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Periodontal disease in adults with untreated congenital growth hormone deficiency: a case–control study

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Abstract

Aim: The aim of this study was to investigate the possible associations between isolated growth hormone deficiency (IGHD) and periodontal attachment loss (PAL) in adults affected by congenital IGHD.

Materials and methods: Forty-five previously identified IGHD subjects were eligible for this study. The final study sample comprised 32 cases (gender:20M/12F; age:44.8 ± 17.5) matched for age, gender, diabetes, smoking status and income to 32 controls (non-IGHD subjects). Participants were submitted to a full-mouth clinical examination of six sites per tooth and were interviewed using a structured, written questionnaire. Periodontitis was defined as proximal PAL ⩾ 5 mm affecting ⩾ 30% of teeth.

Results: No significant differences were observed in the percentage of sites with visible plaque between IGHD and non-IGHD subjects (59.4% *versus* 46.9%, $p = 0.32$). IGHD subjects had significant less supragingival calculus (31.3% *versus* 59.4%, $p = 0.02$) and more bleeding on probing (71.9% *versus* 18.8%, $p < 0.01$) than controls. PAL ⩾ 5 mm was significantly more prevalent (100% *versus* 71.9%, $p < 0.01$) and affected more teeth (30.5% *versus* 6.7%, $p < 0.01$) in cases than in controls. After adjusting for supragingival calculus, IGHD cases had a higher likelihood of having periodontitis than controls (OR = 17.4–17.8, 95% CI = 2.3–134.9, $p = 0.004$–0.005).

Conclusion: Congenital IGHD subjects have a greater chance of having PAL.

Key words: dwarfism; endocrinology; growth hormone; periodontal attachment loss; periodontitis

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Growth hormone (GH) has a fundamental role in postnatal growth, thereby increasing survival capacity, as proper growth provides an adult body that is able to work and reproduce. In addition to stimulating body growth, GH plays an important role in body metabolism and composition, cardiovascular and bone status (Brixen et al. 1995, Carrol & Christ 1998).

In the embryonic tooth development of the rat, GH, GH-binding protein and GH receptor (GHR) are expressed in odontogenic cells that are undergoing morpho-differentiation, histo-differentiation and dentinogenesis (Zhang et al. 1997). GHR's have also been detected in periodontal tissues, influencing cell differentiation and function during root formation, and establishment of the periodontal ligament (Zhang et al. 1992). Animal studies have shown that GH increases the formation of hard tissues in vivo in bone (Rosen et al. 1995), dentine (Young et al. 1993), enamel (Young et al. 1992) and cementum (Clayden et al. 1994). Rats given daily injections of GH for several months develop molar tooth hypercementosis (Becks et al. 1948). Furthermore, GH possesses important immune-regulatory functions (Clark 1997) that may influence chronic inflammatory responses. Indeed,

Conflict of interest and source of funding statement

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GH deficiency (GHD) has been associated with defective antibody and cell-mediated immunity (Kiess et al. 1986, Edwards et al. 1991).

Isolated growth hormone deficiency (IGHD) is a rare disease (1/3480 to 1/10,000 live births) (Vimpani et al. 1977, Lindsay et al. 1994) characterized by proportional post-natal growth retardation, doll facies, a high-pitched voice and central obesity (Aguiar-Oliveira et al. 2010). Most IGHD subjects in developed countries are treated with GH therapy during childhood (Juul et al. 1997). Therefore, collecting a cohort of individuals with untreated unequivocal severe IGHD to assess the periodontal impact of IGHD in humans is a difficult task. In a low socio-economic status population in northeastern Brazil, we have identified a large extended kindred with IGHD due to a homozygous *null* mutation (IVS1+1G → A) in the GHRH receptor (GHRHR) gene (*GHRHR)*. These IGHD subjects have severe short stature and very low serum GH and IGF-I levels throughout life (Aguiar-Oliveira et al. 1999, Salvatori et al. 1999). Most adult subjects have never been treated with GH. This study investigates the possible associations between IGHD and periodontal attachment loss (PAL) in these adults affected by congenital IGHD.

Materials and Methods

Study population and design

The study population was identified in a large extended IGHD pedigree we have previously drafted. This includes 105 subjects (over seven generations) in a total population of 32,000 (Salvatori et al. 1999), residing in Itabaianinha county, in the northeastern Brazilian state of Sergipe. Farming, pottery making, handcraft and small business are their main activities in this area. The spread of the mutation in this area was facilitated by the limited population mobility, and the high prevalence of consanguineous marriages (21.7% of 322 unions examined) (Salvatori et al. 1999). From the 105 subjects detected in the original pedigree in 1999 (Salvatori et al. 1999), the full names of 75 were identified. Of these, 53 were still alive (Aguiar-Oliveira et al. 2010). Some subjects ($n = 4$) could not be traced. Exclusion criteria were age <18 years ($n = 4$), being edentulous ($n = 5$) and having previously received periodontal treatment and/or GH replacement ($n = 8$).

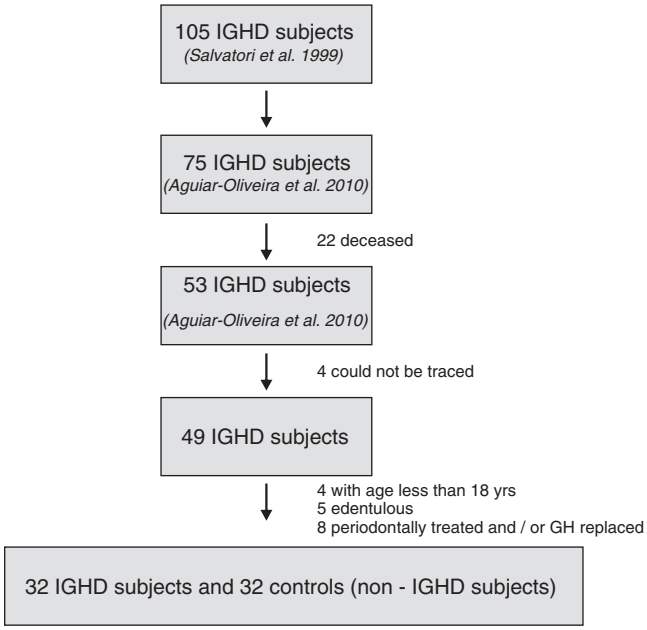

Fig. 1. Flowchart describing the sampling strategy used.

Therefore, from the 49 IGHD subjects who were contacted, the final eligible study sample comprised 32 subjects (20 males and 12 females) (Fig. 1). Thirty-two non-IGHD subjects from Itabaianinha were matched by age (± 5 years), gender, monthly income, diabetes and smoking status [for current smokers: duration of current smoking (± 5 years) and the number of items (cigarettes) smoked on a daily basis (± 10); for former smokers: how many years they had smoked (± 5 years), how many years since stopping smoking (± 5 years) and the number of items (cigarettes) smoked on a daily basis (± 10)]. Potentially eligible controls were selected at the same time as the cases using a neighbourhood approach (expecting that both cases and controls were representative of similar living conditions) (Miettinen 1985).

When a potential IGHD case was contacted by telephone or in person for an interview (through data provided by the medical group), she/he was asked to identify neighbours (known for a long time) with approximately the same demographic characteristics. Of these neighbours, two or three were sorted and contacted. If they fit the eligibility criteria and matched characteristics (age, gender, socio-economic condition assessed by monthly income, diabetes and smoking status), they were invited to participate in the study. Based on the strict matching procedures (including several matching characteristics) and the eligibility criteria, some of the subjects were examined (to provide dental care, if needed) but were not included in the study as control subjects. Finally, 32 non-IGHD subjects from Itabaianinha comprised the control group.

Data collection

Demographic, anthropometric and biochemical data

The fieldwork was conducted between June 2009 and January 2010. All eligible and consenting subjects were invited to participate in an interview performed by a trained dental assistant based on a structured written questionnaire tested in another Brazilian population with similar socio-economic status characteristics (Corraini et al. 2008). The following information was collected: age (in years), gender, occupation, address, household size, income (yes/no) and, if applicable, its approximate monthly amount in Brazilian Reais. Subjects were also asked whether they could read and write (yes/no), how many years of education they had received and whether they had ever received dental treatment (yes/no), and the frequency of tooth cleaning. Current smoking status (yes/no), former smoking status (yes/no), the duration of current and former smoking (in years), time since cessation for former smokers (in years), the type of tobacco-containing items used and the number of items smoked on a daily basis for current and former smokers

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were also collected. Height was measured using a portable stadiometer (Portable stadiometer, Welmy Indústria e Comércio Ltda, Santa Bárbara d'Oeste, São Paulo, Brazil), with subjects standing on the centre of the base with the back to the stadiometer. A portable weighing scale (Portable weighing scale, Filizola S.A. Pesagem e Automação, São Paulo, Brazil) was used to measure their weight. All individuals underwent fasting morning glucose check. The diagnosis of diabetes mellitus was established if the glucose value was >126 mg/dl (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 2003) (Accu-Chek Active, Roche, Brazil).

Clinical Data

The clinical examinations were carried out by a single trained periodontal specialist (I. B.) assisted by a recorder (L. A. O. N.). All clinical examinations were performed under field conditions. Most of them were conducted in the Dwarves Association building and some were conducted in the subjects' homes, always using a Portable Dental Doctor's Office (Consuldent, Rio de Janeiro, Brazil). Probing depth (PD) was measured as the distance (in millimetres) from the free gingival margin to the bottom of the pocket; bleeding on probing (BOP) was recorded as present if bleeding occurred within 15 s after the assessment of PD; and gingival recession (GR) was measured as the distance from the cemento-enamel junction to the free gingival margin. All parameters were determined at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual and mesio-lingual) in all permanent teeth, excluding third molars, using a mouth mirror (Mirror #5, Hu-Friedy, Chicago, IL, USA) and a manual periodontal probe (PCPUNC-15, Hu-Friedy) positioned parallel to the long axis of each tooth. The measurements were rounded to the lowest whole millimetre. When the cemento-enamel junction was located apical to the free gingival margin, it was recorded as a negative value.

PAL was calculated as the sum of the PD and GR values for each site. Two sites per tooth (mid-buccal and mid-lingual) were assessed for the presence of visible plaque (yes/no) and supragingival calculus (yes/no). Supragingival calculus was defined as calcified deposits located on exposed crowns and root surfaces extending up to 1 mm below the free gingival margin. Excessive amounts of supragingival calculus compromising the assessment of the periodontal conditions were removed using periodontal curettes (Gracey curettes 5/6, 11/12, 13/14 Hu-Friedy) before probing.

Measurement reproducibility

The interview questions were tested before the survey through a pre-test comprising eight inhabitants from Itabaianinha county. We tested them in IGHD individuals who had previously received a 2-year treatment with GH completed 10 years before this study (and, therefore, could not be part of the study). The questionnaire was then refined to use a more adequate language. Standardization procedures were used by the interviewer to ensure better consistency of the data obtained (Corraini et al. 2008), including similarity regarding information elicited for cases and controls.

The clinical examiner (I. B.) was trained and calibrated in the clinical examinations 15 days before and 4 months after the beginning of the study. The training and the intraexaminer reproducibility assessments were carried out under the same field conditions as those used in all clinical examinations. Intraexaminer reproducibility of PD and PAL was assessed by double recordings of two contra lateral quadrants in four subjects. The repeat recordings were made 7 days after the first clinical examination. The intraclass correlation coefficients for PD and PAL at the site level were 0.94 and 0.97, respectively, and ranged between 0.93 and 0.96 for PD and between 0.96 and 0.97 for PAL.

Data analysis

The purpose of the statistical analyses was to study the association between PAL and IGHD. Furthermore, the prevalence (of IGHD and controls) was described for PAL$\geqslant$5 mm, $\geqslant$7 mm and for 30% of teeth with proximal PAL$\geqslant$5 mm. Extent, defined as the percentage of sites affected per person, was also described for PAL$\geqslant$5 mm, $\geqslant$7 mm. Other matched and unmatched demographical, behavioural and clinical characteristics were also described among cases and non-cases. Differences in the means and proportions between groups were analysed using paired Student's *t*-test and the McNemar test, respectively.

To explore and quantify the association between untreated IGHD and PAL, a series of conditional logistic regression analyses (Hosmer & Lemeshow, 2000) were carried out using the procedure clogit in a statistical package (STATA version 10.0 for Windows, Stata, College Station, TX, USA). For this purpose, the group variable ''pair'' was created in order to assume that subject outcomes are independent within matched strata. Two periodontal outcomes were considered: the prevalence of 30% of teeth with proximal PAL$\geqslant$5 mm (Tonetti & Claffey 2005) and the presence of one or more sites with PAL$\geqslant$7 mm. Regarding the inclusion of independent variables, all the matched variables in the design were treated as potential confounders; hence, if a factor for which control affected the outcome estimates in prior univariate χ^2-tests at an important degree (significant at $p\leqslant 0.25$), this variable was considered as an independent variable in the multiple model (Rothman et al. 2008). The independent variables considered for inclusion in the conditional logistic regression analysis included: IGHD status (yes/no), years of education ($\leqslant$5/$>$5 years), household size ($<$four people/$\geqslant$four people), subjects who were ever treated by a dentist (yes/no), or ever received oral healthy instructions (yes/no), and percentage sites with supragingival calculus ($\leqslant$25%/$>$25%). All independent variables associated with the outcome at $p\leqslant 0.25$, as evidenced in a univariable logistic regression analysis (Hosmer & Lemeshow 2000), were included in the full model. A variable was considered a confounder if its removal from the model resulted in changes in the β-estimates $>10\%$.

Ethical considerations

Permission to carry out this study was obtained from both the IRB's of the Federal University of Sergipe and of the University of São Paulo and the Itabaianinha' Dwarves Association. All subjects who agreed to participate signed a written informed consent.

Results

Subject characteristics

All the IGHD subjects contacted answered the structured questionnaire and received the full-mouth clinical examination. When a control could not

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be contacted or was not willing to participate, the next living neighbour was sorted and contacted.

Table 1 describes the demographic and behavioural characteristics of cases and controls. By definition, the age, gender, smoking habits and diabetes status did not differ between the groups. Years of education, household size, ever being treated by a dentist and ever receiving oral health instructions were also similar between the groups. As expected, the mean height and weight of IGHD subjects were significantly lower than controls.

Clinical characteristics

The mean number of teeth was similar in the two groups (Table 2). Figure 2 shows the cumulative frequency distributions of PAL$\geqslant$5 mm (Fig. 2a) and $\geqslant$7 mm (Fig. 2b), in the two groups. A trend towards an increase of sites recorded with PAL $\geqslant$5 and $\geqslant$7 mm could be observed, with differences in grouping (IGHD and controls).

When compared with controls, IGHD presented the same amount of visible plaque ($p = 0.32$), significantly less amount of supragingival calculus ($p = 0.02$) and more BOP ($p < 0.01$). PAL prevalence $\geqslant$5 mm, $\geqslant$7 mm and of 30% of teeth with proximal PAL$\geqslant$5 mm was higher in the IGHD group than in the control group ($p = 0.0012$, $p < 0.0001$ and $p < 0.0001$, respectively), and 24 and 25 out of 32 IGHD cases, and six and four out of 32 controls were diagnosed with periodontitis (PAL$\geqslant$5 mm in $\geqslant$30% teeth and PAL$\geqslant$7 mm in one or more sites, respectively) (Table 2)

In the univariate analyses, none of the matched variables in the design affected the outcome estimates in prior univariate χ^2-tests to a significant degree (significant at $p \leqslant 0.25$). Regarding the unmatched variables, the independent variables included in the multiple models were percentage of sites presenting supragingival calculus ($p = 0.04$ and $p = 0.12$) for the prevalence of PAL$\geqslant$7 mm and the prevalence of 30% of teeth with proximal PAL$\geqslant$5 mm, respectively; household size ($p = 0.12$); and subjects who were ever treated by a dentist ($p = 0.18$) for the prevalence of 30% of teeth with proximal PAL$\geqslant$5 mm (Table 3). Table 4 shows that IGHD subjects had a higher likelihood of having a prevalence of 30% of teeth with proximal PAL$\geqslant$5 mm in the final multiple conditional regression models (OR = 17.4, 95% CI = 2.3–131.2, $p = 0.004$), and a prevalence of PAL$\geqslant$7 mm (OR = 17.8, 95% CI = 2.3–134.9, $p = 0.005$). However, the percentage of sites with supragingival calculus had to be retained in the model because of confounding.

Table 1. Selected matched and unmatched demographic, biological and behavioural characteristics of IGHD ($N = 32$) and Control ($N = 32$) subjects

Characteristics	IGHD ($N = 32$)	Controls ($N = 32$)
Mean age	44.8 ± 17.5	44.6 ± 17.2
Individually matched characteristics		
Age group [years; *N*(%)]		
20–29	6 (18.8%)	6 (18.8%)
30–39	6 (18.8%)	7 (21.9%)
40–49	6 (18.8)%	6 (18.8%)
50–59	8 (25.0%)	7 (21.9%)
60+	6 (18.8%)	6 (18.8%)
Gender, males [*N* (%)]	20 (37.5%)	20 (37.5%)
Current or former smokers [*N* (%)]	11 (34.4%)	11 (34.4%)
Mean (SD) pack-years	4.95 (3.31)	5.30 (5.41)
Diabetes mellitus	1 (3.1%)	1 (3.1%)
Unmatched characteristics		
>5 years of education [*N* (%)]	15 (57.7%)	16 (53.3%)
Household size [$\geqslant$four people, *N* (%)]	16 (50.0%)	22 (68.8%)
Subjects ever treated by a dentist [*N* (%)]	19 (59.4%)	21 (65.6%)
Subjects ever received oral health instructions [*N* (%)]	14 (43.8%)	13 (40.6%)
Mean (SD) height (in metres)	1.23 (0.08)*	1.64 (0.10)
Mean (SD) weight (in kg)	34.7 (8.1)*	66.0 (11.7)

*$p < 0.01$.
IGHD, isolated growth hormone deficiency; SD, standard deviation.

Table 2. Selected clinical characteristics of IGHD ($N = 32$) and Control ($N = 32$) subjects

Characteristics	IGHD ($N = 32$)	Controls ($N = 32$)
Mean *N* teeth present (95% CI)	16.0 (13.2–18.7)	18.8 (16.8–20.7)
Prevalence PAL$\geqslant$5 mm (%)	100%*	71.9%
Extent PAL$\geqslant$5 mm [mean (95% CI)]	30.5% (20.3–40.7)*	6.7% (1.8–11.6)
Prevalence PAL$\geqslant$7 mm	78.1%*	12.5%
Extent PAL$\geqslant$7 mm [mean (95% CI)]	11.1% (5.6–16.6)*	0.6% (− 0.4–1.7)
Prevalence PD$\geqslant$5 mm	96.9%*	21.9%
Extent PD$\geqslant$5 mm [mean (95% CI)]	11.4% (7.7–15.1)*	0.3% (0.1–0.5)
Prevalence PD$\geqslant$7 mm	46.9%*	0%
Extent PD$\geqslant$7 mm [mean (95% CI)]	1.5% (0.6–2.4)	–
Prevalence 30% teeth interproximal PAL$\geqslant$5 mm	75%*	18.8%
% sites $\leqslant$15%	28.1%	81.2%
Bleeding on probing >15%	71.9%*	18.8%
% sites <50%	40.6%	53.1%
Visible plaque $\geqslant$50%	59.4%	46.9%
% sites $\leqslant$25%	68.7%	40.6%
Supragingival calculus >25%	31.3%	59.4%†

*$p < 0.01$.
†$p < 0.05$.
CI, confidence interval.
IGHD, isolated growth hormone deficiency; PAL, periodontal attachment loss.

Discussion

To date, no study addressing periodontal parameters or destructive periodontal diseases in GHD individuals has been reported. This study provides the first data describing the PAL in the largest cohort of subjects with lifetime,

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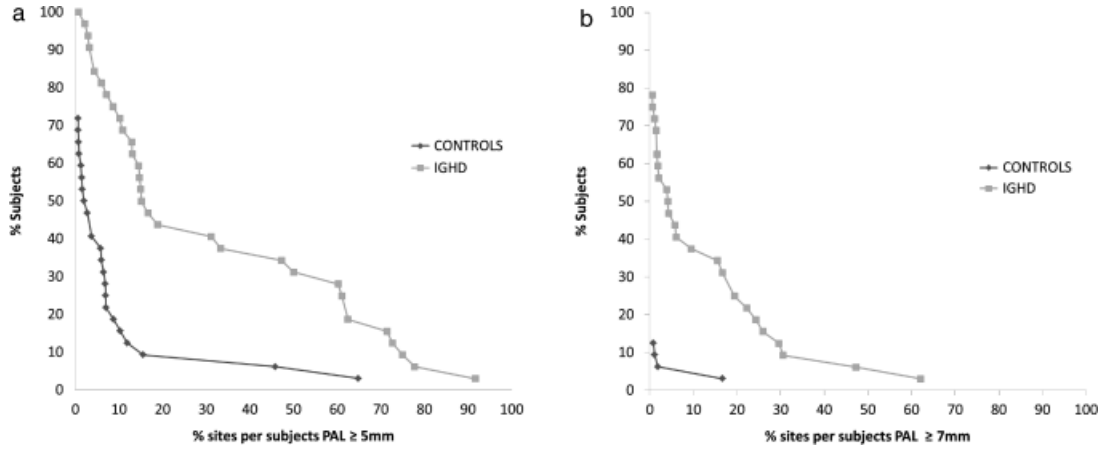

Fig. 2. The cumulative frequency distributions of periodontal attachment loss (PAL) ⩾5 mm (a) and ⩾7 mm (b), according to growth hormone status. IGHD, isolated growth hormone deficiency.

Table 3. Univariable logistic analyses for the 1–1 matched data, unmatched independent variables, $n = 32$ pairs

Independent variable	Levels	Prevalence PAL⩾7 mm				30% teeth interproximal PAL⩾5 mm			
		OR	95% CI	*p*	$(n_{10};n_{01})^*$	OR	95% CI	*p*	$(n_{10};n_{01})^*$
Education (years)	⩽5	Ref	–	–	–	–	–	–	–
	>5	0.67	[0.11;3.99]	0.66	(11;19)	0.67	[0.11;3.99]	0.66	(11;19)
Household size	<4	Ref	–	–	–	–	–	–	–
	⩾4	0.5	[0.13;2.0]	0.33	(13;22)	0.29	[0.06;1.38]	0.12	(15;23)
Ever treated by a dentist?	No	Ref	–	–	–	–	–	–	–
	Yes	0.8	[0.21;2.98]	0.74	(11;22)	0.33	[0.07;1.65]	0.18	(12;22)
Ever received oral health instructions?	No	Ref	–	–	–	–	–	–	–
	Yes	1	[0.29;3.45]	1	(17;15)	1.67	[0.40;6.97]	0.48	(18;15)
IGHD	No	Ref	–	–	–	–	–	–	–
	Yes	22	[2.97;163.21]	0.003	(4;7)	19	[2.54;141.92]	0.004	(6;8)
Supragingival calculus (%)	⩽25	Ref	–	–	–	–	–	–	–
	>25	0.11	[0.01;0.88]	0.04	(17;17)	0.29	[0.06;1.38]	0.12	(16;15)

*Frequency of discordant pairs.
IGHD, isolated growth hormone deficiency; PAL, periodontal attachment loss; Ref, reference category.

Table 4. Final conditional multiple logistic regression analyses model for the outcome variables prevalence of PAL⩾7 mm and prevalence of 30% teeth with interproximal PAL⩾5 mm

Independent variable	Levels	Prevalence PAL⩾7 mm		30% teeth inter-proximal PAL⩾5 mm	
		OR	95% CI	OR	95% CI
IGHD	No	Ref	–	Ref	–
	Yes	17.8*	[2.3; 134.9]	17.4*	[2.3; 131.2]
Supragingival calculus (%)	⩽25%	Ref	–	Ref	–
	>25%	0.3	[0.0; 12.5]	0.5	[0.0; 12.6]

*$p < 0.01$.
IGHD, isolated growth hormone deficiency; PAL, periodontal attachment loss; Ref, reference category.

untreated, congenital IGHD described to date. Our results demonstrate that the prevalence of 30% of teeth with proximal PAL⩾5 mm and of PAL⩾7 mm is higher in IGHD subjects in comparison with matched controls living in the same area.

Regarding the epidemiology of periodontal diseases in GH sufficient Brazilian individuals (historic controls), only two studies, in which a full-mouth assessment of PAL in six sites per tooth was performed, are available. One was conducted in an isolated population of Cajaíba, in the southeastern coastline of Brazil (Corraini et al. 2008), and another one in an urban population in southern Brazil (Susin et al. 2004). The prevalence of PAL⩾5 mm was 83% and 79%, respectively, and for PAL⩾7 mm, it was 52% in both populations. In the present study, the prevalence of PAL⩾5 mm and ⩾7 mm in the control group was 71.9% and 12.5%, respectively. The reason for the reduced prevalence we observed in our controls may be due to environmental, genetic factors or/and a small sample size. However, even on using these two Brazilian populations (Susin et al. 2004, Corraini et al. 2008) as controls, our IGHD cases presented a numerically higher prevalence and extent of PAL⩾5 mm and ⩾7 mm (prevalence: 100% *versus* 79% (Corraini et al. 2008) and 83% (Susin et al. 2004) for PAL⩾5 mm, and 78.1% *versus* 52% (Susin et al. 2004, Corraini et al. 2008) for PAL⩾7 mm; extent: 30.5% *versus* 25% (Corraini et al. 2008) for

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PAL ⩾ 5 mm and 11.1% *versus* 11% (Corraini et al. 2008) for PAL ⩾ 7 mm).

The IGHD group presented significantly less amount of supragingival calculus and more sites presenting BOP than the control group. These observations (especially BOP) could suggest that, in addition to the endocrine effects, immune function (Rapaport et al. 1995) is influenced by the severe lack of GH. This possibility is also suggested by: (1) the reduced body surface area-corrected spleen volume (an organ participating in the immune system) in these IGHD individuals (Oliveira et al. 2008), (2) the observation that GH administration enhances the function of myeloid cells and neutrophils in subjects with adult GHD (Kotzmann et al. 1996, Derfalvi et al. 1998, Sohmiya & Kato 2001), (3) the fact that GHD increased serum pro-inflammatory cytokines (IL-6) (Leonsson et al. 2003) and (4) the GH influence on thymus' function (responsible for the generation of self-tolerant and competent naïve T-cells, as well as of self-antigen-specific natural regulatory T-cells) (Morrhaye et al. 2009).

IGHD was associated with a higher prevalence of 30% of teeth with proximal PAL ⩾ 5 mm and a prevalence of PAL ⩾ 7 mm in the final conditional multiple regression model (OR = 17.4 and 17.8, respectively). This stresses the possible influence of IGHD on periodontal disease. However, the reason for the large confidence interval for the OR values observed in this study may also be due to the relatively low number of subjects (Greenland et al. 2000), especially regarding the discordant pairs of the IGHD variable (Table 3). Despite this, the analysis was possible due to the strict matching procedures (Schlesselman 1982, Rothman et al. 2008), and the OR and 95% CI estimates found still provide meaningful information showing that the IGHD group presented a significantly higher prevalence of PAL compared with the control group.

One limitation of this study was that examiner blinding was obviously not possible. Because of this, measurement bias could have affected the prevalence and extent estimates of disease in the two groups (Schulz & Grimes 2002). On the other hand, the exposure (IGHD) could be assessed with a high level of precision, as it is a lifetime genetic exposure (Salvatori et al. 1999). Furthermore, the 1:1 matched strategy was used in this study because it was very difficult to achieve a higher number of controls due to the eligibility criteria and the strict matching of cases and controls (including several matching variables). Therefore, we opted for a strict matching closest that in control group selection, as the literature shows that when study power under equal allocation is extremely small or large (< 0.1 or > 0.9), increasing the number of controls will not add enough to the study power (Lasky & Stolley 1994). However, this could have led to over-matching by the fact that five variables were used for matching, which can produce bias when one matches for a factor that is associated with exposure but not disease (Schlesselman 1982).

In this population, treatment with GH is offered for free to all IGHD children. But different from western societies, short stature is not considered a handicap by many families in the Itabaianina community, which is why some of the early diagnosed IGHD subjects are not treated.

Regarding the study design, it was impossible to select cases based on the outcome (due to the extremely rare pattern of this exposure); hence, cases were selected by the exposure (Wacholder et al. 1992). As stated, to avoid problems using a case–control design with a cohort method analysis, a strict matching procedure was adopted to avoid controlling for multiple potential causes of periodontal diseases in the analysis, and, in order to confirm these results, follow-up cohort studies in these exposed IGHD would be necessary.

In conclusion, untreated IGHD subjects with a homozygous *GHRHR* mutation have a greater chance of having PAL than matched local controls. The reason for this finding can only be speculated, and may be due to the effect of GHD on the periodontal tissues or/and may be mediated by the effects of the lack of GH on immune function.

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Clinical Relevance

Scientific rationale for the study: This study provides the first publication with detailed data investigating the possible association between PAL and IGHD. Besides the influence on periodontal tissues formation, GH possesses important immunoregulatory functions. This may also mediate the effect of IGHD on periodontal disease.

Principal findings: This study found that subjects with IGHD have a greater chance of having PAL.

Practical implications: IGHD may increase the risk of having periodontitis, which means that, if cohort studies can confirm this statement, population or clinical intervention by means of GH therapy can prevent periodontal breakdown in these subjects.

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