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Association between periodontal status and idiopathic male infertility

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Abstract: About 30% of male infertility cases are idiopathic. Previous studies reported a positive correlation between deep periodontal pockets and sperm sub-motility, which suggests that periodontitis might have a role in idiopathic semen abnormality pathospermia. We evaluated correlations between periodontal infection parameters and the results of sperm analysis of men with idiopathic infertility. In this observational study, semen quality and periodontal status were analyzed for 95 otherwise healthy men attending an andrology unit for sperm analysis. Half the men in the sperm pathology and normozoospermia groups (50.8% and 50%, respectively) had poor periodontal status. Among the 95 participants, 38% had oligozoospermia, 28% had asthenozoospermia, 16% had cryptozoospermia, and 15% were classified as normozoospermic. Sperm pathology category was not associated with frequency of deep periodontal pockets or calculus. Bleeding on probing was significantly lower among men with asthenozoospermia than among those with normozoospermia. Poor periodontal status was not associated with any sperm pathology category or parameter. In contrast with previous findings, the present results indicate that pathospermia and poor semen quality are not associated with periodontal infection in men with idiopathic infertility. (J Oral Sci 58, 247-253, 2016)

Keywords: idiopathic male infertility; pathospermia; periodontal status; periodontitis.

Introduction

Gingivitis and chronic periodontitis are common chronic diseases in adults worldwide (1-3). Poor dental health can adversely affect a number of systemic conditions and diseases, such as cardiovascular diseases (4), diabetes (5), respiratory diseases (6), and inflammatory bowel diseases (7), and can even lead to preterm delivery (8,9) and systemic disease (10-12). However, the associations between periodontal disease and sperm abnormalities have not been adequately investigated.

The estimated prevalence of infertility among couples of reproductive age is 15% (13), and almost half of such cases are attributable to disturbances in male fertility (14). Although the causes of male infertility have been extensively investigated, 25% of male infertility cases are idiopathic (14). Periodontitis might have a role in subfertility (15). A direct causal relationship was suspected between bacterial colonies in dental foci and therapy-resistant bacteriospermia (15). Furthermore, other studies reported a positive correlation between

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deep periodontal pockets and sperm submotility (16). However, no previous study focused on men with idiopathic infertility. Hence, we investigated the relationship between periodontal status and spermiogram parameters in men with idiopathic infertility.

Materials and Methods

Male patients seeking infertility evaluation were recruited at the Andrology Outpatient Clinic in the Department of Obstetrics and Gynaecology of the University of Szeged, Hungary, between 1 October 2010 and 30 July 2013. Sociodemographic data (age, place of residence, education level, and profession) and information on lifestyle factors (smoking, alcohol consumption, and drug abuse) were collected via a self-reported questionnaire, followed by andrological and periodontal examinations.

Semen collection and analysis

Semen was analyzed and classified according to the World Health Organization (WHO) criteria (17). After 3-5 days of abstinence, semen samples were obtained by masturbation and ejaculation into glass containers in a private room close to the laboratory. The samples were handled at room temperature (22-25°C), and semen analysis began within 1 h after ejaculation. Sperm concentration, total sperm count, total sperm motility, and progressive and nonprogressive motility were assessed with phase-contrast optics at ×200 magnification in a Makler counting chamber (FertiCAD Kft., Budapest, Hungary), as described in the WHO laboratory manual for the examination of human semen (17). Sperm morphology was determined at ×1,000 magnification with oil immersion after Diff-Quik staining (Diff-Quik Staining Set, Medion Diagnostics AG, Düdingen, Switzerland). Progressive motility was specified as the proportion of actively moving spermatozoa. Nonprogressive motility was defined as the percentage of moving spermatozoa with no evidence of progression. Total motility was the sum of progressive and nonprogressive motility. Normozoospermia was classified as a normal ejaculation, as defined by WHO reference values, namely, a sperm cell concentration of 15×10^6/mL or greater, a total sperm count of 39×10^6 per ejaculate or greater, total motility of at least 40%, and progressive motility of 32% or greater. Cryptozoospermia was diagnosed when spermatozoa were absent from a fresh preparation but were observed in a centrifuged pellet. Teratozoospermia was diagnosed when the proportion of sperm cells with normal morphology was less than 4%. In cases of oligozoospermia (sperm concentration $<15 \times 10^6$/mL) and asthenozoospermia (progressive motility <32%), a blood sample was collected for measurement of hormone levels, and an ultrasound examination of the testes was performed. When sperm concentration was less than 1×10^6/mL, screening for karyotyping and azoospermia factor microdeletion of the Y chromosome (AZFa /sY84,86/; AZFb /sY127,134/; AZFc /sY254,255/ regions) was also performed.

Only men with idiopathic infertility were enrolled in the study. Men were excluded from the study if they had a varicocele or testicular microlithiasis (confirmed by ultrasound examination), hypogonadism (verified by hormonal measurements), a genetic disorder (determined by chromosome analysis or molecular genetic investigations), or symptoms of genital infection. Patients with azoospermia (no spermatozoa in ejaculate) were also excluded because of the possibility of seminal duct obstruction or serious testicular abnormality.

Periodontal examination

Where it was impossible to measure probing depth (PD), wisdom teeth and radices were excluded from periodontal charting. The amount of plaque was recorded on a 0 to 3 scale on the "Ramfjord teeth" (#16, 21, 24, 36, 41, and 44) (18) at four surfaces per tooth, according to the criteria developed by Silness and Löe (19). If a Ramfjord tooth was missing, the adjacent molar, premolar, or central incisor was examined. PD was measured at six sites on each tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual) with a millimeter-scale Michigan periodontal probe (Hu-Friedy, Chicago, IL, USA). PD values were recorded in millimeters and rounded down to the nearest whole millimeter. Sulcus bleeding on probing (BOP) was classified as positive if bleeding occurred within 15 s after probing at any tooth site. Dental calculus was recorded dichotomously as present or absent. The number of missing teeth was also recorded. Poor periodontal status was defined as a probing depth of ≥4 mm for at least at one tooth site and BOP at 50% or more of teeth (8). Periodontal examination was repeated for 10 patients after an interval of 30 min, and the intraclass correlation coefficient was 0.90.

The dental characteristics of the oligozoospermia, asthenozoospermia, cryptozoospermia, and sperm pathology (men with any sperm abnormality) groups were compared with those of normozoospermic men (control group). Patients with oligo-asthenozoospermia were included in both the oligozoospermic and asthenozoospermic groups. The dentist was blinded to the andrological status of patients. All dental examinations were performed by one of two authors (M.R. and K.K.), both of whom are experienced in periodontal charting.

Table 1 Characteristics of men with idiopathic male infertility treated at the Department of Obstetrics and Gynecology, University of Szeged (1 October 2010 through 30 July 2013)

Age	*n* = 95 (100%)	
Mean		35.1 ± 5.7 years
Minimum		23.9 years
Maximum		52.1 years
Place of residence	*n* = 95 (100%)	
City		74 (77.9%)
Village		21 (22.1%)
Educational level	*n* = 95 (100%)	
Primary school		1 (1.1%)
Technical school		28 (29.5%)
Grammar school General secondary school		33 (34.7%)
Higher education		33 (34.7%)
Occupation	*n* = 95 (100%)	
Unemployed		2 (2.1%)
Manual worker		41 (43.1%)
Other		23 (24.2%)
Intellectual White-collar worker		29 (30.7%)
Smoking	*n* = 95 (100%)	
No		69 (72.6%)
Yes		26 (27.4%)
5 cigarettes/day		10 (10.5%)
10 cigarettes/day		10 (10.5%)
20 cigarettes/day		6 (6.3%)
Past smoking		44 (46.3%)
Quit >5 years before		13 (13.7%)
BMI		
>30		16 (16.8%)
≤30		79 (83.2%)

Data are presented as number (%), unless otherwise indicated.

The study protocol was approved by the Regional and Institutional Human Medical Biological Research Ethics Committee of the University of Szeged, Hungary, in 2010 (Protocol No. 97/2010). Informed consent was obtained from all study participants.

Statistical analyses

Statistical analyses were performed with the SPSS for Windows program, Version 15 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test confirmed that data for our study samples were not normally distributed. Continuous variables were expressed as mean ± SD. The Mann-Whitney *U* test was used for comparison of continuous variables, depending on case-control status (pathospermia vs. normal cases; poor periodontal status vs. healthy periodontium). Univariate comparisons of categorical variables were done with the χ^2 test. The correlation between the sperm parameters and total number of teeth was determined by using Spearman rank correlations. Statistical significance was defined as a two-sided *P* value of 0.05 or lower. Repeatability of the periodontal examination was assessed using intraclass correlation coefficients. Odds ratios for continuous variables were evaluated by univariate logistic regression and were adjusted for confounding factors (age, smoking status, and body mass index) in multiple logistic regression analysis.

Results

During the study period, 95 men were consecutively recruited into the study. Their sociodemographic characteristics are summarized in Table 1. The average age of the participants was 35.1 years (range: 23-51 years). Overall, 26 men (27.4%) smoked and 16.8% were obese, and 36 (37.9%) were oligozoospermic and 27 (28.4%) were asthenozoospermic (15 patients had both disorders). Cryptozoospermia was diagnosed in 15 (15.8%) of the men, and 32 (33.7%) men were normozoospermic. Some type of sperm abnormality was noted in 63 (66.3%) men. Teratozoospermia was diagnosed in five men (5.2%).

Periodontal examination results were analyzed in relation to spermiogram group (Table 2). The mean plaque index was 0.69 in the sperm pathology group and 0.63 in the control group. Among men with any type of sperm abnormality, average PD was 2.19 mm and BOP was observed at 55.5% of teeth; in normozoospermic men the respective values were 1.99 mm and 53.9%. A PD of ≥4 mm was more frequent in men with a sperm abnormality than in the control group, although the difference was not significant (44 [69.8%] vs. 18 [56.3%]). Sixty-two

Table 2 Dental characteristics of men with idiopathic male infertility, according to sperm pathology, treated at the Department of Obstetrics and Gynecology, University of Szeged (1 October 2010 through 30 July 2013)

	Crypto-zoospermia (*n* = 15)	*P* value	OR (95% CI)	AOR (95% CI)	Astheno-zoospermia (*n* = 27)	*P* value	OR (95% CI)	AOR (95% CI)	Oligozoo-spermia (*n* = 36)	*P* value	OR (95% CI)	AOR (95% CI)	Any sperm-pathology (*n* = 63)	*P* value	OR (95% CI)	AOR (95% CI)	Normo-zoospermia (*n* = 32)
Plaque score	0.66 ± 0.40	0.46	1.32 (0.39-4.39)	2.39 (0.55-10.45)	0.59 ± 0.42	0.79	0.85 (0.30-2.46)	0.98 (0.31-3.06)	0.73 ± 0.43	0.13	1.70 (0.62-4.66)	2.03 (0.68-6.12)	0.69 ± 0.42	0.23	1.43 (0.56-3.66)	1.94 (0.68-5.48)	0.59 ± 0.55
PD (mm)	2.20 ± 0.63	0.72	1.03 (0.37-2.82)	1.04 (0.32-3.38)	1.96 ± 0.73	0.11	0.58 (0.25-1.32)	0.55 (0.23-1.31)	1.90 ± 0.58	0.07	0.44 (0.18-1.04)	0.39 (0.1-0.98)	1.99 ± 0.68	0.17	0.64 (0.33-1.22)	0.61 (0.31-1.20)	2.19 ± 0.61
PD ≥4 mm (*n* (%))	10 (66.7%)	0.50	1.56 (0.43-5.60)	2.28 (0.46-11.23)	21 (77.8%)	0.08	2.72 (0.87-8.55)	3.07 (0.87-10.91)	25 (69.4%)	0.26	1.77 (0.65-4.78)	1.82 (0.64-5.19)	44 (69.8%)	0.19	1.80 (0.75-4.35)	1.89 (0.74-4.83)	18 (56.3%)
Average number of PD ≥4 mm	15.87 ± 22.41	0.50	1.01 (0.98-1.04)	1.01 (0.97-1.04)	10.56 ± 21.57	0.98	0.99 (0.97-1.02)	0.99 (0.96-1.02)	7.86 ± 14.41	0.54	0.98 (0.95-1.01)	0.97 (0.94-1.01)	11.17 ± 20.25	0.66	0.99 (0.97-1.01)	0.99 (0.97-1.01)	13.97 ± 19.59
Frequency of calculus/teeth (%)	20.87 ± 18.71	0.42	0.99 (0.96-1.02)	0.99 (0.96-1.03)	28.87 ± 26.34	0.62	1.00 (0.98-1.02)	1.00 (0.98-1.03)	26.29 ± 20.89	0.70	0.99 (0.98-1.02)	1.00 (0.98-1.02)	26.85 ± 22.80	0.73	1.00 (0.98-1.02)	1.00 (0.98-1.02)	26.87 ± 24.84
Frequency of calculus (*n* (%))	11 (73.3%)	0.24	0.39 (0.08-1.85)	0.49 (0.08-2.98)	20 (74.1%)	0.32	0.41 (0.10-1.58)	0.49 (0.12-2.04)	28 (77.8%)	0.35	0.50 (0.14-1.85)	0.61 (0.16-2.36)	49 (77.8%)	0.41	0.50 (0.15-1.67)	0.61 (0.17-2.13)	28 (87.5%)
Missing teeth	3.40 ± 3.16	0.86	1.00 (0.82-1.23)	0.90 (0.67-1.22)	3.67 ± 3.04	0.72	1.03 (0.87-1.23)	0.97 (0.80-1.19)	3.78 ± 3.44	0.76	1.04 (0.89-1.21)	1.01 (0.84-1.21)	3.48 ± 3.15	0.95	1.01 (0.88-1.16)	0.97 (0.81-1.15)	3.38 ± 2.95
Frequency of BOP/teeth (%)	17.33 ± 7.95	0.21	1.05 (0.97-1.14)	1.04 (0.94-1.14)	10.37 ± 7.73	0.046	0.94 (0.88-1.01)	0.93 (0.87-1.00)	11.69 ± 7.53	0.14	0.96 (0.90-1.02)	0.94 (0.88-1.01)	13.17 ± 8.07	0.44	0.98 (0.93-1.04)	0.97 (0.91-1.03)	14.22 ± 8.13
Frequency of BOP (%)	63.27 ± 30.89	0.46	1.01 (0.99-1.03)	1.01 (0.99-1.03)	52.04 ± 29.39	0.57	0.99 (0.98-1.01)	1.00 (0.98-1.01)	48.00 ± 30.43	0.29	0.99 (0.98-1.01)	0.99 (0.98-1.01)	53.90 ± 30.40	0.74	1.00 (0.98-1.01)	1.00 (0.98-1.01)	55.47 ± 34.63
Frequency of BOP ≥50% (*n* (%))	10 (66.7%)	1.00	1.20 (0.33-4.36)	1.19 (0.26-5.49)	14 (51.9%)	0.44	0.65 (0.23-1.83)	0.58 (0.19-1.77)	17 (47.2%)	0.23	0.54 (0.20-1.42)	0.48 (0.17-1.36)	35 (55.6%)	0.66	0.75 (0.3-1.79)	0.70 (0.28-1.76)	20 (62.5%)
Frequency of BOP ≥50% + PD ≥4 mm (*n* (%))	7 (46.7%)	0.55	1.13 (0.33-3.86)	1.09 (0.26-4.62)	11 (40.7%)	1.00	0.88 (0.31-2.49)	0.74 (0.24-2.28)	14 (38.9%)	0.81	0.82 (0.31-2.15)	0.62 (0.22-1.78)	26 (41.3%)	10.83	0.90 (0.38-2.14)	0.81 (0.33-2.00)	14 (43.8%)

Data from all sperm pathology groups were compared with those from the normozoospermia group (the controls). *P* values were calculated with the Mann-Whitney *U* test, for continuous variables (expressed as mean ± SD), and differences between categorical variables, expressed as number (*n*) and rate (%), were assessed by the χ^2 test. Odds ratios for continuous variables were estimated by univariate logistic regression, and adjusted odds ratios were calculated by multiple logistic regression adjusted for age, smoking status, and body mass index. Statistical significance was defined as a two-sided *P* value of 0.05 or lower.
(A)OR: (adjusted) odds ratio; 95% CI: 95% confidence interval; PD: probing depth; BOP: bleeding on probing.

men had a probing depth ≥4 mm and 15 had a probing depth ≥6 mm. Thus, two-thirds of participants had deep pockets and almost one-sixth had very deep pockets. In addition, the frequency of poor periodontal status, defined as concomitant bleeding on probing at ≥50% of teeth and presence of at least one tooth with a PD ≥4 mm (POB ≥50% + PD ≥4 mm in Table 2) was similar in the sperm pathology group and normozoospermia groups (50.8% and 50%, respectively). Almost all periodontal characteristics studied did not significantly differ between the controls and any spermiogram group (i.e., men with diagnoses of crypto-, astheno-, or oligozoospermia). Surprisingly, BOP per tooth was significantly lower among men with asthenozoospermia, as compared with the other groups. In addition, teratozoospermia was not significantly associated with adverse periodontal status (data not shown in tables). Poor periodontal status was not significantly associated with any sperm abnormality or sperm parameter (Table 3).

Discussion

We found no correlation between poor periodontal status and any form of idiopathic pathozoospermia in the present study. Poor periodontal status had no significant effect on semen quality. Two previous studies reported evidence of a possible connection between periodontal characteristics and sperm pathology (16,20). Klinger et al. (16) investigated 75 men attending an *in vitro* fertilization clinic. Deep periodontal pockets and clinical attachment loss were significantly correlated with sperm submotility. However, there was no such correlation with oligozoospermia or normozoospermia. A similar study, by Nwhator et al. (20), investigated 76 infertile men and found a significant association between deeper periodontal pockets and suboptimal sperm count in men aged 33-38 years, and a correlation between poor oral hygiene and low sperm concentration. However, there was no correlation between oral health and sperm motility. It is important to note that neither study screened potential participants on the basis of cause of infertility (16,20). In addition, spermiogram results were categorized by using earlier reference values for human semen analysis, which were subsequently revised in 2010 (17). Thus, it is difficult to draw conclusions from these conflicting results on associations of chronic gingivitis/periodontitis with male infertility.

Because the duration of spermatogenesis (i.e., from spermatogonium to spermatozoa production) is 74 days in humans (21), we did not assess clinical attachment loss (CAL) in our study. CAL mainly reflects previous periodontal processes and may have a noninfectious cause (e.g., unsatisfactory tooth brushing technique). In addition, not all gingivitis sites develop CAL (22). Our statistical analyses included BOP and PD as the main periodontal parameters. The selection of these variables was based on the design of previous studies. BOP is a known marker of periodontal inflammation (23-25). A PD ≥4 mm is considered "critical probing depth" (26), while lesser PD values are regarded as normal (27). Moreover, BOP and PD are significant factors in

Table 3 Periodontal status and sperm parameters of men with idiopathic male infertility treated at the Department of Obstetrics and Gynecology, University of Szeged (1 October 2010 through 30 July 2013)

	Poor periodontal status (n = 48)		Healthy periodontium (n = 47)		P value	OR (95% CI)
	n	%	n	%		
Cryptozoospermia	9/27	33.3	6/20	30.0	1.00	1.17 (0.33-4.06)
Asthenozoospermia	11/29	37.9	16/30	53.3	0.299	0.53 (0.19-1.51)
Oligozoospermia	15/33	45.5	21/35	60.0	0.33	0.56 (0.21-1.46)
Any sperm pathology	30/48	62.5	33/47	70.2	0.516	0.707 (0.30-1.66)
Normal morphology (%) (mean ± SD)	50.06 ± 19.42		49.71 ± 13.13		0.599	
Motility (%) (mean ± SD)	51.56 ± 22.90		50.33 ± 18.40		0.484	
Progressive motility (%) (mean ± SD)	47.41 ± 23.17		46.23 ± 18.94		0.570	
Nonprogressive motility (%) (mean ± SD)	4.95 ± 4.96		3.82 ± 3.34		0.133	
Sperm concentration	48.36 ± 48.59		44.612 ± 47.02		0.899	
Total sperm number (concentration × semen volume) (millions)	175.70 ± 190.82		202.97 ± 231.52		0.470	

P values were calculated with the Mann-Whitney U test, for continuous variables (expressed as mean ± SD), and differences between categorical variables, expressed as number (n) and rate (%), were assessed by the χ^2 test. Statistical significance was defined as a two-sided P value of 0.05 or lower.
95% CI: 95% confidence interval.

assessing the risk of periodontitis recurrence and activity (24). In this study, we elected to record plaque amount on the Ramfjord teeth only, as Löe and colleagues reported that plaque is not the only factor associated with progression of chronic periodontitis (28). In their study, most patients (81%) exhibited only moderate progression, despite poor plaque control and gingival inflammation in all participants. Moreover, the present partial-mouth examination reduced examination time, which increased patient convenience and reduced examiner fatigue (29). Data for other variable were collected under full-mouth conditions.

Smoking is one of the most common lifestyle factors that adversely affects periodontal and andrological characteristics. It is a main risk factor for periodontal disease (30) and may have a negative impact on fertility. Tobacco exposure has a detrimental effect on sperm production and motility (31,32) and induces sperm DNA damage (33). In our study sample, 27.4% of patients were smokers, but there was no significant difference in the proportion of smokers between study groups. Previous studies found that smoking cessation improves periodontal health (34) and has beneficial effects on sperm characteristics (35); thus, patients should be strongly advised to quit smoking.

A number of pathological mechanisms by which periodontal inflammation affects semen quality have been proposed. A previous study found a correlation between antibiotic-resistant bacteriospermia and presence of focal dental bacterial colonies resulting from bacteriemia in the oral cavity (15). Bacteriospermia was eliminated by dental treatment, and post-treatment sperm parameters were better than those in a control group. In contrast, another study found no association between presence of bacteria in semen and sperm parameters (36). Hence, bacteriospermia was not investigated in our research.

Pathogens can cause a remote pathogenic effect by direct action via bacteriemia and by inducing production of several circulating cytokines (37). Increased levels of circulating cytokines, caused by periodontal disease, can trigger C-reactive protein production in the liver (38,39). Similar inflammatory molecules are important in regulating the blood-testis barrier, which affects the function of Sertoli cells. Tumor necrosis factor-alpha, transforming growth factor-β3 (40), and interleukin-1 can disturb blood-testis barrier dynamics (41). Interleukin-6 alters blood-testis barrier function by inhibiting protein degradation (42). It should be noted however that because systemic inflammatory markers are not specific to periodontal disease and reflect inflammation from all sources, it is difficult to determine the specific effects of periodontitis. Eberhard et al. (43) found a systemic increase in inflammatory markers, while a recent study by Kinane et al. (44) did not show a similar correlation between short-term experimentally induced gingivitis, bacteriemia, and systemic cytokine levels. In any case, our results indicate that poor periodontal status has no remote effect on sperm production or sperm parameters, since poor oral hygiene did not appear to be linked to adverse sperm parameters in idiopathic infertility. The differences between past (16,20) and present findings

might be attributable to the stricter diagnostic criteria used for periodontal conditions and pathospermia in our study.

Because some evidence supports a link between inflammatory molecular mechanisms and the pathological profile of spermiograms, it was logical to examine the potential association between periodontal status in infertile men and the results of sperm analysis. In conclusion, although there is a connection between male infertility and other oral pathologies, poor periodontal status was not associated with infertility among men without genital infections or other apparent causes of infertility.

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