Periodontal status and serum biomarker levels in HFE haemochromatosis patients. A case-series study

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Periodontal status and serum biomarker levels in HFE hemochromatosis patients. A case series study.

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Conflict of interest

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Clinical relevance

Scientific rationale: HFE-related hereditary hemochromatosis (*HFE*-HH) is the most common form of genetic iron overload in Caucasians. As iron is an essential growth factor and a regulator of the virulence of *Porphyromonas gingivalis* and *Prevotella intermedia*, two common periodontal pathogens, increased severity of periodontitis was hypothesized in *HFE*-HH patients.

Principal findings: *HFE*-HH patients presented severe form of periodontitis associated with high transferrin saturation.

Practical Implications: Dental examination should be included in the initial assessment of all *HFE*-HH patients.

Abstract

Aim. To investigate the association between periodontal status and serum biomarkers in patients with *HFE* hemochromatosis.

Material and Methods. This clinical case series included 84 *HFE*-C282Y homozygous patients. Periodontal evaluation was performed using clinical attachment level, probing depth, gingival bleeding index, visible plaque index and gingival index. Serum markers of iron metabolism were collected from medical records. The relationship between serum biomarkers of iron burden and the severity of periodontitis was investigated.

Results. The study population consisted of 47 men and 37 women, routinely treated in the Unit of Hepatology, University Hospital, Rennes. All patients presented with periodontitis (mild: n=1, moderate: n=37 and severe: n=46). There was a positive association between transferrin saturation > 45% and the severity of periodontitis (adjusted odds ratio=5.49, p=0.002).

Conclusion. Severe periodontitis is associated with the severity of iron burden in patients with *HFE*-related hereditary hemochromatosis. Dental examination should be included in the initial assessment of all these patients.

Introduction

In France, 45% of adults over 40 years of age have at least one tooth with localized periodontitis (Bourgeois et al. 2007). Severe forms of periodontitis (*i.e*. with periodontal pockets ≥ 5 mm) affect 11.2% of adults worldwide (Kassebaum et al. 2014). Periodontitis is associated with risk factors, including age, gender (Harris & Glassell 2011, Silva-Boghossian et al. 2011), tobacco smoking (Bagaitkar et al. 2011, Kumar & Faizuddin 2011), and obesity (Linden et al. 2007, Morita et al. 2011). Recent studies indicate that bacteria involved in periodontitis could be associated with systemic diseases, including diabetes (Ide et al. 2011), rheumatoid arthritis (Hayashi et al. 2010, Sezer et al. 2013) and atherosclerotic cardiovascular disease. Diabetes and arthritis are classical features in patients with hemochromatosis (Powell et al. 2016).

HFE-related hereditary hemochromatosis (*HFE*-HH), the most common form of genetic iron overload in Caucasians, is mainly due to homozygosity for a major mutation, p.Cys282Tyr (C282Y) on the *HFE* gene which is involved in the production of a key regulator of plasma iron, hepcidin. The allelic frequency of the C282Y mutation is 6.2% in the general European population, but it can reach 12% in Brittany or Ireland (Jouanolle et al. 1998, Powell et al. 2016). C282Y homozygosity is responsible for a hepcidin-deficient state leading to an increase in serum transferrin saturation, which potentially results in increased body iron stores (EASL guidelines 2011). The classical treatment of *HFE*-HH patients is venesection to remove iron excess.

Iron lies at the center of a battle for nutritional resource between higher organisms and their microbial pathogens. The iron status of human hosts affects the pathogenicity of numerous infections (Drakesmith & Prentice 2012). It has been reported that non-transferrin bound iron, an abnormal biochemical form of iron that occurs when transferrin saturation exceeds 45%, could modulate bacterial growth and virulence (Jolivet-Gougeon et al. 2008). Most cases of *HFE*-HH are diagnosed during adulthood, between 40 and 50 years of age. Iron burden might represent a favorable background for the development of periodontitis. First, iron is both an essential growth factor and a regulator of the virulence of *Porphyromonas gingivalis* and *Prevotella intermedia*, two common periodontal pathogens (Byrne et al. 2013). Second, as hepcidin is known for its antimicrobial activity (Ganz 2013), low hepcidin production could be a favorable factor for the development of periodontal disease. Finally, bone loss is a frequent complication of *HFE*-HH (Guggenbuhl et al. 2005), which is likely to aggravate alveolar bone damage in periodontitis.

The main goal of the present study was to investigate the association between periodontal status and serum biomarker levels in *HFE*-HH patients and to seek a putative relationship between markers of iron burden and the severity of periodontitis in these patients.

Patients and Methods

Participants

A case-series study was carried out after approval by the local ethical committee (CPP Ouest V - 10/02-744). All participants were recruited, either during phlebotomy therapy or at diagnosis, in the unit of hepatology, University hospital, Rennes between June 2011 and June 2012. They gave an informed written consent and were instructed on the prevention and treatment of periodontitis, as well as on oral hygiene procedures.

Inclusion criteria were homozygosity for the *HFE* C282Y mutation, age between 35 and 65 years and the presence of at least 10 natural teeth. Non-inclusion criteria were pregnancy, presence of another systemic disease (e.g., diabetes), periodontal therapy within the last 12 months, and treatment with either systemic antibiotics or drugs known to cause gingival hyperplasia (*i.e.*, hydantoins, ciclosporin, diltiazem, dihydropyridine) within the last 3 months. In the absence of data on periodontitis in *HFE*-HH patients, the number of patients to be included was based on the precision (± 10%) of the estimation of prevalence of periodontitis (50%) in the French population (Bourgeois et al. 2007).

Interview and collection of clinical and laboratory data

Clinical data recorded at the time of dental examination included body mass index (BMI), smoking status (non-smoker and current or former smoker), teeth-brushing habits (once, twice, or more per day), and dental visits per year (< 1 or ≥ 1). Serum ferritin, iron, and transferrin levels and transferrin saturation were collected from medical charts using routine procedures at the time of both diagnosis of hemochromatosis and dental examinations. The time elapsed between the diagnosis of hemochromatosis and dental examination was also registered.

Evaluation of periodontal status

Full-mouth periodontal examination was performed by one examiner (VM), using a sterile U.S Williams PDT sensor probe at a pressure of 20g (ZILA-PRO-DENTEC) positioned parallel to the long axis of the tooth. The following parameters were evaluated: probing depth (PD) as the distance (mm) between the gingival margin and the bottom of periodontal pocket; clinical attachment level (CAL) as the distance (mm) between the cemento-enamel junction and the bottom of periodontal pocket. Each of the following sites was recorded mesio-, mid-, and disto-buccal; mesio-, mid-, and

disto-lingual from all present teeth except for third molars. Gingival condition was diagnosed using the gingival bleeding index (GBI), gingival index (GI) (Loe 1967, Schatzle et al. 2003) and visible plaque index (VPI) (Silness & Loe 1964, McClanahan et al. 2001).

Definition of periodontal case

To evaluate the presence and severity of periodontitis, case definitions developed by the Center for Disease Control and Prevention and the American Academy of Periodontology (CDC/AAP) were used: mild periodontitis was defined as "≥2 interproximal sites with CAL ≥3 mm, and ≥2 interproximal sites with PD ≥4 mm (not on same tooth) or one site with PD ≥5 mm; moderate periodontitis as ≥2 interproximal sites with CAL ≥4 mm (not on same tooth), or ≥2 interproximal sites with PD ≥5 mm (not on same tooth); severe periodontitis as ≥2 interproximal sites with CAL ≥6 mm (not on same tooth) and ≥1 interproximal site with PD ≥5 mm" (Eke et al. 2012).

Statistical analysis

Data management and statistical analysis were performed using R (version 3.3.1). Results of descriptive analysis are presented as frequencies and mean ± standard deviation (SD). Distribution of continuous variables was assessed using the Shapiro-Wilk test. To analyze the association between the severity of periodontitis and the level of serum biomarkers, Student's t and Mann-Whitney tests were used as appropriate. The Chi-squared test was used for the analysis of categorical variables. $P < 0.05$ was considered as significant. The association between the severity of periodontitis (mild or moderate versus severe periodontitis) and transferrin saturation greater than 45% was assessed using logistic regression analysis adjusted for confounding variables (age, gender, smoking habit, plaque index, dental visit and time elapsed between the diagnosis of hemochromatosis and dental examination). Results are presented as odds ratio (OR) and 95% confidence intervals (CI).

Results

Characteristic of HFE-HH patients

The review of charts from the 244 C282Y homozygous patients regularly phlebotomized in the unit of hepatology resulted in the identification of 87 patients fulfilling inclusion criteria (Figure 1). From these, aged from 35 to 64 years (mean age 50.7 ± 8.4 years), 54% were males.

At the time of the diagnosis of hemochromatosis, one-third of the participants presented with chronic asthenia, 29.8% with osteo-arthropathy, 5.8% with clinical and biochemical liver disease, and 2.4% with cardiopathy. Two males had hypogonadism. Mean serum ferritin (SF) levels were 815 ± 728 µg/L (range: 86 – 4021, normal range: 20-150 µg/L in pre-menopausal women and 20-400 µg/L in post menopausal women and men), transferrin saturation was greater than 60% (normal range: 20-40) in 75% of patients, and mean hepatic iron concentration assessed by MRI was 195 ± 79 µmol/g dry liver (upper limit of normal = 36). All patients had normal C reactive protein levels (*i.e.* < 5 mg/L). Twenty-six patients had liver biopsy. Two presented significant fibrosis (bridging fibrosis or cirrhosis). Liver biopsy was not performed in the other patients because of serum ferritin levels < 1000 µg/L, which indicates the absence of cirrhosis (Guyader et al. 1999).

During the initial treatment of iron excess by weekly phlebotomy, the amount of removed iron ranged from 0.5 to 24 g (mean 4.6 ± 3.9 g).

At the time of dental examination, 82.2% of patients were already treated by regular phlebotomies and 17.8% had not been previously phlebotomized. Serum ferritin was 174.5±418.1 µg/L, serum iron 26.7±9.1 µmol/L, serum transferrin 2.1±0.3 g/L, and transferrin saturation 51.5±18.3%. All patients had normal serum C reactive protein. The mean time that had elapsed between the diagnosis of hemochromatosis and dental examination was calculated as 10.1±7.65 years.

Prevalence and severity of periodontitis (Table 1)

All patients underwent complete periodontal examination. Based on the CDC/AAP case definition, all presented periodontitis, 44% and 54.8% had moderate and severe periodontitis, respectively. Characteristics of patients did not differ according to the severity of periodontitis. Proportions of sites per mouth with CAL ≥ 3 mm, CAL ≥ 5 mm and PD ≥ 4 mm were 50.1±17.4, 10.3±11.8 and 11.9±9.7 percent, respectively. Individuals with severe periodontitis showed the worst values of PD, CAL, as well as for bleeding on probing and GI, with statistically significant differences ($p < 0.001$) when compared to those with mild to moderate periodontitis. There was no difference between both groups with respect to visible plaque. Men and women with *HFE*-HH were equally affected in terms of both frequency and severity of periodontitis. All patients reported brushing their teeth at least once daily and 80 % reported visiting the dentist at least once a year. Sixty four per cent were current or former smokers.

Periodontitis severity and serum iron biomarkers at the time of dental examination

As indicated in table 2, transferrin saturation was significantly higher in the group with severe periodontitis when compared to the group with mild or moderate periodontitis (55.1% versus 47.2% - $p = 0.029$). Subjects with transferrin saturation ≥45% had a 4-fold higher risk (OR= 4.27 - 95%, CI = 1.68–11.37, p=0.003) of severe periodontitis than those with transferrin saturation < 45%. After adjustment for confounding variables, this risk remained elevated (OR= 5.49 - 95%, CI = 1.85–16.28, p=0.002).

Discussion

HFE-HH is a hepcidin-deficient disease resulting in parenchymal iron overload. It is associated with complications commonly found in patients with periodontitis (*i.e.* diabetes and bone loss). The relationship between iron metabolism and periodontitis was previously investigated and no significant serum iron and serum hepcidin modification was found (Carvalho et al. 2016) in patients with periodontitis compared to healthy subjects. To the best of our knowledge, the present study is the first to explore the periodontal status of patients with hemochromatosis.

Serum ferritin level is considered a reliable indicator of body iron stores. However, it can overestimate iron burden in chronic inflammatory conditions such as periodontitis or chronic liver disease. A relationship between serum CRP and ferritin levels has been reported in periodontitis (Chakraborty et al. 2014), but, in the present series, it is unlikely that inflammation could explain, even partly, the rise in serum ferritin levels since serum CRP levels were normal in all patients and initial serum ferritin levels were markedly elevated and well correlated with the amount of iron removed. Then we can assume that serum ferritin levels were truly representative of iron excess and validated the iron-overloaded status of the patients studied.

It is noteworthy that all patients had increased body iron stores at the time of the diagnosis of hemochromatosis and all had periodontitis at the time of dental examination, which was performed after iron removal in 74 out of 87 patients.

To investigate whether indicators of body iron stores were associated with the severity of periodontal disease, the population was divided into two groups (mild and moderate compared to severe periodontitis). General characteristics of both groups were similar with respect to general and iron-related features except for transferrin saturation at the time of dental examination, which

was significantly increased in the group with severe periodontitis. The risk of severe periodontitis was 5.49 times greater when transferrin saturation exceeded 45%, even after adjustment for age, gender, smoking habits, plaque index and time elapsed between the diagnosis of hemochromatosis and dental examination. The threshold of 45% was chosen because it corresponds to the threshold above which non-transferrin bond iron, a highly toxic form of iron occurs (Brissot et al. 2012).

Periopathogens live within periodontal pockets in a complex, mixed, and interacting microbial community bathed in the gingival crevicular fluid, an anaerobic and inflammatory environment. Iron concentration is increased in the crevicular fluid of periodontal diseased sites (Wang et al. 1990). Iron regulates 10% to 20% of bacterial genes and is necessary for bacterial growth. It also favors the virulence of numerous bacteria, some of which are periopathogens (Khan et al. 2007). As soon as transferrin saturation exceeds 45%, non-transferrin bound iron is increased in patients with hemochromatosis (Loreal et al. 2000). This highly reactive form of iron may be more bioavailable for bacteria. Moreover, the relative hepcidin-deficient state characterizing *HFE*-HH may enhance bacterial virulence and/or proliferation because hepcidin has a physiological antimicrobial effect. These data are in line with a direct role of iron in chronic periodontal infection (Lewis 2010, Xie & Zheng 2012, Byrne et al. 2013).

Finally, bone loss is a common feature in human *HFE*-HH, with a frequency ranging from 25.3% to 34.2% (Guggenbuhl et al. 2005). It results from both increased bone resorption due to elevated osteoclast activity and decreased osteoid thickness due to diminished osteoblast activity (Conte et al. 1989). In *Hfe*$^{-/-}$ male mice, a phenotype of osteoporosis develops associating low bone mass and altered bone microarchitecture with a relationship between bone iron load and the increase in the number of osteoclasts (Guggenbuhl et al. 2011). This supports our findings that all of *HFE*-HH patients had periodontitis – of whom 54.8% presented a severe form of the disease with an average of 15% of sites having CAL≥5mm.

The present study has three main limitations. First, it is a case series with no control group available, which precludes assessment of whether the prevalence of periodontitis is really increased in *HFE*-HH as suggested by the finding that all *HFE*-HH patients had periodontitis compared to 50 % in the general French population (Bourgeois et al. 2007). Second, only one dental odontologist was implicated and it may be conceivable that he tended to overestimate periodontal damage. However, this senior associate professor in the periodontal department is well trained in routine examination of patients with periodontitis (ICC >0.9)) and used a widely accepted definition of periodontitis (Eke et al. 2012). This should not have influenced the main result indicating that severity of periodontitis was independently associated with increased transferrin saturation. Third, although significant, adjusted Odd ratios lacked precision as indicated by wide confidence intervals. In addition, some possible confounders, for example socio-economic status, were not taken into account.

In conclusion, the present study suggests that the severity of periodontitis is increased in *HFE*-HH patients and demonstrated an association between the severity of periodontitis and the degree of iron burden, especially elevated transferrin saturation. Dental examination should be part of the initial management of these patients.

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TABLES

Table 1. Distribution of periodontal clinical parameters and general characteristics of the population in accordance to the severity of periodontitis (N = 84).

Table 2. Serum biomarkers levels (mean ± sd) in the 84 *HFE*-HH patients in accordance to the severity of periodontitis.

TABLE

Table 1. Distribution of periodontal clinical parameters and general characteristics of the population in accordance to the severity of periodontitis (N = 84).

Periodontal Clinical Parameters		Total N=84, 100%	Type of Periodontitis		*p-Values*
			Mild (n=1) to moderate (n=37) 45.2%	Severe(n=46) 54.8%	
		Mean ± SD	Mean ± SD	Mean ± SD	
CAL	Mean (mm)	2.82 ± 0.68	2.45 ± 0.32	3.13 ± 0.74	**0.0001**
	CAL ≥ 3 mm (%)	50.1 ±17.4	40.2 ±14.6	58.2 ± 15.2	**0.0001**
	CAL ≥ 5 mm (%)	10.3 ± 11.8	3.7 ± 2.8	15.8 ± 13.6	**0.0001**
PD	Mean (mm)	2.29 ± 0.35	2.06 ± 0.21	2.49 ± 0.34	**0.0001**
	PD ≥ 4 mm (%)	11.9 ± 9.7	5.6 ± 3.4	17.1 ± 10.1	**0.0001**

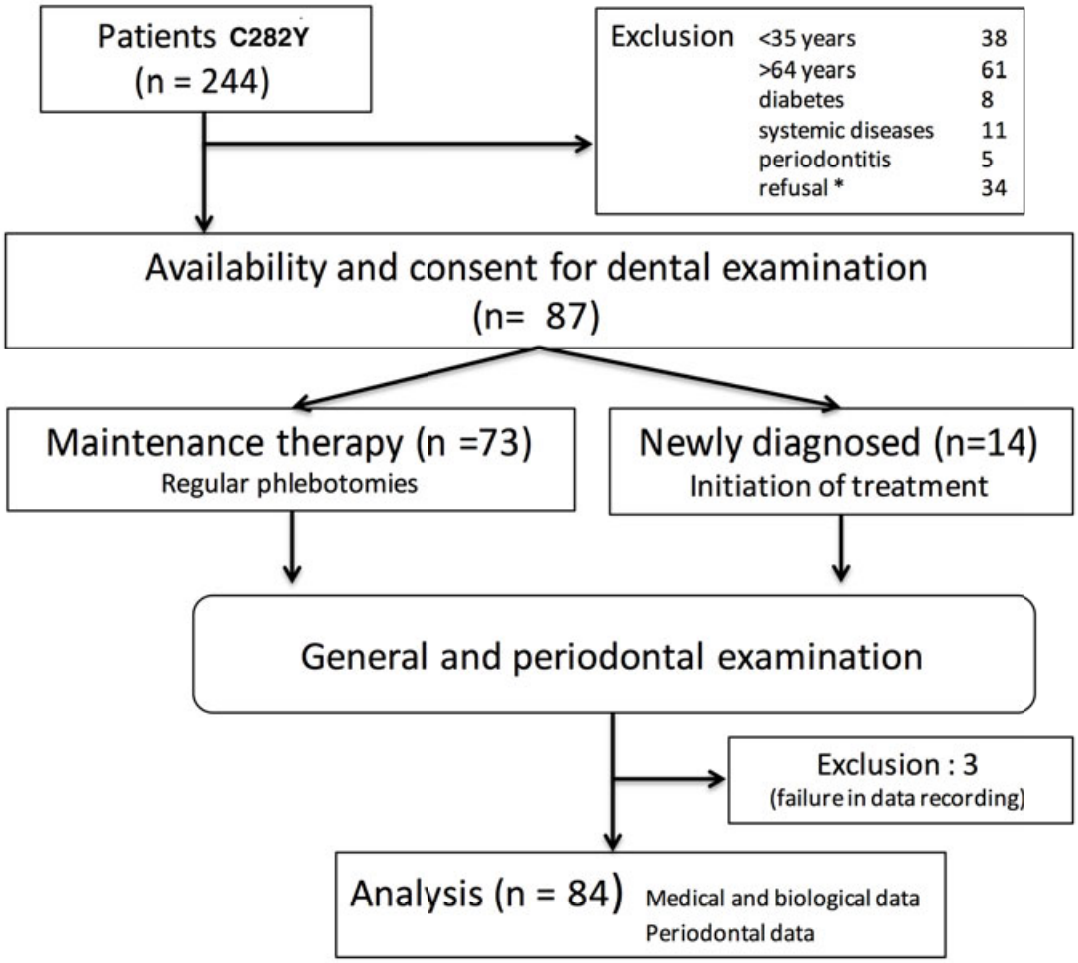
PD ≥ 6 mm (%)	0.9 ± 1.7	0.1 ± 0.3	1.6 ± 2.1	**0.0001**
GBI	0.40 ± 0.30	0.29 ± 0.20	0.48 ± 0.30	**0.001**
PI	0.18 ± 0.20	0.16 ± 0.20	0.20 ± 0.25	0.879
GI	0.40 ± 0.30	0.30 ± 0.21	0.47 ± 0.29	**0.005**
Body Mass Index (Kg/m^2)	25.46 ± 3.61	25.64 ± 3.24	25.35 ± 3.88	0.798
Time elapsed from *HFE*-HH diagnosis	10.4 ± 7.6	11 ± 6.7	9.9 ± 8.4	0.277
Gender				
Male	47 (55.9)	23 (48.9)	24 (51.1)	0.443
Female	37 (44.1)	15 (40.5)	22 (59.5)	
Smoking habits				
Non smoker	30 (35.7)	12 (31.6)	18 (39.1)	0.472
Current or former	54 (64.3)	26 (68.4)	28 (60.9)	
Frequency of dentist visits				
< 1/years	17 (20.2)	7 (18.4)	10 (21.7)	0.752
≥ 1/year	67 (79.8)	31 (85.6)	36 (78.3)	

Table 2. Serum biomarkers levels (mean ± sd), in the 84 *HFE*-HH patients in accordance to the severity of periodontitis.

Measure of periodontitis	Serum biomarkers			
	Iron (µmol/L)	Transferrin (g/L)	Transferrin saturation (%)	Ferritin (µg/L)
Mild/Moderate	25 ± 9	2.2 ± 0.3	**47.2 ± 17.9**	136.2 ± 227.6
Severe	27.8 ± 9.2	2.1 ± 0.3	**55.1 ± 18**	205 ± 524.4
p-value	0.234	0.101	**0.029**	0.916
Statistical test	t.test	t.test	Mann–Whitney test	Mann–Whitney test

FIGURE

Figure 1: Flowchart of participant recruitment



* 14 declared current gum bleeding