INTERACTION BETWEEN ORAL DISEASES AND OTHER HEALTH-RELATED FACTORS

Ph.D. Thesis

Krisztina Kárpáti, DMD

Supervisors:

Márta Radnai, DMD, Ph.D., Prof.

Zoltán Rakonczay, Ph.D.,Dsc., Prof.Emeritus

Department of Orthodontics and Pediatric Dentistry

Faculty of Dentistry

University of Szeged

Szeged

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I. INTRODUCTION

Oral health is considered as an important indicator of well-being, quality of life (QoL) and overall health status of persons. The World Health Organization defines oral health as a state of being free from chronic mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal (gum) disease, tooth decay, tooth loss, and other diseases and disorders that limit an individual's capacity in biting, chewing, smiling, speaking, and psychosocial wellbeing. In the last several decades, it has been extensively studied that poor dental health can adversely affect a number of systemic conditions and disease. Namely, significant correlation has been found between the emergence of gingivitis and periodontitis and other systemic conditions and diseases, such as cardiovascular diseases, diabetes type II, respiratory diseases, inflammatory bowel diseases, and can even lead to preterm delivery and other systemic inflammatory diseases.

ABO blood group antigens (ABGAs) are an integral part of the red cell membrane and these antigens are also expressed into the body fluids. About 70-80% of the population secretes ABGAs in the saliva and other body fluids . Patients may be characterized by so-called "secretor status", namely non-secretors and secretors. The percentage of secretors in Caucasian populations has been estimated to be around 70-80%. Secretor status of a patient may possibly be a factor influencing the development of systemic oral disease. According to several authors, the ABO blood groups and the Rh factor may constitute a risk factor in the development of periodontal diseases. Nevertheless, other studies revealed a connection between certain other diseases and secretor status, including myocardial infarction, neuroses, depression, retinoblastoma, transitional cell carcinoma of the bladder, pancreatic cancer, gastric cancer and various oral malignancies.

Infertility affects more, than 45 million couples worldwide, which corresponds to a prevalence of 15% of couples of reproductive age. Recent estimates showed that in around 40%-50% of the cases, male factors of infertility (MFI) are responsible for the failure to conceive. Diagnosis of male infertility remains a challenge, as in around one-third of the cases, the cause of infertility remains unexplained despite thorough investigation. It has also been hypothesized that periodontal disease affects the physiological function of the reproductive system, which may be associated with infertility. It has been proposed that periodontal disease has a possible contributing role to idiopathic pathospermia, reduced sperm quality, subfertility and infertility.

II. AIMS OF THE STUDY

The aim of my research was to investigate the association of various health-related factors and dental and oral health, specifically corresponding to factors that have not been previously determined in Hungary. During our studies, the associaton between ABO-secretor status and dental health of younger patients was assessed, to enhance our understanding of this phenomenon with data from with a local context.

Similarly, the potential association between the periodontal diseases and pathospermias has been scarcely investigated worldwide. In addition, no previous studies have focused on the subgroup of patients with idiopathic male infertility in correlation with periodontal status, hence, the investigation to reveal the potential relationship between periodontal status and spermiogram parameters of men with unexplained infertility was chosen as a second hallmark of this thesis.

The specific goals of the study were the following:

1. Determination of the caries prevalence and oral hygiene among children and adolescents in Southern Region of Hungary

2. Assessment of potential effects of ABGA expression (i.e. secretor status) into saliva on caries experience among the tested population

3. Seminal analysis of samples of adult male patients with idiopathic male infertility

4. Examination of the potential connection between sperm abnormalities and periodontal status of men with idiopathic male infertility

III. MATERIALS AND METHODS

A. Ethical considerations

Both study protocols were approved by the Regional and Institutional Human Medical and Biological Research Ethics Committee of the University of Szeged, Hungary (Protocol number: 0420/2009 for children/adolescents and 97/2010 for adult male patients) and followed the Declaration of Helsinki Ethical Principles for Medical Research (2013). Participants (and the parents in case of children and adolescents) were provided information regarding the goals, risks and the procedures involved in the study in both oral and written form. Upon receiving this information, participants (in case of adult male patients) or the parents of the participants (in case of children and adolescents) were asked to give their consent to the participation by signing an informed consent form.

B. Study site and population

A sample of healthy schoolchildren (including children and adolescents aged between 6-18 years) participated in this study, who presented for an annually organized dental screening. The sample size of this study was determined by the turn-up rate (as annual screening is mandatory in Hungary), willingness to participate and the compliance with the exclusion criteria. The study was carried out at the Department of Pediatric Dentistry and Orthodontics of the Faculty of Dentistry, University of Szeged (Hungary), between 1st of January and 31st of December 2011.

Adult male patients (>18 years of age) seeking infertility evaluation were recruited between 1st of October 2010 and 30th of July 2013. The study site was the Andrology Outpatient Clinic of the Department of Obstetrics and Gynecology, University of Szeged (Hungary). Socio-demographic data (age, place of residence, highest level of education and current profession) and information pertaining to lifestyle factors (body mass index, smoking, alcohol consumption and substance abuse) were collected via a self-administered questionnaire, followed by andrological and periodontal examinations.

C. Exclusion criteria for participants

During the enrollment for this study, only healthy subjects with no existing oral or systemic diseases were eligible for the study. In addition, taking any kind of medications *per os* at the time of saliva sampling (due to fears of distorting the results) was also a criterion for exclusion from the study.

Only adult males with idiopathic infertility were enrolled into the study. The following exclusion criteria were defined: varicocele or testicular microlithiasis (confirmed by ultrasound examination); hypogonadism (verified by hormonal measurements); genetic disorders (determined by chromosome analysis or molecular genetic investigations) or symptoms of urogenital infection. Patients with azoospermia (no spermatozoa in the ejaculate) were also excluded due to the possibility of seminal duct obstruction or serious testicular abnormality.

D. Assessment of caries prevalence and oral hygiene among children and adolescents

Data for the calculation of decayed/missing/filled teeth indices (dmf-t/DMF-T) were collected as part of a routine dental status assessment. Caries experience was assessed by calculating the dmf-t/DMF-T scores according to the WHO criteria (1997). A tooth with more than one carious lesion was scored as one decayed tooth. White spot caries lesions were not included. The simplified oral health hygiene index (OHI-S) was also calculated, based on the guidelines of Greene and Vermillion(1964) prior to the determination of secretor status, therefore, the dentists performing the assessment was blind to subjects' secretor status; thus, a significant potential confounding factor was eliminated.

E. Assessment of periodontal status among adult male patients

The amount of plaque was recorded on a 0 to 3 scale on the "Ramfjord teeth" (16, 21, 24, 36, 41, 44) at four surfaces per tooth, according to the criteria defined by Silness and Löe(1964). If a Ramfjord tooth was missing, the adjacent molar, premolar or central incisor was examined. Probing depth (PD) was measured at six sites of each tooth (mesiobuccal, centrobuccal, distobuccal, mesiolingual, centrolingual, and distolingual) with the help of a millimeter scale Michigan periodontal probe (Hu-Friedy, Chicago, IL, USA). PD values were recorded in millimeters and were rounded down to the whole millimeter. Sulcus bleeding on probing (BOP) was classified as positive, if bleeding occurred within 15 seconds after probing at any site of the tooth. Dental calculus was recorded dichotomously as present or absent. The number of missing teeth was also registered. Poor periodontal status was defined as a probing depth of ≥ 4 mm at least at one site and BOP at $\geq 50\%$ of teeth. Repeated periodontal examination was performed in n=10 patients after an interval of 30 minutes, to determine the intra-class correlation co-efficient (ICC), the reliability of ratings or measurements for the cluster of male patients. Based on the results of these ten patients, an intra-class correlation co-efficient value of 0.90 was calculated , which indicated good reliability. Wisdom teeth and radices, where it was impossible to carry out the measurements of the probing depth (PD) were excluded from the periodontal charting. The dental examinations were performed by dentists who were experienced in periodontal charting.

F. Determination of ABO blood groups and secretor status

ABO secretor status was determined based on the protocol described by Vidas *et al*(1999*)*. Unstimulated whole saliva (3-4 mL) was collected from each child/adolescent subject, saliva was collected in test tubes. A glass funnel with a piece of absorbent paper inside was inserted into the test tube and participants were asked to spit into the funnel 1 or 2 times per minute. Using this procedure, it was possible to collect the required amount of saliva. The absorbent paper inside the funnel served to filter contaminants. Saliva collection took place either in the morning hours or early in the afternoon, with at least 2 hours after the last meal or tooth brushing. Saliva samples collected were sealed and placed in boiling water for 10-20 minutes to inactivate enzymes. Samples were then centrifuged at 3000 rpm for 5 minutes and the supernatant was separated and analyzed for ABO blood group antigens (the saliva samples were either processed immediately or stored at -80°C until use) by a hemagglutination inhibition test with appropriate antisera (Blood Grouping Test Reagents Anti-A, Anti-B, Anti-H, Sifin® Berlin, Germany; ALBA clone Anti-A, Anti-B, Anti-H Blood Grouping Reagents, Alba Bioscience®, Edinburgh, United Kingdom).

A plate hemagglutination-inhibition test was employed in the following manner: the saliva samples were diluted in a ratio of 1:2, while A-, B-, and H-antisera were diluted in a ratio of 1:8. Sterile distilled water was used for the dilution of saliva and the reagents. The

diluted saliva samples and antisera were then mixed in test tubes and stored in a wet chamber for 10 minutes. After the incubation period, 1-2 drops of 2-3% erythrocyte solution were added to each sample and the result was recorded. The hemagglutination reaction indicated a lack of ABO antigen production, and a reaction not taking place revealed the presence of antigens in the saliva samples, as in the latter case, the antigen-antibody complexes were already formed when the proper antiserum was added to the saliva sample. In the case of non-secretors, no reaction was seen. The study population was divided into two groups on the basis of dentition (i.e. mixed or permanent), determined within the secretor and non-secretor subgroups. The mixed dentition group had both deciduous and permanent teeth, the permanent dentition group had permanent teeth only. Data analysis was conducted based on this grouping.

E. Semen analysis

Semen collected from adult male patients was analyzed and classified according to the criteria of the World Health Organization laboratory manual for the examination and processing of human semen. 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). The investigation of the semen began within 1 hour following ejaculation. Sperm concentration, total sperm count, total sperm motility, progressive and non-progressive motility were assessed with phase-contrast optics at 200X magnification in a Makler® counting chamber (FertiCAD Kft., Budapest, Hungary) as described in the WHO laboratory manual. Sperm morphology was determined at 1000X 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. Non-progressive motility was defined as the percent of moving spermatozoa with an absence of progression. Total motility was defined as the sum of progressive and non-progressive motility. Normozoospermia was attributed to normal ejaculation as defined by the WHO reference values; sperm cell concentration $15x10^6$/ml or greater; total sperm count $39x10^6$/ejaculate or greater; total motility at least 40%; progressive motility 32% or greater. Cryptozoospermia was diagnosed when spermatozoa were absent from a fresh preparation, but they could be observed in a centrifuged pellet. Criterion for teratozoospermia was the proportion of sperm cells with normal morphology below 4%. In case of oligozoospermia (sperm concentration $<15x10^6$/ml) or asthenozoospermia (progressive motility <32%), blood samples were taken for measurement of hormone levels and ultrasound examination of the testis was also carried out. In case of sperm concentration of $<1x10^6$/ml, karyotyping and azoospermia factor microdeletion of Y chromosome (AZFa /sY84,86/, AZFb /sY127,134/, AZFc /sY254,255/ regions) screening was also performed. The dental parameters of oligozoospermia, asthenozoospermia, cryptozoospermia and of the sperm pathology group (*men with any sperm abnormality*) were compared to men with normozoospermia (*control group*). Patients with oligo-asthenozoospermia were considered in both the oligozoospermic and asthenozoospermic groups. No information about the andrological status of the patient was available for the dentist at the time of the dental examination.

D. Statistical analyses

The normality of variables was tested using Kolmogorov–Smirnov tests. As our data did not fulfill the criteria of normal distribution, nonparametric Mann-Whitney U tests were used for between-groups comparisons. To determine the degree of association between dmf-t/DMF-T and OHI-S, the χ^2 test was used. Wherever our dataset violated the assumption of the χ^2 test , we used Fisher's exact test (FET). The level of statistical significance was set at $p<0.05$. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) Software (IBM SPSS Statistics for Windows 17.0, IBM Corp., Armonk, NY, USA). Mann–Whitney U tests were used for comparison of continuous variables depending on the case-control status (pathospermia vs. normal cases and poor periodontal status vs. healthy periodontium). Statistical significance was defined at the two-sided $p=0.05$ level. Odds ratios for continuous variables were evaluated by univariate logistic regression. Odds ratios were adjusted for confounding factors (age, smoking status and body mass index) in multiple logistic regression analyses. In addition, to assure that the resulting sample size yielded statistically meaningful results, a post-hoc power analysis was also conducted. *Post-hoc* power analysis for the dmf/DMF comparisons was conducted in G*Power (Universität Kiel, Kiel, Germany).

IV. RESULTS

Overall, n=130 healthy schoolchildren (including n_{male}=60, n_{female}=70 participants) were enrolled in the study. The participants were all Caucasian from a homogenous socioeconomic background. Data were analyzed by dentition type and secretor status. Out of the n=130 subjects, n=95 (73.0%) turned out to be secretors, out of which n=54 (56.8%) had permanent dentition (mean age: 15.63 years; range: 14-18 years) and n=41 (43.2%) had mixed dentition (mean age: 9.86 years; range: 6-13 years). Of the n=35 (26.9%) non-secretors, n=17 (48.6%) had permanent dentition and n=18 (51.4%) had mixed dentition. In both dentition types, around 40 percent of the subjects were caries-free (defined as dmf-t/DMF-T=0), however, the ratios were considerably different if secretor status was considered. The ABO antigen distribution in secretors was also determined from the n=130 samples of saliva. The distribution of the examined secretor population overall was as follows: A: 44.2% (n=41); B: 16.8% (n=16); AB: 15.8% (n=15); O: 23.2% (n=22).

The association between OHI-S and dmf-t/DMF-T in the mixed dentition group was not statistically significant, while in the group with permanent dentition, significant association was found between the these two factors ($\chi^2(252, N=71)=346.93$, $p<0.0001$). Significant differences in oral hygiene between the different sexes were found only in the mixed dentition group (n_1=33, n_2=30, U=322, p=0.017, in Mann-Whitney U test). Consequently, girls between 6-12 years generally exhibited better oral hygiene measured by the OHI-S index than boys of the same cohort (mean OHI-S indices were 0.83 vs. 1.21, respectively). In the permanent dentition group, such difference was not observed (n_1=27, n_2=40, U=393, p=0.061, in Mann-Whitney U test). The association between sex and secretor status in mixed dentition was significant ($p<0.0001$, in Fisher's exact test), while in permanent dentition no significant association was found (p=0.458, in Fisher's exact test). To verify that the dmf-t/DMF-T comparisons were not influenced by different oral hygiene as related to sex, OHI-S between the sex subgroups of the secretor/non-secretor groups were also compared. No significant difference was found for either sex (secretors versus non-secretors: [girls: n_1=54, n_2=15, U=397, p=0.914, in Mann-Whitney U test]; [boys: n_1=39, n_2=20, U=345, p=0.479, in Mann-Whitney U test].

There were no statistically significant differences in dmf-t/DMF-T status between secretors and non-secretors in permanent dentition (n_1=17, n_2=54, U=454.5, p=0.952, in Mann-Whitney U test). However, statistically significant difference was found in dmf-t between secretor and non-secretor statuses in mixed dentition (n_1=18, n_2=41, U=234, $p< 0.05$, in Mann-Whitney U test). To determine whether this effect was linked to any secreted antigen in particular, association between dmf-t/DMF-T status and antigen types was computed, but no significant association was found: $\chi^2(30, N = 41)=23.16$, p=0.809. In particular, the mean dmf-t/DMF-T values in mixed dentition were significantly lower in the secretor group (2.1 ± 3.46), compared to the non-secretor group (3.8 ± 3.94). When only dentition types were compared without taking secretor status into account, this difference could no longer be observed (2.60 ± 3.76, 2.59 ± 3.13, mixed and permanent, respectively).

During the study period, n=95 men were recruited consecutively into the study. The average age of the participants was 35.1 years (range: 23-51 years). n=26 men (27.4%) were smokers and 16.8% of the men were obese (BMI>30).

Out of the ninety-five participants, n=36 (37.9%) men were considered to have oligozoospermia, n=27 (28.4%) of patients had asthenozoospermia (including n=15 patients, who had both disorders). Cryptozoospermia was diagnosed in n=15 (15.8%) cases and n=32 (33.7%) of men were normozoospermic. N=63 (66.3%) of the patients had at least one type of sperm parameter disorder. In five cases (5.2%) teratozoospermia was found.

The mean plaque index was 0.69 in the sperm pathology group, while 0.63 in the control group. The average PD was 2.19 mm and BOP occurred at 55.5% of the teeth among those who had any type of sperm abnormality, whereas these periodontal factors among men with normozoospermia were 1.99 and 53.9%, respectively. Men with spermiogram disorders tended to have a PD ≥4mm more frequently than in the control group, although the difference was not significant (44/69.8% and 18/56.3%, respectively). 62 men had ≥4 mm, and 15 men had ≥6 mm probing depths indicating that two thirds of the participants had deep, and almost one sixth of the patients had very deep periodontal pockets. In addition, the frequency of having poor periodontal status, characterized by having bleeding on probing at ≥50% of the teeth and having at least one ≥4mm at the same time (POB≥50% + PD ≥4mm), was practically the same in the sperm pathology group and in the normozoospermia groups (50.8% and 50.0%, respectively).

Poor periodontal status (i.e. studied periodontal parameters) did not show any significant association with any of the sperm abnormalities (crypto-, astheno- and oligozoospermia) and did not correlate significantly with any of the sperm parameters, as compared to controls. Teratozoospermia did not exert any significant correlation with the adverse periodontal status. Interestingly, men with asthenozoospermia had the lowest BOP per teeth among all subgroups tested.

V. DISCUSSION

A. Association between oral health and ABO secretor status

In our present study, a sample of 130 children and adolescents were examined in the Southern Region of Hungary, in order to identify if an association exists between caries experience and secretor status. It is important to emphasize that this study-to our knowledge, the first to address this specific issue-was designed as exploratory, and therefore results are to be interpreted as preliminary. Nevertheless, the results of our study may be regarded as an addition to this scientific debate.

In terms of the percent-wise distribution of blood types (as determined indirectly from antigens secreted into the saliva), our secretor sample was representative of the Hungarian population, as based on previously published reports. The ratio of secretors to non-secretors was also in accordance with previously published data. Caries experience was assessed by the dmf-t/DMF-T scoring system and the simplified oral hygiene index (OHI-S) was also calculated for the study population. The Mann-Whitney U test was used to compare dmf-t/DMF-T and OHI-S between secretors and non-secretors divided into mixed and permanent groups on the basis of dentition. The only significant, secretor status-related difference was found in dmf-t/DMF-T, which was significantly higher in non-secretors, which is one of the main findings of this thesis. Significant difference between sexes was also found in oral hygiene in the mixed dentition group, however, no association was found between dmf-t/DMF-T and OHI-S in either groups.

The main finding of the abovementioned study, is that ABO secretors of mixed dentition exhibit lower caries experience, is fairly difficult to explain at the present level of our knowledge, especially considering that studies focusing on ABO secretion and caries in children are limited. Our results, however, show this effect is associated with mixed dentition, that is, the presence of primary teeth. Considering that primary teeth are more caries-prone due to the lower mineral content of their enamel layer, their morphology, and narrow interdental spaces, it might be hypothesized that secretion of ABO antigens into the saliva may provide some sort of additional protection to primary teeth. The extent of influence of ABO secretor status on adult dental caries is yet to be determined.

B. Association between periodontal health and idiopathic male infertility

In our present study, ninety-five adult males with idiopathic infertility were enrolled to assess any potential interactions between periodontal status and sperm pathologies. Based on our findings, there was no correlation between the poor periodontal status of the participants and any form of idiopathic pathozoospermia in the present study. Our statistical analyses included the BOP and PD results as main periodontal parameters: this was based on previous studies, because BOP has been proven to be a substantial sign of periodontal inflammation. The PD ≥4mm is considered as a “critical probing depth” size, while smaller PD is accepted as normal. Moreover, BOP and PD are considered as significant factors in the risk assessment of recurrence or activity of periodontitis. In our investigation, it seemed to be suitable to record plaque amount on the Ramfjord teeth only, as Löe *et al.*(1964) has shown that plaque is not the solely factor in the progression of chronic periodontitis. In their study, the majority

of patients (81%) had only moderate progression, despite of poor plaque control and gingival inflammation of each individual member of the study population. Moreover, this partial mouth examination is eligible to reduce examination time, which is more convenient for the patients and helps to prevent the fatigue of examiners.

VI. NEW FINDINGS

a. Caries experience and oral hygiene among children and adolescents in light of secretor status: In the studied sample, 73.0% of participants were secretors, around 40% of participants were caries-free. Girls between 6-12 years exhibited significantly better oral hygiene practices based on the OHI-S measurement in the mixed dentition group. The association between OHI-S and dmf-t/DMF-T parameters was only significant in participants with permanent dentition.

b. Assessment of the effects of secretor status on caries experience: There were no statistically significant differences in dmf-t/DMF-T status between secretors and non-secretors in permanent dentition, a significant protective effect was shown for secretors in mixed dentition.

c. Seminal analysis of samples from adult male patients with idiopathic male infertility: Out of the ninety-five participants in our sample, n=63 (66.3%) of the patients had at least one type of sperm parameter disorder detected.

d. Connection between periodontal status and idiopathic male infertility: Poor periodontal status did not show any significant association with any of the sperm abnormalities (crypto-, astheno- and oligozoospermia) and did not correlate significantly with any of the sperm parameters, as compared to controls. Men with asthenozoospermia in our sample had the lowest levels of bleeding on probing (BOP) among all subgroups.

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VIII. PUBLICATIONS

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