

The Impact of Oral Contraceptives on Women's Periodontal Health and the Subgingival Occurrence of Aggressive Periodontopathogens and *Candida* Species

María Isabel Brusca,* Alcira Rosa,* Olatz Albaina,† María D. Moragues,‡ Fernando Verdugo,†§ and José Pontón†

Background: The purpose of this study is to evaluate the influence of oral contraceptive (OC) use on the subgingival occurrence of specific periodontopathogens and the host's periodontal status.

Methods: Ninety-two females aged 19 to 40 years were included in the study. They were divided into two groups, OC users and non-users, and subgrouped according to the most severe periodontal condition and duration of OC usage. A pooled subgingival sample from each subject was cultured to investigate the presence of *Candida* species, *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*), and *Prevotella intermedia*.

Results: OC users, particularly smokers, show a statistically significant increase in the prevalence of severe periodontitis. OC users had deeper probing depths (≥ 5 mm) than non-users. Moreover, OC users had higher gingival index scores and clinical attachment loss, ≥ 2 and ≥ 5 mm, respectively, than non-users ($P < 0.01$). Patients taking OCs had significantly higher numbers of cultures positive for *Candida*. Seven *Candida* species were isolated. Subgingival *Candida* was associated with *P. gingivalis* and *P. intermedia* in 82.9% and 85.4%, respectively, in patients taking OCs. *A. actinomycetemcomitans* was isolated in patients with moderate and severe periodontitis and was associated with subgingival *P. gingivalis*, *P. intermedia*, and *Candida*.

Conclusions: OC use may increase the risk of severe periodontitis and seems to cause a selection of certain *Candida* species in periodontal pockets. OC users showed a higher prevalence of *P. gingivalis*, *P. intermedia*, and *A. actinomycetemcomitans* compared to non-users. *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. tropicalis*, and *C. glabrata* were the species with the ability to survive in the conditions created by the sex hormones after 3 years. *J Periodontol* 2010;81:1010-1018.

KEY WORDS

Actinobacillus actinomycetemcomitans; *Candida*; oral contraceptives; periodontitis; *Porphyromonas gingivalis*; *Prevotella intermedia*.

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monal contraceptive usage by females of child-bearing age has been suggested as a potential risk factor for the progression of destructive periodontitis.¹⁻⁶ However, recent data from large cross-sectional studies (National Health and Nutrition Examination Surveys I and III) failed to validate this hypothesis.⁷

Gingival inflammation seems to be associated with high concentrations of sex steroids in females taking birth control pills.^{8,9} The new generations of oral contraceptives (OCs) containing 30 to 35 μ g of estradiol present fewer associated vascular systemic risks.¹⁰ Other authors support the notion that increased gingival inflammation depends on the duration of OC use.^{9,11}

Variations in female endogenous sex hormones during menstruation or pregnancy or use of OCs have long been associated with gingivitis.^{1-6,8-11} An in vitro study showed that polymorphonucleocyte chemotaxis was enhanced by progesterone and reduced by estradiol. Such polymorphonucleocyte

* Department of Microbiology and Parasitology, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina.

† Department of Immunology, Microbiology and Parasitology, School of Medicine and Odontology, University of Basque Country, Leioa, Spain.

‡ Department of Nursing I, University of Basque Country.

§ Veterans Affairs Hospital, Greater Los Angeles Healthcare System, Los Angeles, CA.

hormonal-induced impairment may translate into gingival inflammation.¹² A recent study¹³ could not confirm a specific cyclic pattern of bacterial colonization in a group of 20 systemically and periodontally healthy females who were not taking OCs during one full menstrual cycle. Interestingly, the levels of *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*) reportedly increased between the onset of menstruation and 2 weeks ($P < 0.05$). These differences were not significant at the $P < 0.001$ level set in the aforementioned study.¹³

Adriaens et al.¹⁴ recently showed in a group of 20 pregnant females (mean age, 31.5 years), that total bacterial counts decreased between week 12 and postpartum. Although the counts of *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, *Fusobacterium* spp., *Parvimonas micra* (previously *Peptostreptococcus micros* or *Micromonas micros*), and *Prevotella intermedia* decreased, *A. actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia* (previously *T. forsythensis*), and *Treponema denticola* did not change for this specific population between week 12 of pregnancy and postpartum. Furthermore, Dinas et al.¹⁵ reported that from a pool of 425 pregnant females, 47% presented symptoms of gingivitis.

The importance of smoking as a risk factor associated with periodontal disease progression is underscored in different studies.¹⁶ Kamma et al.¹⁷ compared the microbial profiles of smokers and non-smokers in a group of patients with early onset periodontitis and reported that smokers harbored a greater number of bacteria in total and presented deeper pockets (>5 mm). The study also showed that a number of oral bacteria and fungi, including *Candida albicans*, were more frequently isolated in smokers.

The pathogenesis of *Candida* species in the progression of periodontal disease has not been fully addressed. On the contrary, the bacterial role of common periodontopathogens, such as *P. gingivalis*, *A. actinomycetemcomitans*, and *P. intermedia*, has been studied for decades. More studies are needed to understand the putative role of yeast as periodontopathogens. A number of studies recently reported that *Candida* species are isolated in subgingival samples of patients with periodontal disease.¹⁸⁻²⁰ *C. albicans* is usually the most frequently isolated species of yeast in patients with periodontal disease. Yet, *C. dubliniensis*, a recently described oral pathogen very similar structurally to *C. albicans*, has also been associated with periodontitis.²¹

There are specific receptors within the periodontal tissues for estrogens and progesterone.²² Some researchers^{23,24} have shown specific patterns of bacterial colonization in the presence of estrogens and progesterone in periodontal pockets, whereas a differ-

ent group²⁵ found no association between low-dose OCs and gingivitis. It is also reported that sex hormones (estrogens and progesterone) could alter the gingival vasculature and local immune response, reducing the capacity of the periodontium to repair.²⁶ A limited number of animal studies also show that sex hormones have the ability to alter the gingival microvasculature, increasing its permeability and cellular immune response, which could predispose to periodontitis.²⁷⁻²⁹ A recent human study on 27 non-smoking, healthy females without signs of periodontitis showed that the fluctuation of sex steroid hormones during menstruation had an impact on gingival inflammation. Bleeding on probing (BOP) and interleukin-1 β were significantly increased under good plaque control.³⁰ There seems to be evidence suggesting that sex hormonal therapy may modify the gingival inflammatory response and alter the subgingival microbiota.^{8-13,22-29}

Little is known about the influence of OCs on the patterns of subgingival colonization, severity and extent of periodontal disease, and its potential association with duration of use. Furthermore, scarce scientific data are available regarding the presence of *Candida* species in subgingival tissues. The purpose of this study is to determine the influence of OC usage on the distribution of specific periodontopathogens (*Candida* spp., *P. gingivalis*, *A. actinomycetemcomitans*, and *P. intermedia*) in females of child-bearing age. Moreover, a further aim is to find associations between OC use, age, and duration of the drug administration and severity of the disease.

MATERIALS AND METHODS

The study was approved by the School of Odontology, University of Buenos Aires Ethical Committee, Buenos Aires, Argentina. All patients signed informed consent forms. Ninety-two females with an average age of 30 years (range, 19 to 40 years) were included in the study. Patients were recruited between March 2007 and February 2009 from the university and private clinic offices. They were divided into those taking OCs ($n = 41$) and an age-matched control group not taking OCs ($n = 51$). Moreover, the test and control groups were matched for socioeconomic characteristics and oral habits without variations in ethnicity, occupation, or educational level. Subjects were excluded from the study if they presented with a metabolic or systemic disorder (e.g., diabetes, epilepsy, hypertension, or metabolic syndrome) that could affect the periodontium, had taken antibiotics in the 6 months previous to the study, or were pregnant. Therefore, all subjects were systemically healthy and had not received antibiotic therapy or professional cleaning in the 6 months previous to the study. A detailed questionnaire was completed by each

patient recording a full medical history, smoking habits, and contraceptive pill usage before clinical examination. Specifically, the type of OC and the duration of the medication were recorded. All patients were examined by an experienced periodontist (MIB). The medical history data were withheld from the examining clinician to prevent potential influence of bias. At the beginning of the study, the examining clinician underwent a period of training to achieve a reproducibility of 98% for probing depths and 96% for attachment level measurements to within ≤ 1 mm.

Clinical Parameters

Plaque and gingival inflammation were measured for each site using the indices proposed by Silness and Løe³¹ and Løe and Silness,³² respectively. The data for these measurements are presented as the percentage of sites for each patient exhibiting plaque (i.e., plaque index ≥ 1 and ≥ 2) and gingival inflammation (gingival index [GI] ≥ 1 and ≥ 2).

After a comprehensive periodontal examination including probing depths (six sites per tooth), number of teeth, and radiographic evaluation, the study population was grouped according to the most severe periodontal condition at any one site and the usage of OCs. Full-mouth radiographs were analyzed by one experienced periodontist (MIB) to assess interproximal bone loss and confirm clinical measurements. The following criteria were used: healthy/gingivitis subjects presented no radiographic bone loss and probing depths ranging from 2 to 4 mm. Mild chronic periodontitis exhibited radiographic bone loss and clinical attachment loss (CAL) of 1 to 2 mm. Moderate chronic periodontitis described patients with CAL of 3 to 4 mm and severe chronic periodontitis with CAL ≥ 5 mm. Clinical attachment levels were calculated using the cemento-enamel junction or margin of a crown as the reference landmark. For instance, whenever a gingival recession was present, CAL was measured by adding the probing depth to the distance from the cemento-enamel junction to the free gingival margin.

Specimen Collection

Each subject provided a pooled subgingival sample from the deepest pockets in each quadrant using a minimum of four and a maximum of eight sterile paper points per patient. After supragingival scaling, paper points were inserted into the gingival sulcus/pockets for 20 seconds and placed thereafter in 0.5 ml of reduced transport Göteborg, anaerobically prepared III (VMGA III) medium. After homogenization, a serial dilution was performed and aliquots of 20 μ L of each dilution were placed into different media (anaerobic blood agar supplemented with vancomycin and kanamycin, trypticase soy agar with bacitracin and vancomycin,³³ and a medium for *Candida*^{||}).

Anaerobic incubation was performed^{||} at 36°C for 7 days. *Candida* agar plates were incubated at 37°C for up to 7 days. Identification of the periodontopathogenic species *P. gingivalis*, *A. actinomycetemcomitans*, and *P. intermedia* was performed by means of morphologic and biochemical properties.[#] *Candida* species were identified by colony color and morphology in a medium for *Candida* and carbohydrate assimilation.^{**} Isolates yielding green colonies on agar for *Candida* were further characterized to differentiate *C. albicans* from *C. dubliniensis*. Definitive identification of *C. dubliniensis* was done by means of their colony color and morphology in medium,³⁴ analysis by multiplex polymerase chain reaction,³⁵ and reactivity with a latex agglutination test specific for *C. dubliniensis*^{36,††} and an anti-*C. dubliniensis* antiserum.³⁷

Statistical Analyses

Descriptive analyses of data were expressed as mean \pm SD. Significance of group comparisons was determined by the Student *t* test or the chi-square test with the Yates' correction when appropriate. Statistical significance was set at $P < 0.05$.

RESULTS

Ninety-two females entered this study. They were divided into those taking OCs ($n = 41$) and a control group of those not taking OCs ($n = 51$). The mean age for the two groups did not significantly differ, being 30.34 ± 6.24 (range, 19 to 40) years for the OC group and 30.31 ± 4.7 (range, 20 to 38) years for the control group.

Females on hormonal OCs used three types of brands that contained different concentrations of ethinyl estradiol, gestoden, and drospirenone. Brand one was used by 24 patients and contained 0.015 mg of ethinyl estradiol and 0.06 mg of gestoden.^{††} Brand two was used by 13 patients and contained 0.03 mg of ethinyl estradiol and 3 mg of drospirenone.^{§§} Brand three was used by four patients and contained 0.02 mg of ethinyl estradiol and 3 mg of drospirenone.^{|||} No statistically significant differences in periodontal health were found among patients using any of the three brands (data not shown).

The prevalence of periodontal diseases in OC users is shown in Table 1. Individuals had an average of 27 permanent teeth (range, 26 to 32). Most of the patients (89.13%) did not wear a removable partial denture or prosthesis (data not shown). Patients on OCs

|| CHROMagar, BioMerieux, Marcy l'Etoile, France.

¶ Anaerocult system, Merck KGaA, Darmstadt, Germany.

API 20 A and API NH, BioMerieux.

** API 20 C AUX, BioMerieux.

†† Bichro-Dubli Fumouze, Fumouze Diagnostics, Levallois-Perret, France.

‡‡ Mirelle, Bayer HealthCare, Buenos Aires, Argentina.

§§ Yasmin, Bayer HealthCare.

||| Yasminelle, Bayer HealthCare.

Table 1.
Prevalence of Periodontal Disease in OC Use or Smoking Habit

Clinical Status	Contraceptive				Smoker				OC user				Non-OC user			
	Users		Non-Users		Yes		No		Smoker		Non-Smoker		Smoker		Non-Smoker	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Health/gingivitis	7	17.1*	17	33.3	7	17.1†	17	33.3	2	9.6‡	5	25	5	25§	12	38.7
Mild periodontitis	15	36.6	22	43.1	16	39	21	41.2	7	33.3	8	40	9	45	13	41.9
Moderate periodontitis	9	21.9	7	13.8	8	19.5	8	15.7	5	23.8	4	20	3	15	4	12.9
Severe periodontitis	10	24.4*	5	9.8	10	24.4†	5	9.8	7	33.3‡	3	15	3	15	2	6.5
Total	41	100	51	100	41	100	51	100	21	100	20	100	20	100	31	100

* Chi-square test ($P < 0.01$ versus contraceptive non-user group).
 † Chi-square test ($P < 0.01$ versus non-smoking group).
 ‡ Chi-square test ($P < 0.01$ versus non-smoking OC-user group).
 § Chi-square test ($P < 0.05$ versus non-smoking non-OC-user group).

Table 2.
Clinical Parameters in OC Users Versus Non-Users

Clinical Parameter	Contraceptive			
	Users		Non-Users	
	n	%	n	%
Gingival index ≥ 1	39	95*	42	82
Gingival index ≥ 2	26	63*	22	43
Plaque index ≥ 1	39	95†	43	84
Plaque index ≥ 2	25	61*	15	29
Probing depth ≥ 5	34	83*	34	66.7
Probing depth ≥ 6	19	46.3*	12	23.5
Clinical attachment level ≥ 3	19	46.3*	12	23.5
Clinical attachment level ≥ 5	10	24.4*	5	9.8

* Chi-square test ($P < 0.01$ versus the contraceptive non-user group).
 † Chi-square test ($P < 0.05$ versus the contraceptive non-user group).

showed a statistically significant increase in the prevalence of severe periodontitis. A similar situation was recorded for smokers versus non-smokers ($P < 0.01$; Table 1). The OC group ($n = 41$) was comprised of 21 smokers (51.2%): nine (42.8%) smoked < 10 cigarettes a day and 12 (57.2%) ≥ 10 . From these last 12 subjects, seven (33.3%) had severe periodontitis and five (23.8%) had moderate periodontitis. The OC non-users ($n = 51$) had 20 smokers (39.3%), 13 of them (65%) smoked < 10 cigarettes a day and seven (35%) ≥ 10 . From these last seven subjects one (5%) had severe periodontitis; two (10%) had

moderate periodontitis; three had mild periodontitis (15%); and one (5%) had gingivitis (data not shown).

The increase in prevalence of severe periodontitis correlated with the age of patients and plaque index but not with BOP (Tables 2 and 3). OC users presented a higher proportion of GI scores ≥ 1 (95%) compared to non-users (82%) (Table 2). Furthermore, OC users had higher proportions of GI scores ≥ 2 (63%) compared to non-users (43%) and this difference was statistically significant ($P < 0.01$; Table 2). OC users (83%) had significantly deeper probing depths ≥ 5 mm than non-users (66.7%). OC users (46.3%) had more areas with CAL ≥ 3 mm than non-users (23.5%). Moreover, OC users (24.4%) presented more areas with CAL ≥ 5 mm than non-users (9.8%; Table 2). Control patients > 26 years showed a statistically significant ($P < 0.01$) increase in periodontal disease (Table 3). OC users < 25 years had a higher rate of periodontitis compared to controls (Table 3).

The duration of OC use also had an influence on periodontal health. The mean \pm SD of duration of OC was 33.7 ± 25.7 months (data not shown). In general, patients on OCs for > 3 years showed a higher prevalence of moderate and severe periodontitis. Subjects taking OCs > 3 years presented 25% and 41.7% of moderate and severe periodontitis, respectively, versus 20.7% and 17.2% in the OC group of < 3 years of duration (data not shown). The prevalence of gingival health/gingivitis and mild periodontitis decreased in the group taking OCs > 3 years (0% and 33.3%, respectively) compared to OC users of < 3 years (24.1% and 37.9%, respectively) (data not shown).

Fungal cultures yielded 80 isolates of seven *Candida* species including 25 *C. albicans*, 15 *C. tropicalis*, 14 *C. parapsilosis*, nine *C. krusei*, nine *C. dubliniensis*, seven *C. glabrata*, and one *C. guilliermondii*. In most

Table 3.
Prevalence of Periodontal Disease According to Age and OC Use

Clinical Status	Age					
	19 to 25 years		26 to 35 years		>36 years	
	n	%	n	%	n	%
<i>Contraceptive Users</i>						
Health/gingivitis	4	33.3	2	11.7	1	8.3
Periodontitis	8*		15		11	
Mild periodontitis	8	66.7	4	23.6	3	25
Moderate periodontitis	0	0	6	35.3	3	25
Severe periodontitis	0	0	5	29.4	5	41.7
Subtotal	12	100	17	100	12	100
<i>Contraceptive Non-Users</i>						
Health/gingivitis	7	100	8	24.2	2	18.2
Periodontitis	0		25†		9†	
Mild periodontitis	0	0	15	45.5	7	63.6
Moderate periodontitis	0	0	6	18.2	1	9.1
Severe periodontitis	0	0	4	12.1	1	9.1
Subtotal	7	100	33	100	11	100

* Chi-square test ($P < 0.01$ versus the contraceptive non-user group).

† Chi-square test ($P < 0.01$ versus the 19- to 25-year group).

cases, one *Candida* species was isolated from the sulcus. Two *Candida* species (*C. albicans* and *C. dubliniensis*) were recovered only from one patient with severe periodontitis and not taking OCs. The prevalence of *Candida* colonization was 95.1% in the OC group and 78.4% in the control group (Table 4). Patients on OCs had a statistically significant ($P < 0.05$) higher number of cultures positive for *Candida*. The number of *Candida* species was the same in both groups of patients. However, some species were isolated more frequently in one of the groups (Table 4). *C. dubliniensis* was only isolated in control patients ($P < 0.01$), and *C. guilliermondii* was only isolated in patients on OCs. *C. parapsilosis* and *C. tropicalis* were isolated more frequently in the OC group (13 versus 1 and 10 versus 5, respectively), but only in the case of *C. parapsilosis* were differences statistically significant ($P < 0.001$). *C. parapsilosis* was isolated more frequently in patients with periodontitis (12 versus 1) ($P < 0.05$). *C. albicans* was isolated more frequently in control patients (19 versus 6) ($P < 0.01$). *C. krusei* and *C. glabrata* were isolated in similar numbers in both groups (Table 4).

Time of OC usage did not show a significant influence on the distribution of *Candida* species. Patients on OCs for >3 years showed a less varied species profile than patients on oral hormonal contraceptives for ≤3 years (five versus six, respectively). Only five *Candida* species (*C. albicans*, *C. parapsilosis*, *C. krusei*, *C.*

tropicalis, and *C. glabrata*) were isolated in patients on OCs for >3 years (data not shown). The patient's age did not show statistically significant differences in the number of *Candida* species isolated from patients taking OCs (data not shown). However, control patients with ages ≤25 years showed only two *Candida* species (*C. albicans* and *C. tropicalis*) compared to the more varied species profile of patient groups with ages between 26 and 35 years and >36 years (five *Candida* species each).

Candida isolation was accompanied by subgingival presence of *P. gingivalis* and *P. intermedia* in 82.9% and 85.4%, respectively, of patients taking OCs (Table 5). In most cases, *P. gingivalis* and *P. intermedia* were isolated in patients with periodontitis. Patients on OCs and isolation of *P. gingivalis* or *P. intermedia* showed a statistically significant ($P < 0.01$) increase in the prevalence of severe periodontitis compared to controls. *A. actinomycetemcomitans* was isolated in patients with moderate and severe periodontitis ($P < 0.01$) and in association with *P. gingivalis*, *P. intermedia*, and *Candida*. Control patients showed a lower prevalence of the three periodontopathogens, especially in the case of *P. intermedia* and *A. actinomycetemcomitans*, compared to the OC group (Table 5). In six patients taking OCs, isolation of *A. actinomycetemcomitans* was accompanied by a *Candida* species, three of them *C. parapsilosis*.

In 16 patients there was no isolation of periodontal bacteria from the periodontal pockets; 11 of them were OC non-users. The clinical presentations in patients with no isolation of periodontal bacteria in the periodontal pockets ranged between gingival health/gingivitis and moderate periodontitis (data not shown). OC usage lowered the percentage of patients with gingival health and gingivitis but increased the percentage of patients with mild and moderate periodontitis. *Candida* species were isolated in 10 patients negative for periodontal bacteria in the periodontal pockets (data not shown). Six of them were OC non-users. *Candida krusei* was isolated in patients negative for periodontal bacteria irrespective of OC usage. However, *C. albicans* and *C. dubliniensis* were isolated in the periodontal pockets of OC non-users negative for periodontal bacteria, whereas *C. tropicalis* and *C. glabrata* were grown from the periodontal pockets of OC users without isolation of periodontal bacteria.

DISCUSSION

The present study aims to assess the influence of OC usage on the distribution of specific periodontopathogens (*Candida* spp., *P. gingivalis*, *A. actinomycetemcomitans*, and *P. intermedia*) in women with an average age of approximately 30 years (range, 19 to 40). A further objective was to find associations

Table 4.
Prevalence of *Candida* spp. Isolation on Periodontal Diseases

Clinical Status	Contraceptive					
	Users (n = 41)			Non-Users (n = 51)		
	n	Species	%	n	Species	%
Health/gingivitis	3	<i>C. tropicalis</i>	7.69	8	<i>C. albicans</i>	19.51 [§]
	1	<i>C. parapsilosis</i>	2.56 [†]	2	<i>C. dubliniensis</i>	4.88*
	1	<i>C. glabrata</i>	2.56	2	<i>C. krusei</i>	4.88
	1	<i>C. albicans</i>	2.56	1	<i>C. tropicalis</i>	2.44
	1	No growth	—	4	No growth	—
Subtotal	6		15.37	13		31.71
Mild periodontitis	5	<i>C. parapsilosis</i>	12.82 ^{††}	6	<i>C. albicans</i>	14.63
	4	<i>C. krusei</i>	10.27	5	<i>C. dubliniensis</i>	12.20*
	2	<i>C. tropicalis</i>	5.13	3	<i>C. krusei</i>	7.32
	2	<i>C. albicans</i>	5.13	3	<i>C. tropicalis</i>	7.32
	1	<i>C. guilliermondii</i>	2.56 [†]	1	<i>C. glabrata</i>	2.44
	1	<i>C. glabrata</i>	2.56	4	No growth	—
Subtotal	15		38.47	18		43.91
Moderate periodontitis	3	<i>C. parapsilosis</i>	7.69 ^{††}	1	<i>C. tropicalis</i>	2.44
	3	<i>C. tropicalis</i>	7.69	1	<i>C. parapsilosis</i>	2.44
	2	<i>C. albicans</i>	5.13	1	<i>C. albicans</i>	2.44 [§]
	1	<i>C. glabrata</i>	2.56	1	<i>C. glabrata</i>	2.44
				3	No growth	—
Subtotal	9		23.07	4		9.76
Severe periodontitis	4	<i>C. parapsilosis</i>	10.27 ^{††}	4	<i>C. albicans</i>	9.75 [§]
	2	<i>C. glabrata</i>	5.13	2	<i>C. dubliniensis</i>	4.88*
	2	<i>C. tropicalis</i>	5.13			
	1	<i>C. albicans</i>	2.56			
	1	No growth	—			
Subtotal	9		23.09	6		14.63
Total	39 [‡]		100	41		100

* Chi-square test, Yates correction ($P < 0.01$ versus the contraceptive user group).
 † Chi-square test, Yates correction ($P < 0.001$ versus the contraceptive non-user group).
 ‡ Chi-square test, Yates correction ($P < 0.05$ versus the contraceptive non-user group).
 § Chi-square test ($P < 0.01$ versus the contraceptive user group).
 || Chi-square test ($P < 0.01$ versus the health/gingivitis group).

between duration of drug use, age, and severity of the disease.

OC use increased the risk for severe periodontitis and seemed to cause a selection of certain *Candida* species subgingivally. *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. tropicalis*, and *C. glabrata* were the species presenting the ability to survive subgingivally after 3 years. Moreover, OC users showed a higher prevalence of *P. gingivalis*, *P. intermedia*, and *A. actinomycetemcomitans* compared to non-users.

The influence of OC use on oral health has been addressed in several studies.¹⁻⁷ Although it is widely accepted that female sex hormones have an impact on oral structures and there is evidence suggesting that sex hormones may produce gingival inflammation by altering the microvascular component of the gin-

giva,^{38,39} the influence of OC usage by females of child-bearing age on the progression of periodontitis is controversial.^{1,7}

OC users had 20% higher proportion of GI scores ≥ 2 (63%) compared to non-users (43%) in the present study ($P < 0.01$). Regardless of smoking status, BOP (GI ≥ 2) was significantly higher in OC users. Smoking ≥ 10 cigarettes a day in the OC group accounted for the most severe forms of periodontitis compared to OC non-users. Smoking is a well-known risk factor for periodontitis and could have acted synergistically with OC use in its progression. In fact, this study reports that non-OC users who smoke had less severe periodontal disease than OC users who smoke. However, these data should be interpreted with caution because of the small sample size of smokers (≥ 10 cigarettes a day) in this group. Further studies are needed to elucidate the potential synergistic effect of smoking and OC use in the progression of periodontitis.

The results presented in this study suggest that OC use has a role in periodontal health because patients on OCs tend to have poorer periodontal health than non-users and show a statistically significant increase in the prevalence of severe periodontitis and major periodontopathogens. The effect was more marked in patients with >3 years of OC use, who showed a higher prevalence of moderate and severe periodontitis. These

results are in agreement with the work of Mullally et al.¹ who reported that current users of OCs had poorer periodontal health than non-user controls. However, other factors, such as patients' age, may also play a role because control patients >26 years not taking OCs showed an increase in periodontal disease compared to younger controls. Older age was found to be a risk factor for severe periodontal disease in a Swedish adult population.⁴⁰

Candida species, notably *C. albicans*, are frequent colonizers of the oral cavity. A number of studies have reported on the isolation of yeasts, mainly *Candida*, from periodontal pockets in a large number of patients with periodontitis (7.1% to 44.4%).^{18-20,41-43} Higher percentages of *Candida* colonization have been found in HIV-infected patients with periodontal lesions

Table 5.

Prevalence of *P. gingivalis*, *P. intermedia*, *A. actinomycetemcomitans*, and *Candida* in Periodontal Diseases

Clinical Status	Microorganism							
	<i>P. gingivalis</i>		<i>P. intermedia</i>		<i>A. actinomycetemcomitans</i>		<i>Candida</i> spp.	
	n	%	n	%	n	%	n	%
<i>Contraceptive Users</i>	41	100	41	100	41	100	41	100
Health/gingivitis	2	4.9	5	12.2	0	0	6	14.6
Mild periodontitis	15	36.5	12	29.3	0	0	15	36.5
Moderate periodontitis	7	17.1	8	19.5	1	2.4	9	22
Severe periodontitis	10	24.4*	10	24.4*	5	12.2*	9	22
Subtotal	34	82.9	35	85.4	6	14.6	39	95.1
<i>Contraceptive Non-Users</i>	51	100	51	100	51	100	51	100
Health/gingivitis	6	11.8	6	11.8	0	0	13	25.5
Mild periodontitis	18	35.3	12	23.5	0	0	18	35.3
Moderate periodontitis	7	13.7	6	11.8	0	0	4	7.8
Severe periodontitis	5	9.8	5	9.8	1	2	6	11.8
Subtotal	36	70.6	29	56.9	1	2	41	80.4

* Chi-square test ($P < 0.01$ versus the contraceptive non-user group).

(42.3% to 53.7%).^{18,43,44} In the present study, the prevalence of *Candida* colonization was 95.1% in the OC group and 78.4% in the control group. The reason for the high percentage of *Candida* colonization observed in this study is presently unknown but it may be linked to gender and dietary factors. It has been reported that oral *Candida* colonization in patients with periodontitis is significantly higher in females compared to males.⁴⁵

Several yeast species have been isolated from periodontal pockets in patients with periodontitis. *C. albicans* was the species most frequently isolated but other species including *C. dubliniensis*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. lipolytica*, *C. guilliermondii*, *C. sake*, *Saccharomyces cerevisiae*, *Trichosporon mucoides*, and *Rhodotorula* spp. have also been identified.^{18-20,43,44} In the present study, seven *Candida* species were isolated. Six of them have been described in other studies (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*, *C. glabrata*, and *C. guilliermondii*),^{18-20,43,44} but to our knowledge this is the first study reporting on the isolation of *C. krusei* from periodontal pockets.

Although in this study the number of *Candida* species in the group of patients taking OCs versus the control group was very similar, some species were more frequently isolated in one of the two groups. The present findings suggest that OC use may cause a subgingival selection for certain *Candida* species, such as *C. guilliermondii*, *C. parapsilosis*, and *C. tropicalis*. This selection was more evident for individuals on OCs >3 years because only five *Candida* species (*C. albicans*, *C. parapsilosis*, *C. krusei*, *C. tropicalis*,

and *C. glabrata*) were isolated. Conversely, *C. dubliniensis* was not isolated from periodontal pockets of patients on OCs. The absence of *C. dubliniensis* in the periodontal pockets of patients taking OCs was surprising and suggests that changes caused by sex hormones in the periodontium may reduce or limit *C. dubliniensis* growth. A reduction in the number of *Candida* species at subgingival sites compared to oral mucosa has been reported by Urzúa et al.¹⁸

The association between *Candida* and periodontitis is controversial. Although different *Candida* species have been isolated from

periodontal pockets of patients with periodontitis^{18,19,43,44} and hyphae have been found to invade the periodontal connective tissue,⁴⁶ the absolute proof implicating *Candida* in the pathogenesis of periodontitis is still lacking. In our study, a significant association between *Candida* and periodontitis was only found for *C. parapsilosis*. For the rest of the *Candida* species there were no significant differences between the range of species found in patients with gingivitis or periodontal health and patients with periodontitis. However, in most cases *Candida* isolates were accompanied by well-known periodontopathogens, such as *P. intermedia*, *P. gingivalis*, and *A. actinomycetemcomitans*. In those cases, it seems likely that the bacteria co-isolated with *Candida* were the putative agents responsible for the development of periodontitis. In a limited number of patients, *Candida* species were not co-isolated with periodontal bacteria. However, because most of those subjects presented gingivitis or mild to moderate periodontitis, it is plausible to speculate that *Candida* species might have played a secondary role in the pathogenesis and progression of periodontitis.

One of the main limitations of this study is the relatively small sample size. Further studies are needed to elucidate the impact of OC use on women's periodontal health and the subgingival occurrence of not well-known periodontopathogens, such as *Candida* species.

CONCLUSIONS

OC use may increase the risk of severe periodontitis and seems to cause a selection of certain *Candida*

species in periodontal pockets. OC users showed a higher prevalence of *P. gingivalis*, *P. intermedia*, and *A. actinomycetemcomitans* compared to non-users. *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. tropicalis*, and *C. glabrata* were the species with the ability to survive in the conditions created by the sex hormones after 3 years. *C. dubliniensis* seemed to be negatively affected because it was not isolated in the periodontal pockets of OCs users.

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Correspondence: Professor José Pontón, Department of Immunology, Microbiology and Parasitology, School of Medicine and Odontology, University of Basque Country, Apartado 699, 48080 Bilbao, Vizcaya, Spain. Fax: 34-94-6013495; e-mail: jose.ponton@ehu.es.

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