.69

B Initially intermediate sites (PPD 4mm - 6mm)



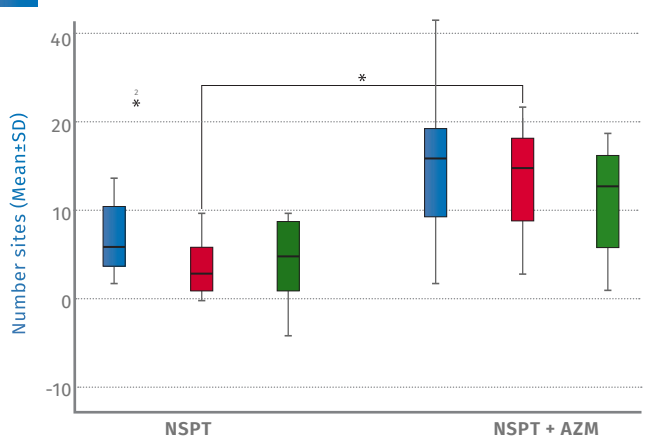
TIME POINT	BASELINE	1 MONTH	3 MONTHS	6 MONTHS
NSPT	28.3±1.3	19.4±9.3	23.8 ±12.2	22.5 ±11.3
NSPT + AZM	27.8±15.1	12.4±26.3	14.2±10.5	17.1±12.7
p-value	0.97	0.16	0.14	0.40

C Initially deep sites (PPD ≥7mm)



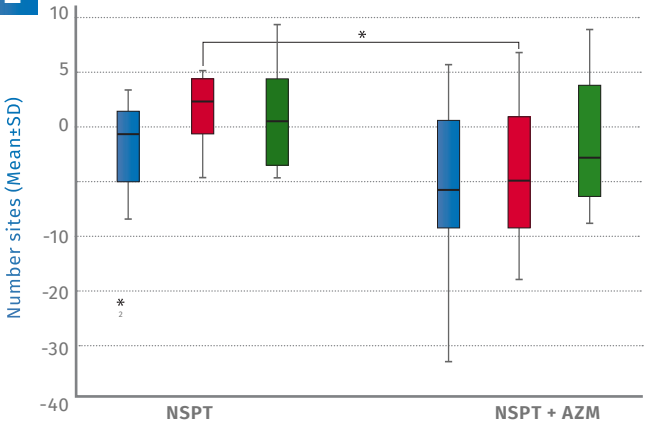
TIME POINT	BASELINE	1 MONTH	3 MONTHS	6 MONTHS
NSPT	1.2±1.3	1.5±1.9	1.7 ±2	2.4±2.5
NSPT + AZM	0.7±0.95	0.7±1.1	0.1±0.3	0.2±0.4
p-value	0.38	0.32	0.07	0.04*

D Δ Initially shallow sites (PPD 1mm - 3mm)



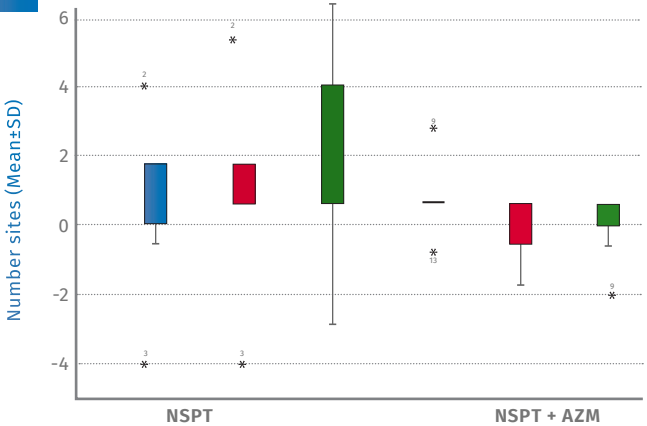
TIME POINT	NSPT	NSPT+AZM	*P*-VALUE
1 MONTH	8.42±7.20	15.42±9.86	0.15
3 MONTHS	3.85±3.67	13.57±6.85	<0.001*
6 MONTHS	4.42±5.41	11.14±7.19	0.07

E Δ Initially intermediate sites (PPD 4mm - 6mm)



TIME POINT	NSPT	NSPT+AZM	*P*-VALUE
1 MONTH	-8.42±7.20	-15.42±9.86	0.15
3 MONTHS	-3.85±3.67	-13.57±6.85	<0.001*
6 MONTHS	-4.42±5.41	-11.14±7.19	0.07

F Δ Initially deep sites (PPD ≥7mm)



TIME POINT	NSPT	NSPT+AZM	*P*-VALUE
1 MONTH	0.28±2.42	0.00±0.57	0.41
3 MONTHS	0.42±2.63	0.57±0.97	0.15
6 MONTHS	1.14±2.67	0.42±0.78	0.15

The significance of differences between groups at each time point was assessed using the unpaired *t*-test ($p>0.05$) or Mann-Whitney U test. AZM: Azithromycin. NSPT: Non-Surgical Periodontal. SD: Standard Deviation. PPD: Probing Pocket Dep. Δ: Delta.*:Statistically significant difference.

ISSN Print 0719-2460 - ISSN Online 0719-2479.

RESULTS.

This study was conducted between March and September 2016. The flow chart of the study, (Figure 1). Of a total of thirty-two patients selected, fourteen patients completed the study. No compliance problems were noted, and all patients followed the protocol of the study. No subjects reported any specific adverse effects.

Demographic and clinical characteristics (Table 2). No significant differences were found between groups at baseline (p>0.05). For the full mouth analysis, a PPD mean (p=0.04) significantly lower and PPD reduction (p=0.02), at 6-months post NSPT versus the control group. Furthermore, PD reduction (Table 2) was greater in the AZM-treated patients (p=0.02).

No statistically significant differences were observed between the groups for the CAL mean or the CAL gain (Table 2). At one month after treatment, a statistically significant decrease was detected in the percentage of sites with BoP in the experimental group versus the control group. This was maintained at 3- and 6-months post intervention. When comparing sites with plaque, OI was significantly lower in the intervention group at 3- and 6-months post treatment (Table 2).

A significant difference was not observed in the mean number of shallow and intermediate sites between treatment groups, in any post-NSPT measurement (Figure 2A and Figure 2B).

However, a significant decrease was observed in deep sites in the intervention group at 6 months post-treatment (Figure 2C).

Regarding changes or delta (Δ) in the number of initial shallow (PPD 1–3mm), intermediate (PPD 4–6mm) or deep sites (PPD ≥ 7mm), at the third

Table 1. Patient selection criteria.

INCLUSION CRITERIA	EXCLUSION CRITERIA
Patients with:	Patients with:
- ≥ 18 years. - Classified by the American Society of Anesthesiologists (ASA) as ASA I or ASA II that were compatible with local anesthe-sia procedures. - At least 10 natural teeth present, excluding semi-erupted third molars. - Periodontitis untreated, stage III generalized, grade B, according to the AAP/EFP classification of 2018 29. For Stage III, the following criteria are considered: Severity: Interdental CAL at the site of greatest loss ≥ 5 mm, radiographic bone loss extending to the middle or apical third of the root, and tooth loss due to periodontitis ≤ 4 teeth). Complexity: PPD ≥ 6mm, vertical bone loss ≥ 3mm, furcation involvement Class II or III and treatment in the previous 3 months. Moderate ridge defect. Generalized: >30% of teethinvolved. Grade B: Indirect evidence of progression (% bone loss/age: 0.25 to 1.0)	- Haemostasis disorders. - Taking medications associated with gingival disorders such as: anticonvulsants (phenytoin), calcium channel blockers (nifedipine), or immunosuppressive drugs (cyclosporine). - Any systemic diseases that affect the immunoinflammatory response. - Treatment with antacids on a regular basis due to chronic gastritis and/or self-medication with antacids. - Treatment with drugs such as: warfarin, digoxin or acetylsalicylic acid. - A history of allergic reactions to local anesthetics. - Orthodontic appliances. - Antibiotic - History of previous periodontal treatment. - Pregnancy. - Valvular prostheses or failures of heart valves, with a risk of endocarditis. - Psychic and Intellectual Disability, in accordance with Chilean law number 20,584, title II, paragraph 8, article 28. - Consumption of more than 10 cigarettes per day. - Allergy to AZM. - Lactose intolerance.

Table 2. Demographic and clinical features of patients with periodontitis. Mean (±SD) of PPD reduction, CAL gain and full-mouth clinical parameters at baseline and at follow-up visits.

VARIABLES	TIME POINT	TREATMENT GROUPS		*p*-value
		NSPT + PLACEBO	NSPT + AZM	
Age (mean ± SD)		50.7 ± 8.93	53.8 ± 6.54	0.42
Gender (male/female)		4/3	2/5	0.28
PPD	Baseline	2.97 ± 0.30	2.85 ± 0.35	0.65
	1 month	2.75 ± 0.28	2.42 ± 0.34	0.08
	3 months	2.82 ± 0.30	2.48 ± 0.28	0.05
	6 months	2.82 ± 0.31	2.44 ± 0.32	0.04*
PPD reduction	6 months	0.14 ± 0.16	0.41 ± 0.24	0.02*
CAL	Baseline	2.58 ±1.28	3.18 ± 1.17	0.80
	1 month	2.3 ± 1.31	2.98 ± 1.14	0.32
	3 months	2.47 ± 1.28	3.04 ± 1.12	0.39
	6 months	2.51 ± 1.36	2.97 ± 1.18	0.51
CAL gain	6 months	0.07 ± 0.13	0.21 ± 0.15	0.09
% of sites with BoP	Baseline	52.8 ± 16.9	41 ± 12.3	0.15
	1 month	24.7 ± 9.6	12.7 ± 4.9	0.01*
	3 months	23.8 ± 7.2	13 ± 3.3	$p<0.001$*
	6 months	24.8 ± 10.1	12.4 ± 5.3	0.01*
% of sites with Biofilm	Baseline	83.5 ± 7.3	81.7 ± 10.3	0.70
	1 month	58.8 ± 9.7	47.8 ± 11.2	0.07
	3 months	47.1 ± 9.2	30.8 ± 5.6	$p<0.001$*
	6 months	65.2 ± 7.2	53.7 ± 5.8	$p<0.001$*

The significance of differences between groups at each time point was assessed using the unpaired *t*-test and x^2 test ($p<0.05$)
AZM: Azithromycin. NSPT: Non-Surgical Periodontal. PPD Probing Pocket Depth. CAL: Clinical Attachment Level. BoP: Bleeding on Probing. SD: Standard Deviation.*:Statistically significant difference.

Table 3. Number and percentage of subjects presenting low, moderate and high risk for periodontitis progression at 6-month post NSPT. Risk for disease progression according to Lang *et al.*,[11] at 6-month post-therapies: Low (≤4 sites with PD ≥5 mm), moderate (5-8 sites with PD ≥5 mm) and high (≥9 sites with PD ≥5 mm).

RISK FOR DISEASE PROGRESSION	NSPT + PLACEBO (%)	NSPT + AZM (%)	*p*-value
Low risk	4 (57.1)	7 (100%)	0.19
Moderate risk	3 (42.9)	0 (0.0)	
High risk	0 (0)	0 (0.0)	

AZM: Azithromycin. NSPT: Non-Surgical Periodontal Therapy.
The significance of differences between groups was assessed using the Fisher's exact test ($p<0.05$).

ISSN Print 0719-2460 - ISSN Online 0719-2479.

Table 4. Inferential analysis between the groups for the presence of periodontopathogens, according to temporality.

VARIABLES	TIME POINT	TREATMENT GROUPS		*p*-value
		NSPT + PLACEBO	NSPT + AZM	
Porphyromona gingivalis	Baseline	2	1	0.52
	3 months	0	0	N/A
	6 months	0	0	N/A
Tannerella forsythia	Baseline	6	6	1
	3 months	7	5	0.13
	6 months	5	3	0.28
Treponema denticola	Baseline	4	5	0.58
	3 months	1	0	0.29
	6 months	5	2	0.11
Fusobacterium nucleatum	Baseline	6	5	0.05
	3 months	5	3	0.28
	6 months	6	7	0.29

AZM: Azithromycin. NSPT: Non-Surgical Periodontal.
The significance of differences between groups at each time point was assessed using the unpaired *t*-test and x^2 test ($p<0.05$)
*:Statistically significant difference.
-:Pg acts as a constant and it was not possible to effect a statistical analysis.
The presence of this microorganism was not observed in either group in the 3rd and 6th month.

month post NSPT, a statistically significant increase in the number of shallow sites was observed (Figure 2D) in association with a decrease in the number of sites with intermediate in the experimental group *versus* the control group (Figure 2E). In the deep sites, there were no significant differences (Figure 2F).

Regarding the risk of disease progression,[11,12] all the subjects presented a "low risk" for disease progression at 6 months post therapy (Table 3). However, no significant differences were found between the groups (p=0.19).

Regarding the presence of periodontal pathogens, inter-group and intra-group comparison revealed no significant differences (Table 4).

DISCUSSION.

The present study evaluated the effect of the systemic administration of AZM as an adjunct therapy to NSPT on the clinical and microbiological variables of patients with periodontitis Stage III grade B.

The utilization of antibiotics as a coadjuvant for the treatment of periodontitis is widely supported in the literature.[13] Nevertheless, due to the overuse of antibiotics, and the development of bacterial resistance, its routine administration as an adjunct to NSPT is not recommended.[1]

Its indication should be considered for specific categories, *e.g.*, periodontitis, stage III generalized, grade B, in patients 55 years or younger, and those with full-mouth NSPT.[14]

The patients in this study had these characteristics. In terms of the study population, no significant differences in demographic or clinical characteristics were found between groups (Table 2), and therefore, a homogeneous sample was studied.

In relation to PPD, the mean and the reduction 6 at months in the AZM group, was significantly lower post NSPT (Table 2). Similarly, previous research reported a significant reduction in the average PPD 6. Contrarily, others studies no significant differences between study groups were observed at any time

points.[10,15,16] In relation to the changes or delta (Δ) in the number of sites, shallow, intermediate and deep, from baseline to the third month post NSPT, a significant increase in shallow sites was observed in the experimental group *versus* control group (Figure 2D), in contrast to previous studies.[3,15,17]

Besides, a significant reduction in intermediates sites was observed (Figure 2E), in accordance with Oteo *et al.*,[6] and contrary to other previous studies.[10,15,18] For the mean of deep sites, a statistically significant decrease was observed at 6 months post treatment (Figure 2C). This result coincides with others findings, but at one month after NSPT.[3,17]

The administration of azithromycin adjunctive to NSPT has led to different results depending on the baseline PPD6. It has been demonstrated to be more effective in patients with severe periodontitis, particularly in the deeper initial pockets (≥6 mm).[5,7,17] In the present study, the selected patients had a mean PPD that was initially low (Table 2). However, favorable results were obtained.

The significant changes in PPD in the intervention group could be a positive effect of AZM in controlling some of the pro-inflammatory processes involved in periodontal disease.[3,19,20] This effect would be produced for its long half-life and high concentrations in human serum , periodontal tissues,[6] fibroblasts, phagocytes and leukocytes.[9,17,19] AZM is absorbed by these cells, which helps them to quickly bring the drug to the site of inflammation and to maintain a high local concentration.[10] Moreover, it exhibits inhibitory effects on oxidant production and it modulates pro-inflammatory cytokine release by these cells.[9]

Nonetheless, this should be interpreted with caution due to some relevant limitations. Just 14 patients (n=7/group) provided data for the 6-month visit, and this may be considered as a limited sample size. Regarding BoP, a significant decrease was detected in the intervention group at all timepoints (Table 2). This coincides with previous studies[18,21,22] at 3 and 6 months, unlike earlier results.[6,10]

These results are consistent with those obtained for PPD since both indicate periodontal stability and are related to the anti-inflammatory role of azithromycin.[7,9] Furthermore, O'Rourke 3 suggested investigating the use of AZM as a complement to NSPT, especially in patients who did not achieve resolution of BoP and PPD.

For OI, a significant decrease in the intervention group compared to the control group during the third and sixth month was observed (Table 2), in contrast to previous work.[6] These results are due to the patient's personal motivation to maintain good oral hygiene. This contributes to the reduction of PPD and BoP obtained, since supragingival plaque control is a key factor in attaining favourable clinical and microbial outcomes, following systemic antibiotic therapy in periodontitis.[23] In addition, supragingival debridement may induce beneficial changes in the subgingival microbiota.[1]

Concerning CAL gain in both groups, no significant differences were observed (Table 2), consistent with that reported by other researchers.[5,7,10,15] It is thought that NSPT by itself is successful in the long term for most patients, since it generates a biocompatible surface that allows for periodontal repair.[24] Contrarily, previous research has reported a significant CAL decrease in the experimental group.[6,17,22]

Regarding the existing NSPT protocols, the present study was based on the FM-SRP[12,25] and the AZM prescription (500 mg/24 h, for 3 days), and was carried out after the last session. Prior research shows better clinical and microbiological results with FM-SRP,[25] in contrast to that reported by Sanz *et al.*,[1] Additionally, it has been recommended that NSPT be completed within a short time-period and for the antibiotic intake to start on the day of therapy completion. This rationale is based on the increase of antibiotic tolerance in biofilms within 24h after initial colonization.[14] In addition, the azithromycin concentration in inflamed periodontal tissues decreases to 50% after seven days.[6]

In this manner, the necessary concentration in the periodontal pockets to obtain the therapeutic effect can be achieved,[14] mainly in the initial deep

ISSN Print 0719-2460 - ISSN Online 0719-2479.

sites. On the contrary, studies reported that the effectiveness of AZM as an adjunct to NSPT does not depend on the type of protocol used.[12,25] The selection of AZM dosage may be controversial, because the approved dosages in the United States (5-day, first dose of 500mg and then 250mm daily) and in Europe (3-day, 500mg daily) are different. The latter is the one most commonly evaluated in periodontal literature.[25] It has demonstrated better results when the bacterial cure rate was analyzed,[6,13] and may improve patient compliance.

On the other hand, an extended period of five days was associated with an additional several adverse events, including diarrhea, headaches, metallic taste and sleepiness, were reported.[10,12] Finally, dosage, NSPT-protocol and administration of the AZM,[13] needs to be identified in additional trials. In relation to AZM adverse effects, they are reported to be very low (in 0.7% of the patients).[6] Nonetheless, one study suggested that systemic AZM may have proarrhythmic effects in patients with heart disease,[17] contrary to that reported by Almalki *et al.*[26] Then, if these antecedents exist, a consultation with the patient's physician is advisable before its prescription. In this study, no subjects reported adverse effects. In this investigation, all the subjects in the intervention group presented a "low risk" for disease progression at 6 months post therapy (Table 3). However, no significant differences were found between groups.

Previous studies suggested that subjects with residual pockets, especially those with PD ≥5 mm, have a greater risk of additional attachment loss.

In this study, inter-group and intra-group comparison revealed no significant differences in the presence of pathogens (Table 4). Previous studies reported in the AZM group a significant reduction in the frequency of detection of Pg at 6 months[6] and of Tf[21] at 1 month follow-up, and of both independent of the usage of placebo or AZM.[10]

Different results were also reported with respect to Pg, Tf or Td.[21,25,27] The frequency of Fn decreased significantly at one month, but began to increase until reaching baseline levels at 6 months . It should be noted that the comparisons with other investigations is complex, due to the differences regarding the quantity and type of bacteria studied. Besides, most of these studies evaluated the presence or absence of a few species in a limited number of sites.[6]

Periodontal treatment is focused on the control of the associated microbiota, removing or reducing the bacterial load of the periodontopathogens associated with the subgingival biofilm,[18] which allows reducing soft tissue inflammation. The endpoint of treatment is pocket closure, defined by PPD ≤4mm and absence of BoP.[1]

These clinical outcomes are achieved when the proportions of periodontal pathogens are reduced and the root surfaces are recolonized with a higher proportion of symbiotic species. This shift is difficult due to the subgingival biofilm protecting resident organisms from periodontal treatment and allowing the survival of strict anaerobe pathogens, even in highly oxygenated areas of the mouth, such as tongue, oral mucosa, saliva and shallow pockets.[20] Although the effectiveness of NSPT is well documented, it does not always induce the ecological changes necessary to achieve and maintain the desired clinical improvements, in all subjects in the long term,[20] especially in deep periodontal pockets difficult to access with microbial invasion at epithelial level and tissue destruction,[18] which induces progression of periodontal disease.[20]

Besides, the recolonization of other oral sites by periodontopathogens also accounts for the failure of NSPT.[18] In all these situations systemic antibiotic therapy complementary to NSPT, is indicated.

On the other hand, a recent investigation indicated that the etiology of periodontitis is closely related to the bacterial dysbiosis,[28] through the overgrowth of proinflammatory Gram-negatives species.[14] Therefore, periodontitis-associated microbiota is inflammophilic,[28] that is, it survives the inflammation, it benefits from it and promotes it . While systemic azithromycin is well recognized for its antibacterial properties, it has also been shown to possess additional anti-inflammatory and immune-

modulating effects.[7]

The majority of cells involved in both the innate and adaptive immune responses are influenced by its administration[9] and by affecting the production of cytokines, AZM has a dampening effect on the pro-inflammatory response.[17] Consequently, it could be thought that the improvement of PPD and BoP in the experimental group is also due to this effect.

The limitations of this study are: First, the statistical power, since this study could have been too small to detect real differences between the groups in some variables. Second, the microbiological analysis, since four periodontal pathogens were considered, and only conventional PCR was performed.

It is suggested in future research, a larger sample size be used and to compare the clinical and microbiological effects of AZM with other antibiotics, with a follow-up period of at least 1 year, in smoking and diabetic patients. Along with this, evaluate the effect of AZM on cells and molecules involved in the immune response periodontal, to clarify its anti-inflammatory properties and possible immune-modulating effects.

CONCLUSION.

Within the limitations of the present study, AZM as an adjuvant to NSPT provides beneficial additional effects for PPD and BoP compared to NSPT alone, in patients with severe periodontitis, particularly in the deeper initial pockets (≥6 mm).

ISSN Print 0719-2460 - ISSN Online 0719-2479.

Conflict of interests:

Authors report no conflicts of interest in connection with this article.

Ethics approval:

This research was approved by the Dentistry Faculty's Scientific Ethics Committee of the Andres Bello University's (UNAB) (Decision N°. 038).

Funding:

This study was funded by the Andres Bello University's by awarding the competition for Biomedical and Clinical Sciences 2016 (project code: DI-1364-16/CB).

Authors' contributions:

All authors contributed to this manuscript.

Acknowledgements:

American Journal Experts for their editing and proofreading service of the manuscript in English *(https://secure.aje.com/certificate/verify)*, verification code EE8C-C36B-4C98-0BB1-1003.

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