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Association between the Insulin-Like Growth Factor axis in serum and Periodontitis in the Study of Health in Pomerania: An exploratory study

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1. Introduction

Regulation of growth and development of the human body and its specific organs is modulated by a huge network of local and systemic growth factors. Of these growth factors is the IGF family, which plays a very important role in health and disease (Juul, 2003). The IGF family is composed of many components, including also the insulin-like growth factor-I (IGF-I) and its binding protein (IGFBP-3). Beside their anabolic effects in adults and their important role in childhood growth (Attanasio et al., 2002), IGF-I and IGFBP-3 are recently gaining a great clinical importance, as they showed an increasing strong association with mortality (Ajwani et al., 2003, Avlund et al., 2009, Friedrich et al., 2009a). Further, IGF-I and IGFBP-3 serum levels, along with periodontitis, has been associated with many systemic diseases (Geusens and Boonen, 2002, Vasan et al., 2003).

Different studies showed that periodontitis and IGF-I/IGFBP-3 levels in serum are related to many mutual factors. Among these factors are age, psychological factors, diabetes, cardiovascular diseases, waist circumference obesity and physical activity (Clark, 2004, Rarick et al., 2007, Tiryakioglu et al., 2003). However, limited studies have examined the influence of IGF-I levels on periodontitis itself.

Therefore, the need to discover further risk factors for periodontitis, including a possible association with IGF-axis, may open the horizon for a better understanding of the susceptibility, epidemiology, process and treatment of periodontitis. Furthermore, if confirmed, knowing new risk factors could serve as a powerful instrument to improve management and prevention of the disease, consequently improving the quality of life in patients with periodontitis.

1.1. Periodontal disease

1.1.1. Pathogenesis and Etiology

Periodontal disease refers to a number of multifactorial inflammatory diseases affecting the periodontium (Page, 2002). Gingivitis is considered as the pre-stage of periodontitis. It refers to an inflammation, where only the soft tissues surrounding the teeth are affected. The primary cause of gingivitis is the supragingival bacteria (plaque) that accumulate between the tooth crown and the gum. Untreated gingivitis could lead to periodontitis (Page, 2002).

Sub-gingival bacteria multiply in the periodontal pockets and cause inflammation in the gingival tissues. Moreover, periodontitis results from a complex interaction between the bacterial infection and the host response. This interaction is often altered by behavioral factors. Therefore, periodontitis results in an inflammatory degradation of periodontal tissues and alveolar bone (Figure 1) (Loesche and Grossman, 2001). If left untreated, periodontitis causes an irreversible progressive bone loss around teeth, attachment loss and consequently tooth loss (Page et al., 1997).

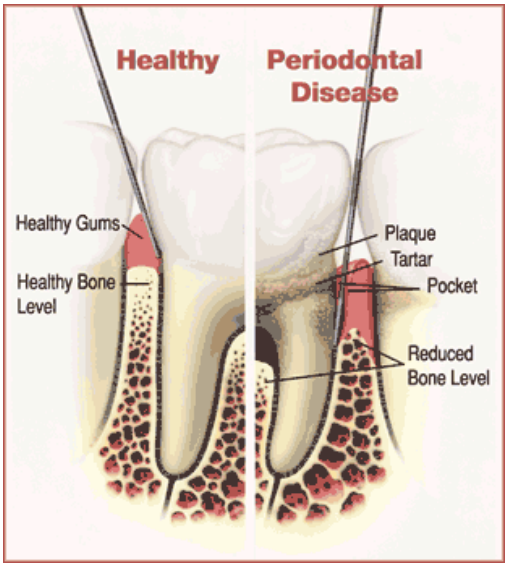

Figure 1: Comparison of a healthy against a periodontally diseased tooth: the bone loss happening around the tooth is irreversible (referring to (Tellman, 2012))

1.1.2. Periodontitis and tooth loss

Tooth loss is a complex outcome that is largely determined by dental caries, periodontitis, behavioral and socio-economic risk factors as well as many other factors (Zaher et al., 2005, Zitzmann et al., 2010). Whereas dental caries is the predominant cause of tooth loss over the whole life span (Eklund and Burt, 1994, Milgrom and Reisine, 2000), especially in younger subjects (Al-Shammari et al., 2005), periodontitis is classically considered as the leading cause of tooth loss in subjects aged 40+ years (Reich and Hiller, 1993).

In Germany, causes for tooth loss were remarkably stable between 1993 and 2007: dental caries (30%) and periodontitis (29%) mainly accounted for tooth extractions; from 40 years onwards, periodontitis became the main cause (Glockmann et al., 2011). Using Study of Health in Pomerania (SHIP) data, it has been shown that a higher extent of periodontal disease is correlated with a greater number of lost teeth (Desvarieux et al., 2004). This finding was previously potentially emphasized from the multiethnic Oral Infections and Vascular Disease Epidemiology Study (INVEST) in New York (Desvarieux et al., 2003), which implied that tooth loss is a marker of past periodontal disease in specific populations. It was also demonstrated in a longitudinal study that, reduced marginal bone level is associated with an increased risk of tooth loss (Dannewitz et al., 2006).

The fact that, periodontitis is the main cause of tooth loss in subjects aged above 40 years old, has been confirmed in the recent years (Bahrami et al., 2008, Bole et al., 2010, Thorstensson and Johansson, 2010, Albandar, 2005). Also in SHIP, periodontitis appeared to play a major role for tooth loss, even in women aged 20–39 years (Houshmand et al., 2012). Tooth loss due to other causes (trauma, abrasion, orthodontic/prosthodontic treatment planning) has a much lower prevalence (Spalj et al., 2004).

1.1.3. Associations of putative risk factors with periodontal disease

Periodontal disease has been associated with various risk factors, such as smoking, demographic factors like as age, gender, socio-economic status (Borrell and Papapanou, 2005), and several medical disorders. The clinical importance of the understanding of these risk factors could be manifested in working out theories of causation and treatment protocols to be used in treating patients (Burt, 2005).

1.1.3.1. Age and Gender

One of the non-changeable risk factors for the periodontitis is ageing (Loe et al., 1986). It is widely known that the prevalence, extent, and severity of periodontal disease have a proportional relationship with periodontal disease, with more severity at older age (Alimskii et al., 2004). More frequently it is argued, whether the disease is accumulated throughout the lifetime till elderhood, or the disease is actually an inevitable aspect of the physiology of aging (Johnson et al., 1989). Periodontal disease is among the most prevalent conditions in adults, elders, as well as all age groups. Therefore, aging alone does not lead to periodontal disease in healthy elderly persons (Huttner et al., 2009). If correctly applied, appropriate self-applied oral hygiene practices could prevent elders and adults as well from periodontal disease (Ship and Crow, 1994).

Gender and oral health are significantly associated with each other (Shiau and Reynolds, 2010). Comparing males to females, several studies reported that males are periodontally less healthy compared to the females (Albandar, 2005, Dolan et al., 1997, Eke and Dye, 2009, Holtfreter et al., 2010, Holtfreter et al., 2009). The reason for those gender-differences is not precisely understood yet, but an explanation may be that the lifestyle behaviors of men are more risky than those of females, for example: smoking, worse oral hygiene, lower health awareness and less professional care are usually observed among males (Shiau

and Reynolds, 2010, Slade and Spencer, 1995). However, this relationship might not be always constant, as there are certain gender-related temporary diseases, like the pregnancy-associated gingivitis, which can only affect females. Nevertheless, and because of the temporality of those diseases, they might not have an effect on the prevalence of periodontal disease.

1.1.3.2. Socio-economic status

The socio-economic status could be represented by the educational level. Subjects from diverse social classes differed regarding their health status (Farkas et al., 2009). In general, people who have higher levels of education, higher income, and those who live in more pleasing conditions, have a better health status than the less educated and people living under poor circumstances (Farkas et al., 2009). A putative association between periodontal disease and socio-economic status was demonstrated in a number of studies (Dolan et al., 1997, Hosseinpoor et al., 2011, Mundt et al., 2007, Treasure et al., 2001). Studies have found that subjects with a higher education have a better oral health (Hosseinpoor et al., 2011, Treasure et al., 2001). More specifically, previous studies reported that subjects from diverse socio-economic levels differed regarding their periodontal health status (Boillot et al., 2011, Borrell et al., 2002, Borrell and Crawford, 2008). Various socioeconomic indicators could affect the periodontal health status: income, educational level, quality of health care and occupational status. Among those, education was found to have the greatest influence on the periodontal health status, with a better periodontal health among people with a higher education (Boillot et al., 2011, Borrell et al., 2002, Borrell and Crawford, 2008).

1.1.3.3. Smoking

One of the major risk factors for periodontal disease is considered to be smoking (Okamoto et al., 2006). Numerous studies have confirmed an association between smoking and alveolar bone loss, attachment loss and tooth loss (Konig et al., 2002, Machtei et al., 1999). The pathogenesis of periodontal disease could be affected through smoking. This association has been comprehensively confirmed through epidemiological studies (Bergstrom and Eliasson, 1987, Erdemir et al., 2010, Norderyd et al., 1999, Susin et al., 2004). Nevertheless, the exact mechanism by which smoking affects periodontal disease is still not totally understood (Cesar Neto et al., 2012). It has been reported that smokers present a significantly greater plaque index than non-smokers (Preber and Bergstrom, 1985). Microbiological studies showed that smokers had a higher prevalence of bacterial species related to periodontal disease in comparison to nonsmokers (Zambon et al., 1996). Despite the fact that some working groups reported no differences between smokers and nonsmokers regarding the detection of periodontal pathogens (Apatzidou et al., 2005, Salvi et al., 2005), recent studies have demonstrated a positive association between smoking and amount of bacteria/probing depth (Gomes et al., 2006, Teixeira et al., 2009). In general, nicotine has been reported to be the key molecule for the massively periodontal destruction observed in smokers (Nociti et al., 2000, Nociti et al., 2001).

Nicotine has been reported to adversely affect proliferation, attachment and chemotaxis of periodontal ligament cells, and induce pro-inflammatory cytokine production in the human gingival fibroblasts (Giannopoulou et al., 1999, Giannopoulou et al., 2001). Moreover, the healing of the tissues after periodontal therapy will be hindered because of smoking (Magnusson and Walker, 1996).

Tobacco nicotine can exert cytotoxic effects on the periodontal fibroblast function, which is critical for maintenance of periodontal tissues and for optimal wound healing. It has been reported that nicotine can be stored in and released from the

fibroblast (Hanes et al., 1991). Also, the interaction between smoking and genetics can play a role in the severity of periodontitis (Meisel et al., 2000).

1.1.3.4. Obesity, Diabetes and metabolic syndrome

Periodontal disease has also been related to obesity in many studies (Meisel et al., 2012, Pischon et al., 2007, Saito et al., 2005), but more studies are still required to increase evidence. The mechanism by which obesity could affect periodontal disease is still unclear, but it is known that obese subjects exhibit increased levels of systemic inflammatory mediators. Moreover, hormones and cytokines derived from the adipose tissues are involved in the inflammatory processes (Pischon et al., 2007).

20 years ago, the association between periodontal disease and diabetes mellitus has been recognized in the dental literature (Genco and Loe, 1993). Also in the current literature, diabetes mellitus has been related to periodontal disease in many studies (Demmer et al., 2010, Genco et al., 2005, Kuo et al., 2008). Periodontal disease severity and prevalence were increased in diabetic subjects and worse in poorly-adjusted-diabetes patients (Lalla et al., 2007, Mattout et al., 2006).

Available data from previous studies demonstrated strong evidence that diabetes mellitus is a risk factor for periodontal disease, and the level of glycemic/metabolic control appears to play an important role in this relationship. (Mealey and Moritz, 2003, Papapanou, 1996). Poor glycemic control can increase the severity of periodontal disease in diabetic children (Gusberti et al., 1983) and adults as well (Cutler et al., 1999), whereas improvement in metabolic control could be associated with decreased periodontal disease in diabetic subjects (Karjalainen and Knuuttila, 1996, Sastrowijoto et al., 1990).

On the other hand, periodontal diseases can have a significant influence on the metabolic state in diabetes. For example, the presence of periodontal disease increases the risk of worsening of metabolic control over time (Taylor et al., 1996). Moreover, several studies of type 1 and type 2 diabetic patients with severe periodontal disease have shown improvements in glycemic control following periodontal therapy (Grossi et al., 1997, Grossi et al., 1996, Miller et al., 1992). In the last mentioned studies, periodontal treatment was associated with a reduction in HbA1c levels of about 10% between pre- and post-treatment values.

Metabolic syndrome is a combination of medical disorders characterized by visceral fat-type obesity (central obesity) involving hypertension, lipid abnormality and hyperglycemia. Few studies have demonstrated the strong association between metabolic syndrome and periodontal disease (Morita et al., 2009, Shimazaki et al., 2007). Previous studies have investigated the relationship between periodontal disease and components of the metabolic syndrome also individually. An association were found for example for triglyceride levels, with hyperlipidemia being associated with periodontal disease (Moeintaghavi et al., 2005).

1.2. IGF-family

1.2.1. IGF-family components

The IGF family consists of insulin, insulin-like growth factor-I & II (IGF-I, IGF-II) and six IGF-binding proteins (IGFBP-1 to IGFBP-6). The binding proteins, traditionally, are well-known to regulate the activity of hormones by extending their half-life, and IGFBPs are no exception for this. They have variable functions and mechanisms of actions. In addition to functioning as simple carrier- protein, serum IGFBPs regulate the endocrine actions of IGFs by determining the amount of IGF available to bind to IGF-I receptors. On the other side, locally produced

IGFBPs act as autocrine/paracrine regulators of IGF action (Mohan and Baylink, 2002).

1.2.2. IGF-I

1.2.2.1. Synthesis and function

IGF-I is a polypeptide hormone, which is mainly secreted by the liver upon stimulation from the growth hormone (GH). IGF-I plays the mediator role for most of the endocrinological actions of the GH (Jones and Clemmons, 1995). It regulates the differentiation of cells, apoptosis, and tumorigenesis of the cells. Moreover, it has anabolic effects in adults, and plays an important role in childhood growth (Attanasio et al., 2002). Moreover, IGF-I is involved in the proliferation of cells, in carbohydrate homeostasis, and in the synthesis of proteins (Hall, 1972).

Further, IGF-I is considered an essential hormone for organogenesis, as well as a very important hormone for the postnatal growth and development (Lelbach et al., 2005). IGF-I has been found to be stored in large amounts in human bone matrix and decreases with aging similar to the decline in circulating IGF-I (Seck et al., 1998). This age-related decrease in IGF-I was associated with bone loss with ageing. In clinical practice, IGF-I represents a biochemical marker of GH deficiency in childhood and adolescence (Zapf et al., 1978, Daughaday et al., 1972) (Figure 2).

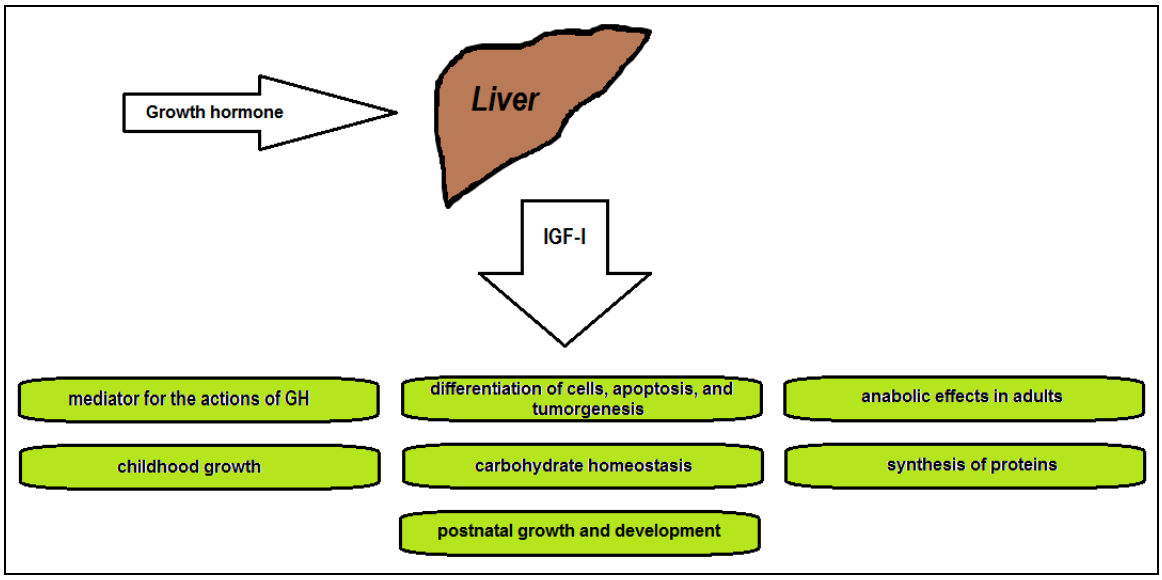

Figure 2. Different functions of the IGF-I hormone, which is secreted from the liver upon stimulation from the GH.

1.2.2.2. IGF-I reference ranges in SHIP

In SHIP, reference ranges for normal serum IGF-I levels differed for each age- and gender- group. Therefore, age- and sex-specific reference ranges for serum IGF-I levels were established. As illustrated in Figure 3, the mean values equal 142 ng/ml for women and 145 ng/ml for men (Friedrich et al., 2008). Those values were found in age- and sex-dependent linear regression models for mean serum IGF-I levels.

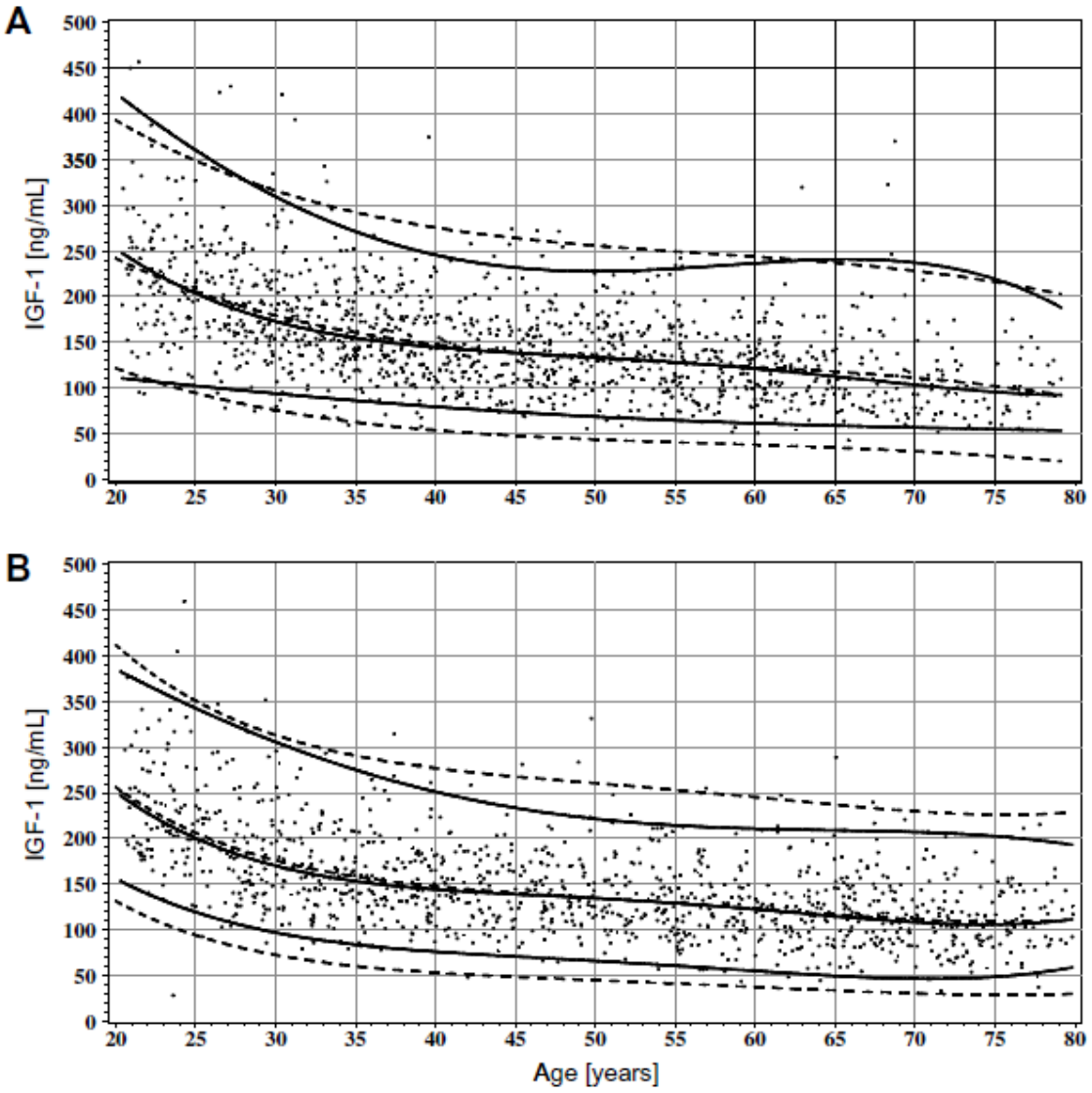

Figure 3. Serum IGF-I reference limits separately for (A) women and (B) men. Individual values of 1307 women and 1288 men are displayed (referring to (Friedrich et al., 2008)).

1.2.2.3. Free IGF-I

It is widely accepted, that free IGF-I in the serum is considered the biologically active form of IGF-I (Frystyk et al., 1994). In addition, free IGF-I has a larger physiological and clinical relevance than its total concentration (Lee et al., 1993). It has been reported, that the IGF-I/IGFBP-3 ratio was found to mirror/measure

the free IGF-I in the serum (Frystyk et al., 1994, Skjaerbaek et al., 1997) . This IGF-I/IGFBP-3 ratio increases in puberty, just as the IGF-I levels do (Juul, 2003).

1.2.3. IGFBP-3

1.2.3.1. Synthesis and function

Free bioavailable IGF is dependent on the regulation by specific binding proteins. Those binding proteins are produced mainly in the liver, and to a little extent in a variety of biological tissues (Juul, 2003). IGF binding protein-3 (IGFBP-3) binds to IGF with high affinity and is widely distributed in the serum, tissue and extravascular fluid. About more than 90% of IGF-I is bound to IGFBP-3. IGFBP-3 enhances or inhibits IGF functions, but also exhibits IGF-independent effects (Juul, 2003).

In experimental studies it was demonstrated that the IGF-I action on osteoblasts in vitro could be stimulated by adding exogenously IGFBP-3 (Ernst and Rodan, 1990). It was also demonstrated that this exogenously added IGFBP-3 increases cortical bone apposition in rats (Bagi et al., 1995). Recent *in vivo* studies demonstrated that higher levels of IGFBP-3 are associated with higher bone mineral density (Gillberg et al., 2002, Pye et al., 2011).

1.2.3.2. IGFBP-3 reference ranges in SHIP

As with IGF-I, reference ranges for normal serum IGFBP-3 levels for subjects within the SHIP-Study differed for each age- and gender- group. For that reason, specific reference ranges for serum IGFBP-3 levels were established for each age- and sex- group. As illustrated in Figure 4, the mean levels for serum IGFBP-3 were 1994 ng/ml for women and 1870 ng/ml for men (Friedrich et al., 2008).

Those values were found in age- and sex-dependent linear regression models for mean serum IGFBP-3 levels.

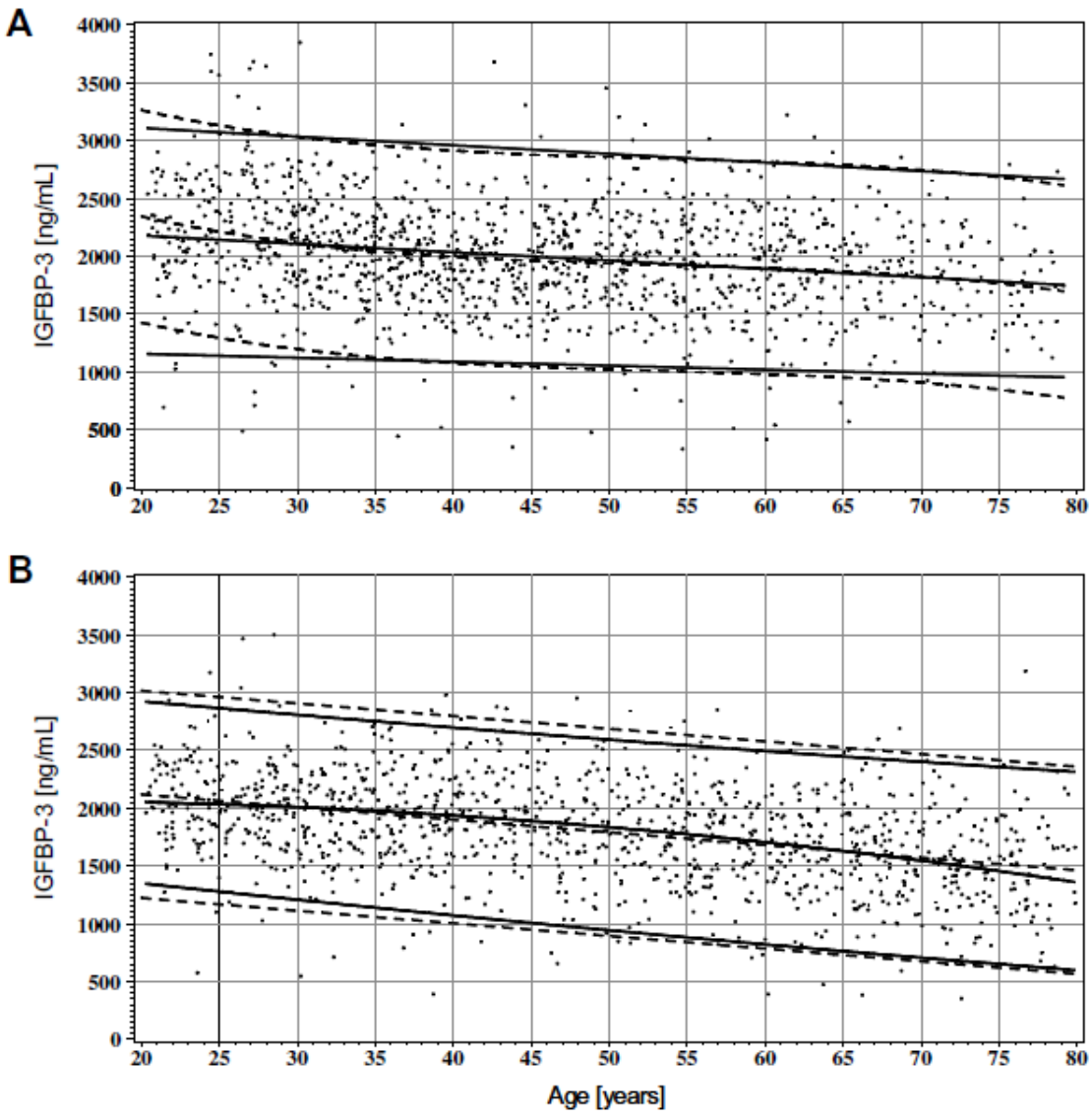

Figure 4. Serum IGFBP-3 reference limits separately for (A) women and (B) men. Individual values of 1307 women and 1288 men are displayed (referring to (Friedrich et al., 2008)).

1.2.4. Effects of serum IGF-I and IGFBP-3 on systemic diseases

During the last ten years, numerous studies investigated the role of serum IGF-I and serum IGFBP-3 in different systemic diseases. IGF-I might represent an important risk factor for systemic diseases. It was demonstrated that low serum IGF-I levels were associated with increased all-cause mortality in men, and low serum levels of IGFBP-3 were associated with increased all-cause mortality in women (Friedrich et al., 2009a). Also, individuals with low IGF-I levels had significantly increased risk of developing ischemic heart diseases (Harrela et al., 2002). In a community-based study, serum IGF-I levels have been shown to be inversely related to the risk for congestive heart failure in elderly people (Vasan et al., 2003) and to cardiovascular disease (Empen et al., 2010).

Moreover, the important relation between the IGF system and the pathogenesis of many types of cancer has been widely investigated (Samani et al., 2007, Baffa et al., 2000). Many epidemiological studies have shown that low levels of IGFBPs were associated with an increased risk for prostate, premenopausal breast, colorectal, and lung cancer (Samani et al., 2007). Further, low levels of IGF-I were associated with osteoporosis in both men and women (Geusens and Boonen, 2002). Most importantly, bone metabolism might be affected by serum levels of IGF-I and IGFBP-3 (Pye et al., 2011, Liu et al., 2008).

Furthermore, the IGF system appears to be involved in a wide range of other diseases, e.g., thyroid disease, diabetes mellitus, and cardiovascular disease (Juul, 2003). In detail, low levels of IGF-I or IGFBP-3 have been detected in adults with anorexia, chronic obstructive pulmonary disease, cirrhosis of the liver, hypothyroidism, type 1 diabetes and seriously ill patients (Juul, 2003). In sum, low IGF-I and IGFBP-3 levels are associated with chronic disease.

1.3. Aims of the study

To our knowledge, this is the first population-based study that examined the relation between the IGF system and periodontal disease. Based on the current literature it might be hypothesized that IGF related factors might also be related to periodontal diseases. Thus, the aim of this study was to investigate the association between the IGF system and periodontal disease in a large population-based cohort. Our hypothesis was that deficient levels of IGF-I and/or IGFPB-3 might be associated with greater periodontal destruction.

2. Materials and Methods

2.1. Study of Health in Pomerania

The Study of Health in Pomerania is a cross-sectional population-based survey in the northeast of Germany. The object of the SHIP is to estimate the prevalence of diseases, recognize potential risk factors in a defined area, and examine the special living situation of this population after the reunification of East and West Germany (Hensel et al., 2003). One of the main goals of the SHIP design is the investigation of the relationship between dental, medical, social and environmentally-and behaviorally determined health factors.

Sampling methods and other study details are given elsewhere (Hensel et al., 2003, John et al., 2001). In brief, a sample of a two-step cluster design, espoused from the World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) project in Augsburg, Germany (Keil et al., 1988), was implemented.

A sample of 7008 women and men aged between 20 and 79 years was drawn from 3 cities in the northeast of Germany (Figure 5), and from 29 communities in

the neighboring region, which is part of West Pomerania. The sample collection was carried out in two steps. Firstly, three cities of the region and twelve larger towns were chosen, and then 17 of 97 smaller villages (<1,500 in habitants) were drawn randomly. In the second stage, from each of these selected communities, German subjects with main residency in the area were drawn randomly, proportionally to each community population size and stratified by age and gender. From the entire study population of 212,157 residents, 7,008 subjects were sampled.

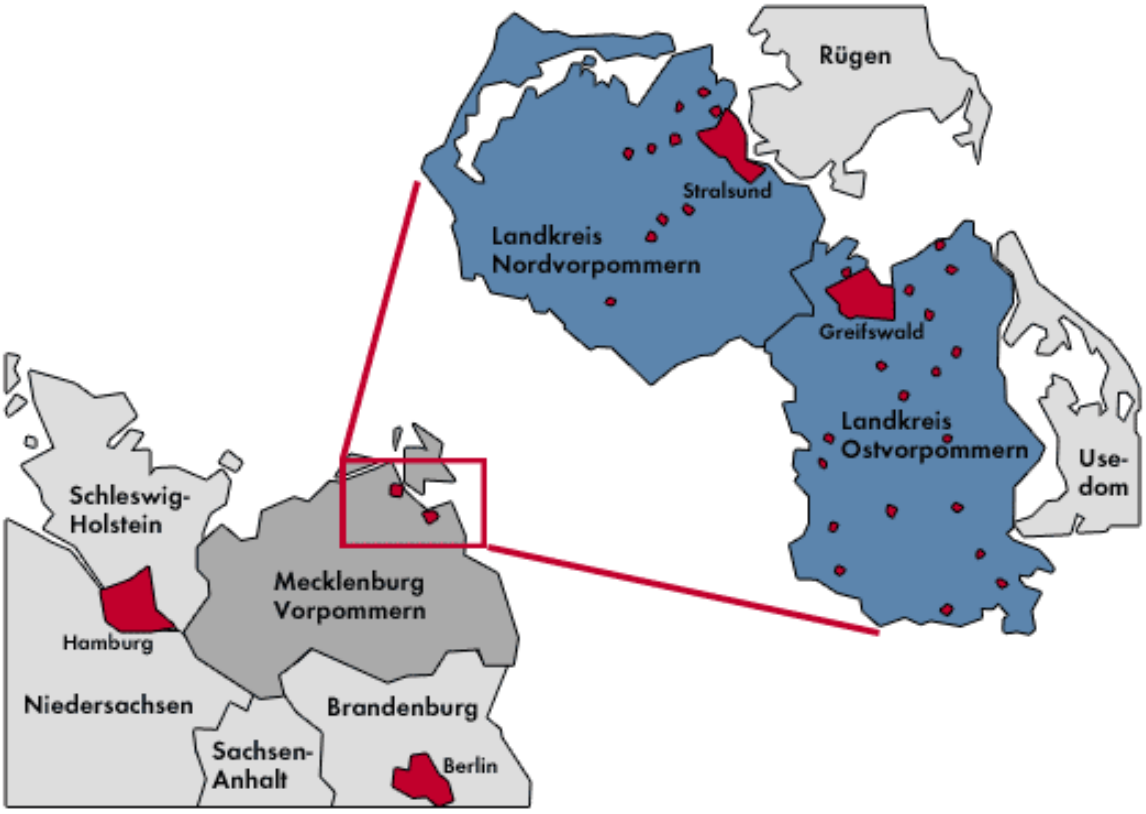

Figure 5. The sample of SHIP was drawn from 3 cities in the northeast of Germany, as illustrated in the map (referring to (Forschungsverbund Community Medicine- Institut für Community Medicine, 2012)).

Because of many reasons (126 died, 615 moved away, and five had severe medical problems) 6262 inhabitants were invited to participate. The net sample included 4308 subjects, reflecting an overall participation rate of 68.8%. The data collection was conducted between October 1997 and May 2001 and consisted of four parts: a medical examination, an oral health examination, a health-related interview, and a self-administrated health- and risk- related questionnaire. For further description of the study population the reader is referred to the attached publication.

2.2. Periodontal measurements

The oral health examinations included the examination of the teeth, the periodontium, the oral lesions (if any), the craniomandibular joint, and prosthodontics (John et al., 2001). Specific details of the clinical periodontal examination are given in the attached publication.

3. Results

In our study, we have investigated the association between the IGF System and the periodontal destruction in a large population-based cohort. It was found that, not all components of the IGF family have the same associations with periodontitis. The study principal finding was that, low serum levels of IGFBP-3 were associated with higher levels of periodontal disease. However, neither IGF-I levels nor IGF-I/IGFBP-3 were associated with periodontitis.

3.1. General characteristics

3.1.1. Distribution of covariates

Subjects with CAL measurements (N=2293) had an average age of 40 years (SD 11, range 20-59 years). The distribution of age is displayed in Figure 6.

Regarding gender distribution, 50.9% of subjects were females. School education was unevenly distributed: 20.7% of subjects reported less than 10 years of school education, 59.0% reported 10 years, and 20.3% reported >10 years of school education. 48.9% of subjects were physically active, i.e. reported physical training during summer or winter for at least 1 h a week. Diabetes mellitus was present in 2.8% of subjects and 18.3% of subjects received hormone replacement therapy.

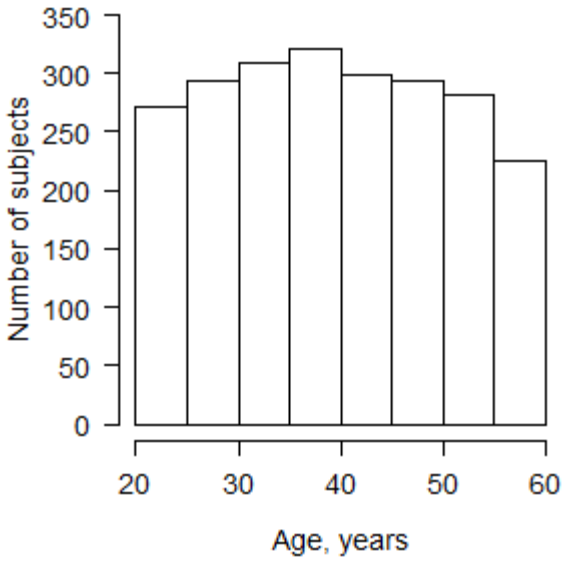

Figure 6. Percentage distributions of subjects' age.

Regarding gender distribution, 50.9% of subjects were females. School education was unevenly distributed: 20.7% of subjects reported less than 10 years of school education, 59.0% reported 10 years, and 20.3% reported >10 years of school education. 48.9% of subjects were physically active, i.e. reported physical training during summer or winter for at least 1 h a week. Diabetes mellitus was present in 2.8% of subjects and 18.3% of subjects received hormone replacement therapy.

The mean number of pack years was 7.7 packs/day * years (SD 12.2; range 0-115.5; Figure 7a). For waist circumference (Figure 7b) the mean was 86.3 cm, with a standard deviation of 13.9 (range 50.5-143.5 cm). Average serum glucose was 5.4 mmol/l, with an SD of 1.4 (Figure 7c). For serum HDL-C (Figure 7d) the mean was 1.48 mmol/l, with a standard deviation of 0.44 (range 0.46-7.32 mmo/l).

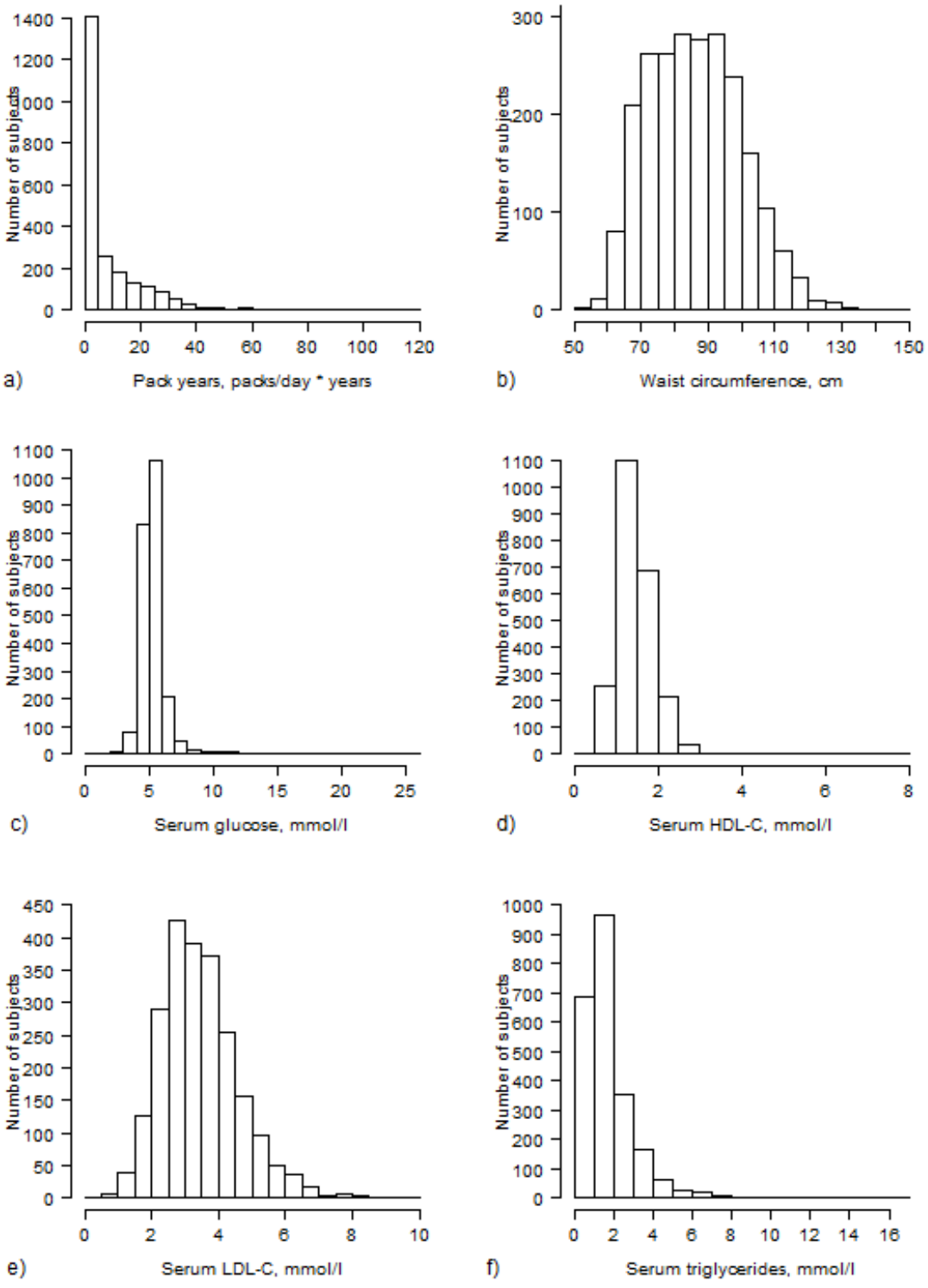

Figure 7. Histograms for the continuous covariates considered in model analyses.

Mean serum LDL-C was 3.4 mmol/l (SD 1.2; range 0.4-9.9; Figure 7e). Average serum triglycerides was 1.7 mmol/l, with an SD of 1.3 (Figure 7f).

3.1.2. Distribution of IGF axis variables

For serum IGF-I, serum IGFBP-3, and IGF-I/IGFBP3 ratio mean levels equaled 158 ng/mL (SD 59; range 32-538), 1992 (SD 462; range 331-3984), and 0.08 (SD 0.04; range 0.03-0.87), respectively. Distributions of IGF-I and IGF-I/IGFBP-3 were slightly skewed to lower values (Figure 8a and c), while serum IGFBP-3 values were approximately normally distributed (Figure 8b).

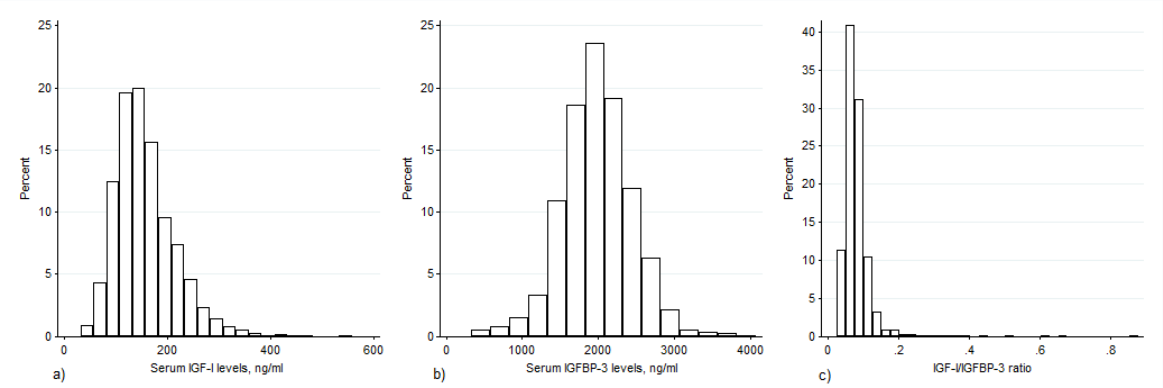

Figure 8. Percentage distributions of a) serum IGF-I levels, b) serum IGFBP-3 levels, and c) IGF-I/IGFBP-3 ratio using increments of 25 ng/ml, 250 ng/ml, and 0.025, respectively.

3.1.3. Distribution of periodontal variables

Mean and standard deviation of mean CAL were 2.16 mm and 1.60, respectively. The distribution of mean CAL had a positive skew (Figure 9a). Thus, square-rooted values of mean CAL were used in regression analyses.

The mean number of missing teeth was 5.9 (SD 6.4). The distribution of the number of missing teeth had a positive skew (Figure 9b). For analyses, subjects were classified as

having a high or low/middle number of missing teeth, considering subjects' age and gender.

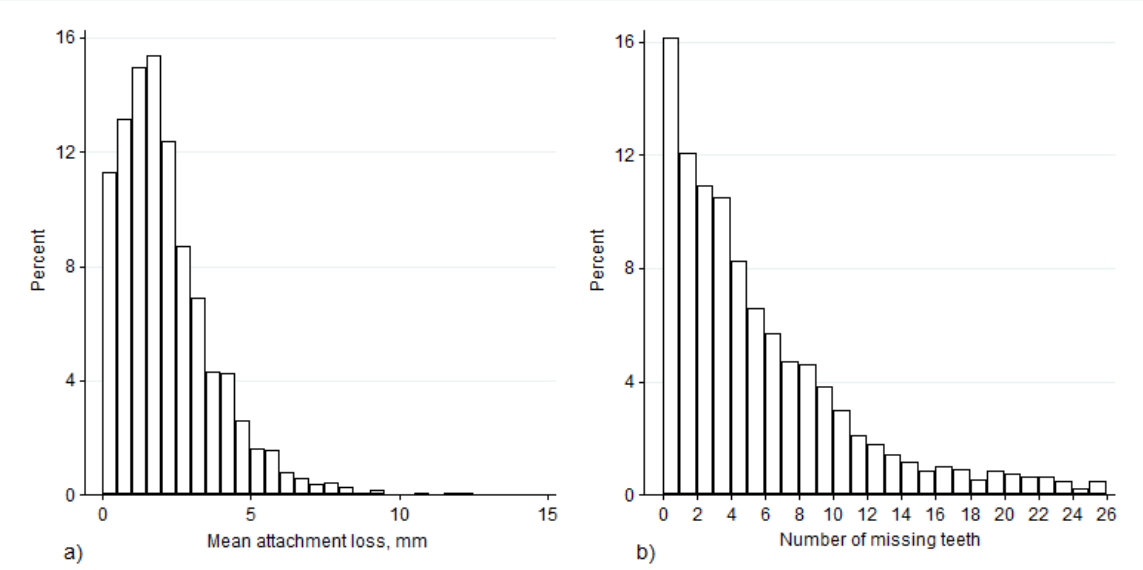

Figure 9. Percentage distributions of a) mean attachment loss and b) the number of missing teeth.

3.2. Association between IGF axis in serum and mean CAL and tooth loss by gender

After stratification by sex, increased serum IGFBP-3 levels were marginally associated with lower mean CAL in females (p=0.055), but not in males (B= 0.003 (95% CI, -0.002; 0.009), p=0.22, Figure 10).

Associations between serum IGF-I or the IGF-I/IGFBP-3 ratio with tooth loss were not significant after full adjustment. Stratifying according to gender (Figure 11), the association between serum IGFBP-3 and increased tooth loss was significant within females (p=0.01), but not within males (p=0.47).

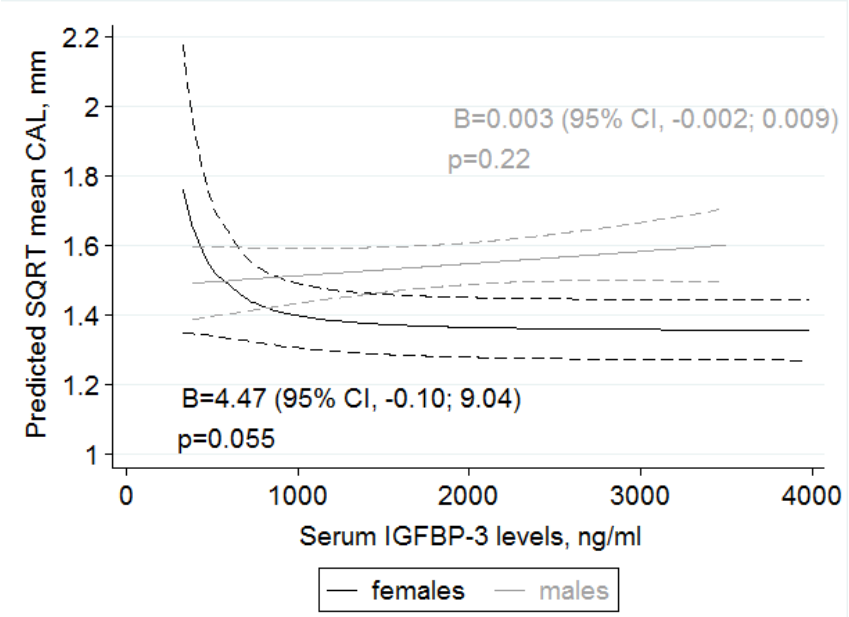

Figure 10. Association between IGFBP-3 and predicted mean clinical attachment loss stratified by gender.

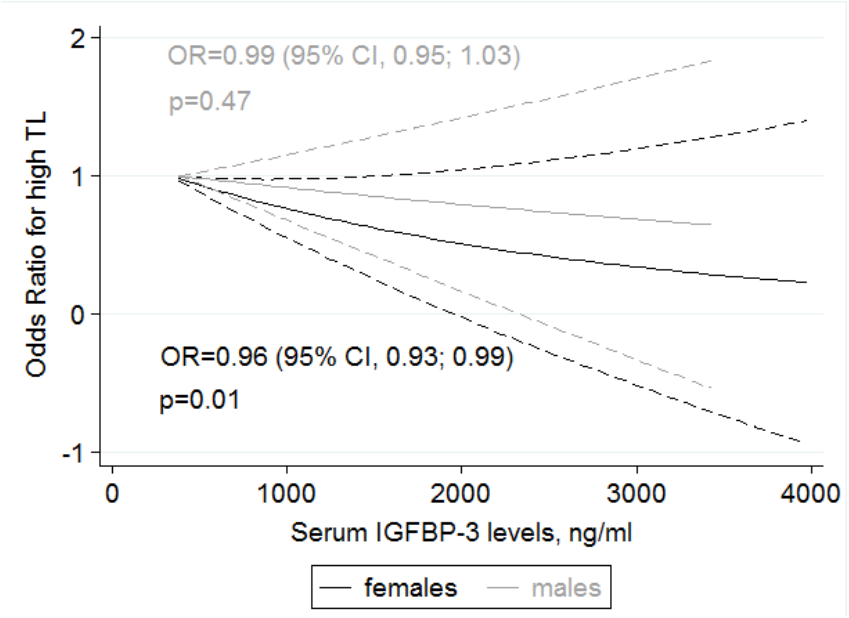

Figure 11. Predicted Odds Ratios for increased tooth loss (TL) compared to reference values for serum IGFBP-3 (ref. 330 ng/ml) stratified by gender.

4. Discussion

4.1. Summary of results

In the present investigation, we studied the association between IGF-Axis and periodontal disease in a large population sample. Our hypothesis was partly confirmed. Our study revealed that low IGFBP-3 levels were associated with more mean CAL and with increased tooth loss, overall as well as for females. Contrary to expectations, we did not find any association for serum IGF-I levels or IGF-I/IGFBP-3 ratio with mean CAL or tooth loss in this study.

4.2. Association between IGF-I and periodontitis

In our study, no significant associations were found between serum IGF-I levels and attachment loss or tooth loss. There are only few exploratory studies evaluating the relation between serum IGF-I levels and periodontitis. An Indian pilot case-control study demonstrated that total IGF-I levels were lowered in periodontitis patients in comparison to healthy controls (Rai et al., 2010). In this Indian study, total IGF-I and free IGF-I levels showed a negative association with age in the whole study population. A significant reduction of total serum IGF-I levels was observed in Periodontitis patients in comparison to the healthy controls, whereas concentrations of free IGF-I were almost comparable in both groups. As a result, a lower amount of bound IGF-I was found in periodontitis patients in contrast to normal healthy controls. The difference between our results and results found in this study was mainly the significant reduction in total serum IGF-I level found in the periodontitis Indian patients. This variation could be attributed to the different parameters used to define periodontitis. We used CAL and tooth loss data as periodontal parameters; whereas the study of Rai et al. (Rai et al., 2010) did not report tooth loss data, but rather pocket depth data.

Nevertheless, the potential role of the IGF-I on the molecular level could not be rolled out. Caton et al. (Caton et al., 2005) reported a proportional relationship between IGFs (IGF-I and IGF-2) and formation of enamel and dentin. This experimental study showed an increase in both dentin and enamel matrix in teeth treated with IGF-I, and an increase in enamel deposition in teeth treated with IGF-II. This indicates an important role for IGF-I in tooth mineralization. The findings indirectly support another studies (Caton et al., 2007, Takahashi et al., 1998), which demonstrated increasing enamel matrix formation by inducing of enamel specific proteins like amelogenin and ameloblastin. These latter results, however, were found in *in vitro* experiments.

Assuming that decreasing IGF-I levels in serum appear in parallel with a decrease in the growth hormone (GH) levels (Chanson and Salenave, 2008, Wacharasindhu et al., 2002), a new Brazilian case-control study reported that subjects with GH deficiency, and consequently decreased serum IGF-I levels, had more periodontal disease in comparison to subjects with normal GH levels (Britto et al., 2011). This study reported that subjects with GH deficiency had more bleeding on probing than controls (Britto et al., 2011). Periodontal attachment loss was significantly more prevalent and affected more teeth in GH deficiency cases than in controls. Subjects with GH deficiency, and a decreased serum IGF-I levels, showed a higher likelihood to have periodontitis than controls. Once again, an important factor which could contribute to the variation of results between our study and the other Brazilian and Indian studies is the big number of subjects examined in our study. Where we examined 2398 subjects, both above mentioned studies (Britto et al., 2011, Rai et al., 2010) comprised a small number of patients (87 and 64 subjects, respectively).

In summary, there was a difference between our results and results found in the above mentioned literature by means of i) number of subjects examined and ii) using different definitions for periodontal disease (absence of tooth loss data in both case-control studies) (Britto et al., 2011, Rai et al., 2010). Thus, the non-

significant associations found in our study regarding IGF-I could only provide a very weak (if any) argument for a supposed causal relationship between IGF-I levels in serum and periodontal disease. To our knowledge, there is no longitudinal study looked after the relationship between IGF-I and periodontal disease.

4.3. Association between IGFBP-3 and periodontitis

In this study, an association between IGFBP-3 and periodontal disease was found in the overall sample and in females. The results went along with our hypothesis, that low levels of IGFBP-3 might be associated with more extensive periodontal disease and tooth loss (Figure 6). Nevertheless, the significant inverse association between serum IGFBP-3 levels and mean CAL was limited to levels between 300 and 1200 ng/ml (Figure 6a). Therefore, those results support the presumption that serum IGFBP-3 levels might be associated with local homeostasis in periodontal tissue.

IGFBP-3 has been reported to have multiple roles both in systemic and in local regulation (Yamada and Lee, 2009) and has been generally accepted to be a local stimulator of IGF action in bone (Govoni et al., 2005). Using SHIP data, we recently also demonstrated the importance of systemic IGFBP-3, showing that low concentrations of IGFBP-3 in serum are associated with higher all-cause mortality in both genders (Friedrich et al., 2009a). Moreover, SHIP data revealed that low IGFBP-3 levels are associated with increased carotid intima-media thickness, a marker of asymptomatic cardiovascular disease (Spilcke-Liss et al., 2011). Another study suggested a reduction in risk of colorectal cancer mortality associated with increasing of IGFBP-3 levels (Haydon et al., 2006).

Takenouchi et al. established a weak, but non-significant, inverse relationship between IGFBP-3 located in the gingival crevicular fluid (GCF) and pocket depths (Takenouchi et al., 2010). Their results go along with our findings, in spite of the

fact that the selected clinical parameters of periodontal disease were different between both studies. We used mean CAL and tooth loss as parameters for periodontal disease, whereas gingival index and probing depth were selected in the Japanese study (Takenouchi et al., 2010). Moreover, the location of the investigated IGFBP-3 was dissimilar. In our study we determined serum IGFBP-3 levels, whereas IGFBP-3 levels of the GCF were investigated in the Japanese study. Nevertheless, their results are consistent with our findings.

Thus, one might speculate that serum IGFBP-3 is in sympathy with the local IGBBP-3 of the GCF, concerning the role of IGFBP-3 in the homeostasis in periodontal disease. And, given the facts that i) IGFBP-3 levels decrease with aging throughout the life time (Juul et al., 1994, Kong et al., 2007, Yu et al., 1999) and ii) periodontal ligament (PDL) cells, cementum, and dentine might carry out the roll as a local reservoir for IGFBP-3 (Gotz et al., 2006a), we might suppose that the gradual decreasing in IGFBP-3 levels with age could contribute to more periodontal disease.

The results of our study implicated minor gender differences (though not statistically significant) in the association between IGFBP-3 and mean CAL or tooth loss, with a more pronounced protective effect of high IGFBP-3 levels in females. In general, studies implemented in this field did not address possible sex differences regarding the association between periodontal status and the IGF-axis.

Regarding sex-differences of IGF-I and IGFBP-3 levels, there was generally no effect of gender on serum IGF-I or IGFBP-3 in adults in most of the studies (Juul, 2003). Only few studies reported sex-specific differences concerning IGFBP-3 levels, demonstrating higher levels of IGFBP-3 in females (Friedrich et al., 2008, Friedrich et al., 2009b, Kaklamani et al., 1999). Therefore, further mechanistic studies are needed to find out, whether gender-specific results found in this study have a biological background.

4.4. Association between IGF-I/IGGFBP-3 ratio and periodontitis

We did not find a clear association between IGF-I/IGFBP-3 ratio and CAL or tooth loss in this study. In a study including a North Indian population, free serum IGF-I levels were directly measured in the laboratory unit ELISA Kit (Rai et al., 2010), whereas we calculated the free IGF-I levels by means of IGF-I/IGFBP-3 ratio. Despite the divergence in the methods of determining free IGF-I levels, neither our study nor theirs found significant associations between free IGF-I and periodontal disease. Thus, our hypothesis regarding an association between free IGF-I and periodontitis has been discarded.

Numerous Studies evaluated IGF-I/IGFBP-3 ratios in normal physiology (Gram et al., 2006, Kong et al., 2007). An American working group found that the increase in the IGF-I/IGFBP-3 ratio led to stimulation of the cells-proliferation because of increasing IGF-I available in mammary (Berry et al., 2001). Further, free IGF-I was also evaluated as a risk factor for systemic diseases including cancer (Douglas et al., 2010, Mattera et al., 2003) and GH-deficiency (Granada et al., 2000). Further, and for up to now unknown reasons, IGF-I/IGFBP-3 ratio was found to be correlated with IGF-I values rather than with IGFBP-3 values (Kong et al., 2007, Mattera et al., 2003). Therefore, it still needs to be illuminated, why IGF-I/IGFBP-3 ratio does or does not act in a similar way compared to IGFBP-3.

4.5. Local vs. systemic IGF-I

Systemic IGF-I, which is produced in the liver, is of great importance in systemic diseases. Systemic liver-derived IGF-I and local bone-derived IGF-I might have some growth-promoting effects in common, stimulating longitudinal bone growth. Also, they may both have the capability to maintain a normal longitudinal bone growth (Ohlsson et al., 2009). Nevertheless it should be clear that, bone-derived IGF-I cannot replace the systemic IGF-I in regulating of the large physiological actions like GH secretion (Ohlsson et al., 2009). At the local level, and although

IGF-I might play a distinguished role in periodontal homeostasis (Gotz et al., 2006b), we could not find an effect of systemic IGF-I itself. It is still to be clarified, whether systematic and local IGF-I correlate with each other; and whether local IGF-I levels would be affected by systemic levels of IGF-I.

4.5.1. Experimental studies

Several experimental studies showed the importance of local IGF-I in periodontal tissues. One study reported an essential role of IGF-I in periodontal structures, where it protects cells from the programmed death of cells through its strong anti-apoptotic activity in periodontium (Werner and Katz, 2004). Giannobile *et al.* demonstrated great effects for locally administrated IGF-I on healing responses to periodontal surgery (Giannobile et al., 1994). In animal models, the local use of IGF-I in parallel to periodontal surgery led to 64% increase in new attachment formation. In contrast, control animals, which did not get locally administrated IGF-I in addition to periodontal surgery, showed only a 34% increase in new attachment formation (Giannobile et al., 1994). The same working group described IGF-I as a proliferative factor for the periodontal ligament and gave it the title “the periodontal engineer” (Giannobile, 1996). Studies which confirmed that IGF-I deserved this title are described in more detail in the following.

Throughout the process of periodontal inflammation, local IGF-I plays a very important role in regulating wound healing (Werner and Grose, 2003). Moreover, treatment of wounds by means of locally administrated IGF-I resulted in acceleration of wound healing through the stimulation of fibroblast collagen synthesis (Jones and Clemmons, 1995). Further, local IGF-I interacts with PDL cells resulting in mineralization of human PDL cells in vitro (Nemoto et al., 2004). Therefore, PDL cells might be considered as a local reservoir for IGF (Gotz et al., 2006a).

While the above mentioned experimental studies revealed an important role for local IGF-I in the inflammation/healing process, our results regarding IGF-I did not reveal a correlation between periodontal disease and systematic IGF-I. Thus, local IGF-I seems to be more important in periodontal disease than systematic IGF-I because of the following important reason:

As previously discussed, local levels of IGF-I are independent from its serum levels. Therefore, the relative importance and differences between systemic and local actions of IGF-I on periodontal disease are still non-consistent and need to be elucidated further.

4.6. Local vs. systemic IGFBP-3

It was demonstrated in the literature that IGFBP-3 has several roles both in systemic and in local regulation (Yamada and Lee, 2009). On the systemic level, and in addition to its role as a systemic IGF-carrier, serum IGFBP-3 control the endocrine actions of IGF-I by regulating the amount of IGF-I available to bind to IGF-I receptors. On the other hand, locally produced IGFBP-3 regulates the paracrine/autocrine actions of local IGF-I and acts upon local tissues (Yamada and Lee, 2009).

Systemic levels of IGFBP-3 were correlated with GH secretion, because patients with GH deficiency had lower serum concentrations of IGFBP-3 compared to healthy controls (Hardouin et al., 1989, Hasegawa et al., 1993). To our knowledge, this is the first study investigating the effects of systemic IGFBP-3 on periodontal disease in such a large population. In the following *in vitro* and *in vivo* studies, it will be demonstrated, whether local and systemic IGFBP-3 correlate with each other.

4.6.1. *In vitro* studies

Several *in vitro* studies have reported the essential role of local IGFBP-3 in the dental healing process and in the oral cavity as a growth factor. Werner and Katz have emphasized the emergent role of the IGF-system, especially IGFBP-3, in oral biology and discussed its functions in tooth development, growth and homeostasis of periodontal ligament (Werner and Katz, 2004). Lately, it has been demonstrated that the IGF-I-axis, including IGFBP-3, may be involved in *in vitro* mineralization of human PDL cells (Nemoto et al., 2004).

Furthermore, exogenously added IGFBP-3 regulated the activity of IGF-I on osteoblasts in *in vitro* cultures (Ernst and Rodan, 1990). It was investigated in *in vitro* cultures of rat osteoblastic cells, whether IGFBP-3 could regulate the biological activity of IGF-I on osteoblasts (Ernst and Rodan, 1990). It was found that IGFBP-3 correlates with a better IGF-I activity on osteoblastic cells, suggesting that IGFBP-3 might act locally to increase the effects of IGF-I in bone. This might indicate potentiating effects of locally produced IGFBP-3 in bone. Periodontal ligament cells, cementum, and dentine may act as a local reservoir for IGFBP-3, from where IGF-I and IGFBP-3 could be provided for cell populations acting in these tissue compartments (Gotz et al., 2006a). Evidently, IGFBP-3 has a very important role in the healing process in periodontal ligament cells.

4.6.2. *In vivo* studies

In the human, members of the local IGF-axis, including IGFBP-3, are regular components of the tissues of the tooth-supporting apparatus (Gotz et al., 2006a). Nevertheless, the significant difference between the periodontally healthy and diseased sites in terms of local IGFBP-3 in GCF of the humans was detected for the first time in a case control study (Sakai et al., 2006), indicating a potential role for IGFBP-3 as a growth factor during the wound healing process in

periodontal disease. The same working group of Gotz et al. found that during wound healing, the early repaired cementum in rats contained mainly IGFBP-3 (Gotz et al., 2006b). Further, in patients with systemic inflammation, circulating IGFBP-3 levels were negatively associated with systemic overexpression of pro-inflammatory cytokine IL-6 in rheumatoid arthritis patients (De Benedetti et al., 1997). Moreover, and in experimental mice, a significant decrease in *in vivo* IGFBP-3 levels was reported after inducing high levels of interleukin-6 (De Benedetti et al., 1997), a conventional inflammation parameter in systemic disease (Mangge et al., 1995). Another experimental study made on diabetic animals has shown, that through suppression of the local IGF-system in the wound, especially IGFBP-3, diabetes can affect the healing process negatively (Bitar, 2000). This might indicate a potential important role for serum IGFBP-3 as a mediator in the healing process in systemic inflammation.

Because IGFBP-3 has a high concentration in serum, the balance between local and systemic IGFBP-3 in periodontal tissue remains to be clarified. Moreover, the majority of studies used models that represent either systemic or local regulation of IGFBP -3 and have not included both systems into their model of study. It is still unknown, whether interaction between these systems has physiological significance (Yamada and Lee, 2009). Despite the relatively novelty of the subject “local vs. systemic IGFBP-3”, our results might support the assumption that the systemic actions of the serum IGFBP-3 are in line with its so far discovered local actions, showing that lower levels of IGFBP-3 are associated with more periodontal disease.

4.7. Strengths and limitations

The major strength of the present study is the large sample size comprising a wide range of social and medical data, permitting comprehensive confounder adjustment and an estimation of the association between different variables of

the IGF-axis and periodontal disease with good statistical precision. One of the strengths of our study is the population-based design. Second, the accurate determination of the periodontal status through certified calibrated dentists. Third, we applied fractional polynomials to explore the relations between IGF-status and periodontal status appropriately, considering both linear and non-linear forms. For non-linear associations, linear splines were presented to facilitate accessibility. However, we must acknowledge that the protective effect of serum IGFBP-3 levels on mean CAL was most obvious at levels between 300 and 1200 ng/mL. Although the number of subjects having serum levels of IGFBP-3 of less than 1200 ng/mL was relatively low, statistical significance of respective associations deliver evidence for a potentially protective effect of serum IGFBP-3 levels on periodontal disease.

4.8. Prospects

In conclusion, lower serum IGFBP-3 levels were associated with more periodontal disease in the overall sample and in females, whereas serum IGF-I and IGF-I/IGFBP-3 ratios were not associated with periodontal disease. In general, the relation between serum IGF-axis components and periodontitis seems to be minor. The cross-sectional study design ruled out drawing any definite causal conclusions of the association between serum IGFBP-3 and periodontal disease. Longitudinal studies are desired to investigate causality more closely. Further, more studies are needed to explore differences between local and systemic IGF-axis in terms of its effect on periodontal disease. If confirmed in further studies, an association between IGF-axis and periodontitis may open the horizon for a better understanding of biological mechanisms related to periodontal disease progression. Moreover, if established in further studies, the association between IGF-axis components and periodontal disease might lead to discover more risk factors for periodontitis. Further, it could help in

widening the therapeutic concepts used IGF-I and IGFBP-3 as wound healing accelerators following periodontal surgery-beside other growth factors.

In a previous work, we have reported that acromegaly patients have higher levels of serum IGFBP-3 in comparison with healthy subjects (Harb et al., 2012). In addition to other available studies, this study indeed cannot give sufficient evidence that such patients should or should not undergo a periodontal treatment. Therefore, further studies could help patients with abnormal IGF-I and IGFBP-3 levels, like acromegalic patients, to better understand and deal with periodontal disease.

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6. Summary

Recent studies reported a ubiquitous role of IGF-I hormone in many physiological and pathological pathways. It has been linked with many serious diseases and also with mortality. The present study was conducted to evaluate the association between Insulin-like Growth Factor (IGF) I related variables and periodontal disease in the population-based Study of Health in Pomerania (SHIP).

Data from the cross-sectional SHIP were evaluated. Serum IGF-I and IGF binding protein (BP)-3 levels were determined by chemiluminescence immunoassays. Periodontal disease was assessed by CAL and tooth loss. Linear and logistic regression models were used to study associations between IGF-related variables and mean CAL or high tooth loss. IGF related variables were assessed as categorical variables and fractional polynomials. Overall, 2293 subjects (1125 males and 1168 females) with clinical attachment loss (Calsina et al.) data and 2398 subjects (1171 males and 1227 females) with tooth count data aged 20-59 years were analyzed.

After full adjustment, for serum IGFBP-3 values ≤1200 ng/mL, mean CAL increased significantly for decreasing serum IGFBP-3 levels (B=-0.027 (95% CI, -0.049; -0.005), p=0.02). The odds for high tooth loss decreased significantly for high serum IGFBP-3 values (OR=0.97 (0.95; 0.99), p=0.02). Neither serum IGF-I nor IGF-I/IGFBP-3 ratios were associated with mean CAL or tooth loss after full adjustment.

Previous literature reported on several functions of IGFBP-3 both on the systemic and the local level. To our knowledge, this was the first population-based study investigating the relationship between serum IGFBP-3 and periodontal disease. Our results support the assumption that the systemic actions of serum IGFBP-3 might go along with its so far discovered local actions, showing that low serum IGFBP-3 levels could be associated with more periodontal disease by means of

CAL and tooth loss. If confirmed in further studies, an association between IGF-axis and periodontitis may open the horizon for a better understanding of biological mechanisms related to periodontal disease progression.

7. Zusammenfassung

Aktuelle Studien berichteten eine ubiquitäre Rolle des IGF-1 Hormons bei verschiedenen physiologischen und pathologischen Vorgängen. Es wurden verschiedene Assoziationen mit schweren Erkrankungen als auch einer erhöhten Morbidität nachgewiesen. Die vorliegende Studie wurde durchgeführt, um den Zusammenhang zwischen verschiedenen mit dem Insulinähnlichen Wachstumsfaktor (IGF) I assoziierten Variablen und Parodontitis in der bevölkerungsbezogenen Study of Health in Pomerania (SHIP) zu untersuchen.

Es wurden Daten aus der Querschnittsstudie SHIP-0 analysiert. Serumspiegel von IGF-I und IGF-Bindeprotein (BP) -3 wurden durch Chemilumineszenz-Immunoassays bestimmt. Der Schweregrad der parodontalen Erkrankung wurde anhand des mittleren Attachmentverlustes und des Zahnverlustes beurteilt. Lineare und logistische Regressionsmodelle wurden verwendet, um die Assoziationen zwischen IGF-Variablen und dem Attachmentverlustes bzw. einer hohen Zahnverlustrate zu evaluieren. IGF-Variablen wurden als kategoriale Variablen und fraktionierte Polynome beurteilt. Insgesamt wurden 2293 Probanden (1125 Männer und 1168 Frauen) mit Attachmentwerten und 2398 Probanden (1171 Männer und 1227 Frauen) mit Zahnzahldaten im Alter von 20-59 Jahren analysiert.

Bei Einschränkung der IGFBP-3-Werte auf einen Bereich von ≤ 1200 ng/mL waren bei abnehmenden IGFBP-3-Werten erhöhte Werte des mittleren Attachmentverlustes zu beobachten (B = -0,027 (95% CI, -0,049; -0,005), p = 0,02). Das Risiko für einen hohen Zahnverlust nahm bei hohen IGFBP-3-Werten signifikant ab (OR = 0,97 (0,95; 0,99), p = 0,02). Nach vollständiger Adjustierung für Confounder zeigten weder Serum-IGF-I noch das IGF-I/IGFBP-3 Verhältnis einen signifikanten Zusammenhang zum mittleren Attachmentverlust oder zur Zahnverlustrate.

In der bisherigen Literatur wurde von mehreren Funktionen des IGFBP-3 sowohl auf der systemischen als auch auf der lokalen Ebene berichtet. Unserem Wissen nach war dieses die erste bevölkerungsbezogene Studie, die den Zusammenhang zwischen Serum-IGFBP-3 und Parodontitis untersucht hat. In dieser Studie konnte gezeigt werden, dass niedrige Serum-IGFBP-3-Level mit mehr Parodontitis gemessen am Attachmentverlust und Zahnverlust assoziiert waren. Daher unterstützen unsere Ergebnisse die Annahme, dass die systemische Wirkung des IGFBP-3 mit seiner bisher entdeckten lokalen Wirkung einhergehen könnte. Sofern dieser Zusammenhang in weiteren Studien bestätigt werden würde, könnte ein Zusammenhang zwischen der IGF-Achse und Parodontitis den Horizont für ein besseres Verständnis der biologischen Mechanismen im Zusammenhang mit parodontaler Progression eröffnen.

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Association between the insulin-like growth factor axis in serum and periodontitis in the Study of Health in Pomerania: an exploratory study

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Abstract

Aim: To evaluate the association of Insulin-like Growth Factor (IGF) I-related variables with periodontitis in the population-based Study of Health in Pomerania (SHIP).

Material and Methods: From the cross-sectional SHIP, 2293 subjects with clinical attachment loss (CAL) data and 2398 subjects with tooth count data aged 20–59 years were analysed. Serum IGF-I and IGF-binding protein (BP)-3 levels were determined by chemiluminescence immunoassays. Linear and logistic regressions with fractional polynomials were used to study associations between IGF-related variables and mean CAL or high tooth loss. For non-linear relations between IGFBP-3 and mean CAL, graphical presentations of fractional polynomials were used to deduce knots for linear splines.

Results: In fully adjusted models, for serum IGFBP-3 values ≤ 1200 ng/ml, mean CAL increased significantly for decreasing serum IGFBP-3 levels [B = −0.027 (95% CI, −0.049; −0.005), $p = 0.02$]. The odds for high tooth loss decreased significantly for high serum IGFBP-3 values [OR = 0.97 (0.95; 0.99), $p = 0.02$]. Serum IGF-I levels and the IGF-I/IGFBP-3 ratio were not related to mean CAL or tooth loss after full adjustment.

Conclusions: Low serum IGFBP-3 levels might be associated with higher levels of periodontal disease. Neither serum IGF-I nor IGF-I/IGFBP-3 ratios were associated with periodontitis.

Key words: clinical attachment loss; periodontitis; serum IGFBP-3; serum IGF-I; tooth loss

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Conflict of interests and source of funding statement

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The IGF family components include insulin, insulin-like growth factor-I & II (IGF-I, IGF-II), and six IGF-binding proteins (IGFBP-1 to IGFBP-6). IGF-I is a polypeptide hormone, which regulates cell proliferation, differentiation, apoptosis and tumorgenesis, has anabolic effects in adults, and plays an important role in childhood growth (Attanasio et al. 2002). IGF-I is mainly secreted by the liver as a result of stimulation by growth hormones (GH) and mediates most of the endocrine actions of GH (Jones & Clemmons 1995). IGF-I decreases with age (Seck et al. 1998). In the Study of Health in Pomerania (SHIP), reference ranges for normal serum IGF-I levels differed by age and gender; mean values equal 142 and 145 ng/ml for women and men respectively (Friedrich et al. 2008).

IGFBP-3 represents the main binding protein which is widely distributed in the serum, tissue, and extravascular fluid. Over 90% of IGF-I is bound to IGFBP-3. IGFBP-3 promotes or inhibits IGF-I functions, but also exhibits IGF-I-independent effects (Juul 2003). Exogenously added IGFBP-3 can stimulate the IGF-I action on osteoblasts in vitro (Ernst & Rodan 1990) and increases cortical bone apposition in rats (Bagi et al. 1995). For SHIP subjects, reference ranges for normal serum IGFBP-3 levels differed by age and gender, with mean levels for serum IGFBP-3 being 1994 and 1870 ng/ml for women and men respectively (Friedrich et al. 2008). It is widely accepted that free serum IGF-I is the biologically active form (Guler et al. 1987). The IGF-I/IGFBP-3 ratio measures free IGF-I.

Periodontitis is a multifactorial inflammatory disease that results in degradation of periodontal tissues and alveolar bone (Loesche & Grossman 2001), attachment loss and, consequently, tooth loss. Periodontitis is considered as the leading cause of tooth loss in subjects aged 40+ years (Reich & Hiller 1993, Bahrami et al. 2008, Bole et al. 2010, Thorstensson & Johansson 2010). Its importance increased in recent decades (Bahrami et al. 2008, Thorstensson & Johansson 2010). Also in SHIP, periodontitis appears to play a major role for tooth loss, even in women aged 20–39 years (Houshmand et al. 2012). Furthermore, causes for tooth loss were remarkably stable in Germany between 1993 and 2007: dental caries (30%) and periodontitis (29%) mainly accounted for tooth extractions; from 40 years onwards, periodontitis became the main cause (Glockmann et al. 2011).

Recently, several studies evaluated the effect of serum IGF-I and serum IGFBP-3 on systemic diseases. Serum IGF-1 levels were also associated with all-cause mortality (Friedrich et al. 2009), risk of congestive heart failure (Vasan et al. 2003), ischaemic heart disease (Juul et al. 2002), and cardiovascular disease (Empen et al. 2010). Moreover, the important role of the IGF system in the pathogenesis of many types of cancer has been widely examined (Baffa et al. 2000, Samani et al. 2007) and many epidemiological studies have shown that low levels of IGFBPs were associated with prostate, colorectal, premenopausal breast, and lung cancer (Samani et al. 2007). Most importantly, serum levels of IGF-I and IGFBP-3 might affect bone metabolism (Liu et al. 2008, Pye et al. 2011).

We are unaware of population-based studies investigating the association between the IGF system and periodontitis. Thus, the aim of this study was to explore the association between the IGF system and periodontal destruction in a large population-based cohort. We hypothesize that deficient serum levels of IGF/IGFPB-3 might be associated with greater periodontal destruction.

Material and Methods

Study population

The SHIP is a cross-sectional population-based survey in the northeast of Germany. Study details including sampling methods are given elsewhere (John et al. 2001, Hensel et al. 2003). Concisely, a two-stage cluster design, adopted from the World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) project in Augsburg, Germany (Keil et al. 1988), was applied. Within selected communities, 7008 Caucasian subjects (20–79 years) with German citizenship and main residency in the area were randomly drawn from population registries, stratified by age and sex. Due to several reasons (126 died, 615 moved away, and five had severe medical problems), 6262 inhabitants were invited to participate, of whom 4308 subjects (response 68.8%) participated in the study. The study was conducted from October 1997 to May 2001. All participants gave informed written consent. The study protocol was approved a priori by the Ethics Committee of the University of Greifswald.

In most subjects aged 60 years and older, periodontal measurements were based on only few teeth, making estimates susceptible to measurement errors. Consequently, only the data of 2902 subjects aged 20–59 years were used. Several chronic disorders affect serum IGF-I levels (Juul 2003). Thus, 307 subjects with one of the following conditions (overlaps exist) were excluded: thyroid diseases ($n = 190$), cancer ($n = 11$), chronic renal diseases ($n = 107$), hepatitis ($n = 11$), or liver cirrhosis ($n = 5$). In addition, subjects with missing IGF-I or IGFBP-3 data ($n = 155$) or subjects with no information for selected confounders ($n = 36$) were excluded. Further exclusion of subjects with missing tooth count data ($n = 6$) resulted in 2398 subjects providing tooth count data for analysis (1171 men and 1227 women). Of those, further 105 subjects with missing CAL values were excluded, resulting in 2293 (1125 men and 1168 women) subjects with CAL data.

Oral health assessment

Eight trained and calibrated dentists carried out oral examinations according to the half-mouth method, either on right or left quadrants in alternate subjects. The periodontal status, assessed by CAL and probing depth (PD), was recorded using a periodontal probe (PCP11; Hu Friedy, Chicago, IL, USA). Probing was performed at four sites per tooth (mesiobuccal, midbuccal, distobuccal and midpalatinal/midlingual), excluding third molars. The number of teeth was counted, excluding third molars.

Each 6–12 months, calibration exercises were performed on persons

not connected with the study. For site measurements of CAL, intra-rater correlations of 0.82–0.91 per examiner and an inter-rater correlation of 0.84 were achieved (Hensel et al. 2003). Between 89.9% and 98.6% of absolute deviations between repeated site measurements of CAL ranged within ±2 mm.

Assays of IGF-I and IGFBP-3

Non-fasting blood samples were drawn from the cubital vein in the supine position. Samples were stored at −80°C for IGF-I and IGFBP-3 analyses. Serum levels of IGF-I and IGFBP-3 were measured using an automated two-site chemiluminescence immunoassay (Nichols Advantage®; Nichols Institute Diagnostica GmbH, Bad Vilbel, Germany). For IGF-I measurements, samples were acidified to separate IGF-I from IGF-binding proteins. The analytical sensitivity was 6 ng/ml, the intra- and inter-assay imprecision was 4.8% and 6.7%, respectively. The analytical sensitivity of the IGFBR-3 assay was 20 ng/ml, the intra- and inter-assay imprecision within the range of 227–2703 ng/ml were 5.8% and 11%, respectively. The IGF-I/IGFBP-3 ratio was calculated.

Assessments of confounders

Information on social and behavioural characteristics and diabetes history were retrieved from computer-aided personal interviews. School education was categorized into three levels (<10, 10, and >10 years). Pack years were calculated as packs per day × years. Alcohol drinking habits were evaluated as beverage-specific alcohol consumption (beer, wine, and distilled spirits) during the last weekend and weekday preceding the examination and the mean daily alcohol consumption was calculated using beverage-specific pure ethanol volume proportions. Subjects who participated in physical training during summer or winter for at least 1 h a week were classified as being physically active. Diabetes was assessed by self-reported physician diagnosis and/or use of anti-diabetic drugs (ATC code A10). To verify the use of anti-diabetic drugs, prescriptions or medications brought during health-related interviews were categorized according to the Anatomical Therapeutic Chemical (ATC) classification system.

From the medical examination, somatometric data were retreived. Height, weight, and waist circumference (WC) were measured using calibrated scales. The Body Mass Index (BMI) was calculated. Information on medication with sex hormones, comprising medication with oral contraceptives (ATC code G03A [hormonal contraceptives for systemic use]) and menopausal hormone therapy (ATC codes G03C, G03D, or G03F) was collected.

Non-fasting serum high-density lipoprotein cholesterol (HDL-C) and serum low-density lipoprotein cholesterol (LDL-C) levels were measured photometrically. Non-fasting serum triglycerides and glucose were determined enzymatically (Hitachi 717; Roche Diagnostics, Mannheim, Germany).

Statistical analyses

Categorical data are presented as percentages; continuous data are presented as median (25%; 75% percentiles) because distributions were skewed and non-normal. To analyse differences in the distribution of variables according to tertiles of serum IGFBP-3, Chi-square tests (categorical data) and Kruskal–Wallis tests (continuous data) were used.

First- and second-degree Fractional polynomials (FP) were applied to explore and graph non-linear associations between IGF-related variables (exposure) and periodontitis (dependent variable) (Royston et al. 1999). To assess goodness of fit, deviances were compared using χ^2 distributions with according degrees of freedom (Royston et al. 1999). If none of the FP models fitted the data significantly better than the linear model, linear regression was applied. In case of non-linear relations, graphical presentations of FPs were used to deduce appropriate knots (best knot was determined by comparison of models using BIC) for linear splines (Harrell 2001). For the association between IGFBP-3 and mean CAL, a linear spline with knot at 1200 ng/ml is presented.

IGF-related variables were automatically scaled using the build-in STATA procedure (100 ng/ml increments for serum IGF-I, 100 ng/ml increments for serum IGFBP-3 and 0.1 increments for IGF-I/IGFBP-3 ratios).

First, using mean CAL as the dependent variable, linear regression models with robust standard errors were evaluated. Linear regression coefficients (B) with their 95% confidence intervals (95% CI) were reported. Predicted mean CAL values with 95% confidence intervals were calculated and displayed graphically. Second, logistic regression models with robust standard errors were evaluated to assess the association between serum IGF status and a high tooth loss. Subjects with high tooth loss were defined as subjects within the highest quintile for tooth loss within each 5-year stratum separated for gender. Odds ratios (OR) with 95% CIs were determined. For graphical visualization of results for serum IGFBP-3, Odds Ratios for varying levels of serum IGFBP-3 were calculated comparing all points with the reference point 330 ng/ml.

Age, gender, school education, pack years, low-density lipoprotein, high-density lipoprotein, total cholesterol, waist circumference, diabetes, alcohol use (g per week), physical activity, and medication with sex hormones (only in women) were considered as potential confounders. We added covariates for adjustment one by one, at each step adding the confounder that makes the most change in the exposure effect estimates among those not yet added. Adding confounders was stopped when change in the exposure estimate was less than 5% (Rothman et al. 2008). Using this approach, age, gender, pack years, school education, high-density lipoprotein, and waist circumference were identified as confounders. In regression models, age was considered as a continuous variable; smoothing plots confirmed that age was linearly associated with mean CAL. Analyses did not account for two-stage cluster study design. The effect of the exposure on periodontal disease was not significantly modified by age or gender, as assessed by interaction terms.

A p value <0.05 was considered statistically significant. All analyses were performed using STATA/SE 12.0 (StataCorp 2011).

Results

General characteristics

Subjects with CAL measurements ($N = 2293$) had an average age of 40 years (SD 11, range 20–59 years); 49% were male. For serum IGF-I, serum IGFBP-3 and IGF-I/IGFBP3 ratio, mean levels equalled 158 ng/ml (SD 59; range 32–538), 1992 ng/ml (SD 462; range 331–3984), and 0.08 (SD 0.04; range 0.03–0.87), respectively (see also Fig. 1).

Across increasing tertiles of serum IGFBP-3, subjects differed significantly regarding age, gender, school education, and pack years ($p < 0.05$, Table 1). The metabolic status was comparable across tertiles. For increasing tertiles of serum IGFBP-3, periodontal status assessed by mean CAL and high tooth loss improved ($p < 0.05$).

Association between IGF axis in serum and mean CAL

Associations between mean CAL (square rooted) and serum IGF-I or IGF-I/IGFBP-3 ratio were modelled using linear regression, while for serum IGFBP-3, linear splines were applied (Table 2). In crude models, all IGF-related variables were significantly associated with mean CAL (M0). After adjustment for age and gender, only the association with serum IGFBP-3 remained statistically significant. After full adjustment, low serum IGFBP-3 levels were significantly associated with high mean CAL (M2). For serum IGFBP-3 values ≤ 1200 ng/ml, mean CAL increased significantly for decreasing serum IGFBP-3 levels (B = −0.027 (−0.049; −0.005), $p = 0.02$, Fig. 2a and Fig. S1, which also includes predicted values). For serum IGFBP-3 levels >1200 ng/ml, mean CAL did not change significantly (B = 0.002 (−0.002; 0.006), $p = 0.26$). No significant associations were found for IGF-I and the IGF-I/IGFBP-3 ratio.

Association between IGF axis in serum and high tooth loss

All associations with high tooth loss were modelled using logistic regression (Table 3). In crude models (M0), risk for high tooth loss was significantly decreased in subjects with high serum IGF-I or IGFBP-3 levels. In the fully adjusted model (M2), the odds for high tooth loss significantly decreased for high serum IGFBP-3 values (OR = 0.97 (0.95; 0.99), $p = 0.02$, Fig. 2b). Serum IGF-I and the IGF-I/IGFBP-3 ratio were not related to tooth loss after full adjustment.

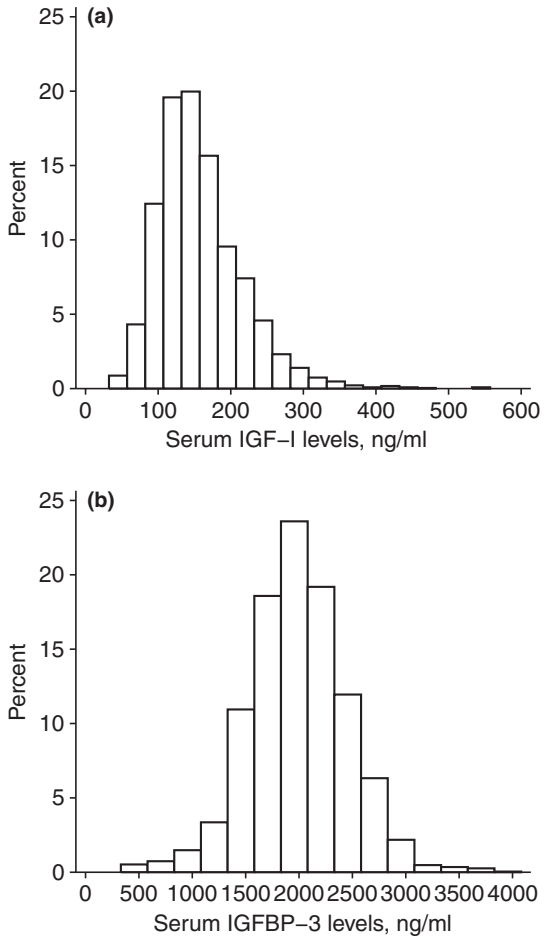

Fig. 1. Percentage distributions of (a) serum IGF-I levels and (b) serum IGFBP-3 levels using increments of 25 and 250 ng/ml, respectively.

Discussion

This study revealed an inverse relation of serum IGFBP-3 levels with clinical attachment loss and number of missing teeth. Contrary to expectations, neither serum IGF-I levels nor IGF-I/IGFBP-3 ratios were associated with attachment loss or tooth loss.

IGF-I

In this study, serum IGF-I levels were not associated with attachment loss or tooth loss. There is only scarce exploratory literature on the association between serum IGF-I levels and periodontitis.

An Indian study demonstrated that total IGF-I levels were decreased in periodontitis patients in comparison with healthy controls (Rai et al. 2012). Under the premise that IGF-I levels in serum decreases in parallel with growth hormone (GH) levels (Chanson & Salenave 2008), a very recent Brazilian case-control study reported that subjects with GH deficiency, and subsequently decreased IGF-I levels, had more periodontal disease (Britto et al. 2011). In summary, use of different periodontal definitions, missing report of tooth loss data in both of those case-control studies, and the rather non-significant associations found in our study regarding IGF-I only provide a weak (if any) evidence for a putative association between serum IGF-I levels and periodontitis.

Table 1. Baseline characteristics of the study population according to tertiles of serum IGFBP-3 levels ($N = 2293$)

	Tertiles of serum IGFBP-3 levels (ng/ml)			
	1st 1562 (1377; 1694)	2nd 1983 (1890; 2062)	3rd 2403 (2270; 2622)	*p* value*
N	766	763	764	
Age (years)	43 (34; 51)	40 (31; 49)	36 (28; 48)	<0.001
Male sex	54.6%	49.9%	42.7%	<0.001
School education				
<10 years	24.1%	18.6%	19.3%	
10 years	58.5%	61.1%	57.3%	
>10 years	17.4%	20.3%	23.4%	0.005
Pack years, packs/day × years	2.9 (0; 16.2)	0.4 (0; 10.7)	0.3 (0; 8.2)	<0.001
Physically active	46.8%	47.2%	52.8%	0.03
Alcohol, g/week	38 (0; 143)	45 (0; 152)	42 (0; 139)	0.71
Diabetes mellitus†	3.0%	3.0%	2.4%	0.67
Waist circumference (cm)	86 (77; 96)	85 (75; 95)	86 (74; 96)	0.34
Serum glucose (mmol/l)‡	5.2 (4.7; 5.6)	5.2 (4.7; 5.6)	5.2 (4.8; 5.7)	0.87
Serum triglycerides (mmol/l)‡	1.3 (0.9; 2.0)	1.4 (0.9; 2.2)	1.4 (1.0; 2.3)	0.005
Serum HDL-C (mmol/l)‡	1.4 (1.2; 1.8)	1.4 (1.2; 1.7)	1.4 (1.2; 1.7)	0.49
Serum LDL-C (mmol/l) ‡	3.3 (2.7; 4.2)	3.4 (2.6; 4.1)	3.3 (2.6; 4.1)	0.78
Hormone replacement therapy	15.3%	16.3%	23.3%	<0.001
Mean clinical attachment loss (mm)	2.05 (1.19; 3.21)	1.81 (1.00; 2.88)	1.67 (0.90; 2.63)	<0.001
Number of missing teeth	4 (2; 9)	3 (1; 7)	3 (1; 7)	<0.001
High tooth loss§	20.8%	16.7%	15.3%	0.01
Serum IGF-I (ng/ml)	124 (100; 157)	148 (122; 180)	173 (140; 218)	<0.001
Serum IGF-I/IGFBP-3 ratio	0.08 (0.07; 0.10)	0.07 (0.06; 0.09)	0.07 (0.06; 0.09)	<0.001

*Chi-square test (categorical data) or Kruskal–Wallis test (continuous data).
†self-reported physician's diagnosis or anti-diabetic treatment (ATC code A10).
‡non-fasting blood samples.
§defined as age- and gender-specific fifth quintile *versus* four lower quintiles; $N = 2398$.
Data are presented as percentages or median (25%; 75% percentile).
N, number; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; IGF-I, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3.

IGFBP-3

Here, an inverse association between serum IGFBP-3 and periodontal disease was consistently found in the overall sample. However, the protective effect of serum IGFBP-3 levels on mean CAL was restricted to levels between 300 and 1200 ng/ml (Fig. 1a). Results support the assumption that serum IGFBP-3 levels might be associated with local homeostasis in periodontal tissue.

IGFBP-3 has several roles both in systemic and in local regulation (Yamada & Lee 2009), and is a local stimulator of IGF action in bone (Govoni et al. 2005). Takenouchi et al. found a weak, but non-significant, inverse association between IGFBP-3 in the gingival crevicular fluid (GCF) and PD (Takenouchi et al. 2010). Their results are in line with our findings, despite the divergence regarding the selected clinical parameters of periodontal disease. Consequently, one might speculate that serum IGFBP-3 correlates with local IGFBP-3 of the GCF, regarding the role of IGFBP-3 in the homeostasis in periodontal disease. And, given the facts that (a) IGFBP-3 levels decrease throughout lifetime (Juul et al. 1994) and (b) periodontal ligament (PAL) cells, cementum, and dentine may act as a local reservoir for IGFBP-3 (Gotz et al. 2006a), we might speculate that the degradation in IGFBP-3 levels with age may contribute to more periodontal disease.

IGF-I/IGFBP-3 ratio

We did not find an association between IGF-I/IGFBP-3 ratio and CAL or tooth loss. In a North Indian population, free serum IGF-I levels were directly measured (Rai et al. 2012), whereas we calculated the free IGF-I levels by means of IGF-I/IGFBP-3 ratio. Despite methodological differences, both studies did not find any significant associations between free IGF-I and periodontal disease. Therefore, our hypothesis regarding a positive association between free IGF-I and periodontitis was discarded.

Numerous studies evaluated IGF-I/IGFBP-3 ratios in normal physiology (Berry et al. 2001, Gram et al. 2006, Kong et al. 2007) and as a risk factor for systemic diseases including cancer (Douglas et al. 2010) and GH deficiency (Granada et al. 2000). Generally, it was found that IGF-I/IGFBP-3 ratios behaved differently compared with IGFBP-3. Furthermore, and for so far unknown reasons, IGF-I/IGFBP-3 ratio was correlated with IGF-I values rather than with IGFBP-3 values (Kong et al. 2007). Therefore, it still needs to be explained, why IGF-I/IGFBP-3 ratios and IGFBP-3 did not act similarly.

Local *versus* systemic IGF-I

Systemic (liver-derived) IGF-I has an emerging importance in systemic diseases. Systemic IGF-I and local bone-derived IGF-I may have overlapping growth-promoting effects, replacing each other with regard to regulation of longitudinal bone growth (Ohlsson et al. 2009). Although IGF-I may

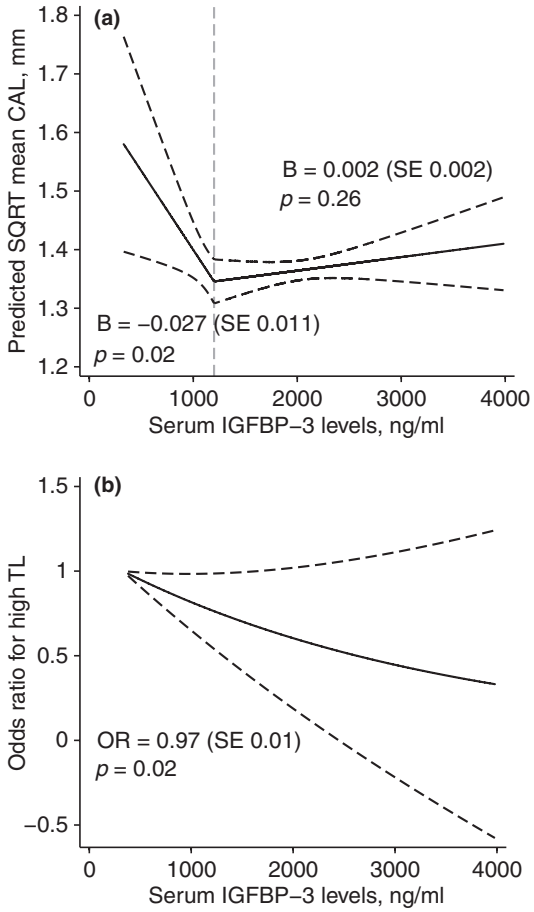

Fig. 2. Associations between serum IGFBP-3 levels and (a) predicted mean clinical attachment loss (CAL; square rooted (SQRT), linear spline, vertical grey line indicates knot at 1200 ng/ml) and (b) Odds Ratios for high tooth loss (TL; Odds ratios compared to reference values for serum IGFBP-3 levels (ref. 330 ng/ml) are given) after full adjustment. Respective *p* values for serum IGFBP-3 based on linear splines (a) and logistic regression (b) are given.

play a prominent role in periodontal homeostasis, we could not demonstrate an effect of systemic IGF-I. Obviously, IGF-I seems to act primarily locally and seems not to be influenced by systemic levels of IGF-I.

A recent study has confirmed the fundamental role of IGF-I in periodontal structures, and showed a strong anti-apoptotic activity of IGF-I in periodontium (Werner & Katz 2004). Giannobile et al. showed that local IGF-I administration in animals resulted in 64% increase in attachment formation (Giannobile et al. 1994). During periodontal inflammation, local IGF-I plays an important role in wound healing and in accelerating healing of inflammation through fibroblast collagen synthesis (Werner & Grose 2003). Periodontal ligament cells may act as a local pool for IGF (Gotz et al. 2006a). Our results regarding IGF-I may be inconclusive because (a) local actions of IGF-I are more relevant with respect to periodontal disease than serum levels of IGF-I and, as previously discussed, (b) local levels are independent from serum levels of IGF-I. Therefore, the relative importance and differences between systemic and local actions of IGF-I on periodontal disease are still inconsistent and need to be further clarified.

Local *versus* systemic IGFBP-3

It was reported that IGFBP-3 has several roles both in systemic and local regulation (Yamada & Lee 2009). IGF-I and IGFBP-3 are correlated with GH secretion, as patients with GH deficiency have lower serum levels of IGFBP-3, and patients with acromegaly have increased levels (Hasegawa et al. 1993). Furthermore, circulating IGFBP-3 levels were associated with systemic over expression of pro-inflammatory cytokine IL-6 (De Benedetti et al. 1997). To our

Table 2. Coefficients (B) and 95% confidence intervals (CI) for associations of serum IGF-I (linear), IGFBP-3 (linear spline, knot at 1200 ng/ml), and IGF-I/IGFBP-3 ratio (linear) with mean clinical attachment loss (square root transformed), adjusted for potential confounders (N = 2293). Linear regressions with robust standard errors were applied

IGF-I (100 ng/ml increments)			IGFBP-3 (100 ng/ml increments)			IGF-I/IGFBP-3 ratio (0.1 increments)		
	B (95% CI)	*p* value		B (95% CI)	*p* value		B (95% CI)	*p* value
				≤ 1200 ng/ml				
M0	−0.302 (−0.341; −0.263)	<0.001	M0	−0.027 (−0.061; 0.007)	0.12	M0	−0.185 (−0.257; −0.113)	<0.001
M1	−0.024 (−0.057; 0.010)	0.17	M1	−0.028 (−0.053; −0.003)	0.03	M1	0.010 (−0.028; 0.048)	0.61
M2	−0.008 (−0.040; 0.024)	0.63	M2	−0.027 (−0.049; −0.005)	0.02	M2	0.012 (−0.023; 0.047)	0.49
				>1200 ng/ml				
			M0	−0.016 (−0.021; −0.011)	<0.001			
			M1	0.000 (−0.004; 0.004)	0.99			
			M2	0.002 (−0.002; 0.006)	0.26			

M0, unadjusted; M1, adjusted for age (continuous) and gender; M2, adjusted for age (continuous), gender, education, number of pack years, high-density lipoprotein (quartiles), and waist circumference; IGF-I, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; *N*, number.

Table 3. Odds ratios (OR) and 95% confidence intervals (CI) for associations of serum IGF-I, IGFBP-3, and IGF-I/IGFBP-3 with high tooth loss (fifth quintile *versus* four lower quintiles), adjusted for potential confounders ($N = 2398$). Logistic regressions with robust standard errors were applied

IGF-I (100 ng/ml increments)			IGFBP-3 (100 ng/ml increments)			IGF-I/IGFBP-3 ratio (0.1 increments)		
	OR (95% CI)	*p* value		OR (95% CI)	*p* value		OR (95% CI)	*p* value
M0	0.82 (0.67; 1.01)	0.06	M0	0.96 (0.94; 0.98)	0.001	M0	0.99 (0.80; 1.22)	0.91
M1	0.88 (0.70; 1.11)	0.29	M1	0.96 (0.94; 0.99)	0.003	M1	1.06 (0.86; 1.30)	0.59
M2	0.92 (0.74; 1.16)	0.50	M2	0.97 (0.95; 0.99)	0.02	M2	1.05 (0.87; 1.28)	0.60

M0, unadjusted; M1, adjusted for age (continuous) and gender; M2, adjusted for age (continuous), gender, education, number of pack years, high-density lipoprotein (quartiles), and waist circumference; IGF-I, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor binding protein-3; *N*, number.

knowledge, this is the first study investigating the relationship, between serum IGFBP-3 and periodontal disease.

It was demonstrated in the literature that the IGF action of osteoblasts in vitro is stimulated through the exogenously added IGFBP-3 (Ernst & Rodan 1990). This might indicate potentiating effects of locally produced IGFBP-3 in bone. Moreover, the important role of local IGFBP-3 in the oral cavity as a growth factor and in dental healing process was previously examined in several in vitro studies: periodontal ligament cells, cementum and dentine may act as a local pool for IGFBP-3 (Gotz et al. 2006a). The same working group found that during wound healing, the early repaired cementum contains mainly IGFBP-3 (Gotz et al. 2006b). Furthermore, the state of hypercortisolemia in diabetes mellitus impairs the healing process through suppression of the IGF-I system, especially IGFBP-3 (Bitar 2000). Obviously, IGFBP-3 plays an important role in the healing process in periodontal ligament cells. Nevertheless, the significant difference between the periodontally healthy and diseased sites in terms of local IGFBP-3 in GCF of the humans was detected for the first time in a case-control study (Sakai et al. 2006).

Because IGFBP-3 has a high serum level, the balance between local and systemic IGFBP-3 in periodontal tissue remains to be elucidated. Our results support the assumption that the systemic actions of serum IGFBP-3 might go along with its so far discovered local actions, showing a protective effect of high serum IGFBP-3 levels on periodontal disease.

As a major strength, we evaluated a large population-based study comprising a wide range of social and medical data, allowing comprehensive confounder adjustment and precise estimation of the association between different variables of the IGF-axis and periodontal disease. We applied fractional polynomials to explore the relations between IGF-status and periodontal status appropriately, considering both linear and non-linear forms. For non-linear associations, linear splines were presented to facilitate accessibility. However, we must acknowledge that the protective effect of serum IGFBP-3 levels on mean CAL was most obvious at levels between 300 and 1200 ng/ml. Although the number of subjects having serum levels of IGFBP-3 of less than 1200 ng/ml was relatively low, statistical significance of respective associations deliver evidence for a potentially protective effect of serum IGFBP-3 levels on periodontal disease.

In conclusion, low serum IGFBP-3 levels might be associated with more periodontal disease, whereas serum IGF-I and IGF-I/IGFBP-3 ratios were not associated with periodontal disease. Altogether, the relation between serum IGF components and periodontitis seems to be minor. The cross-sectional study design warrants from drawing any definite causal conclusions of the association between serum IGFBP-3 and periodontal disease. Longitudinal studies are desired to investigate causality more closely.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Association between serum IGFBP-3 levels and predicted mean clinical attachment loss (CAL; square rooted, linear spline, vertical grey line indicates knot at 1200 ng/ml) after full adjustment. Respective regression coefficients (B), standard errors (SE) and p values for serum IGFBP-3 based on linear splines are given.

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Clinical Relevance

Scientific rationale for the study: IGF-I-axis plays an important role in differentiation and proliferation of periodontal ligament fibroblasts and contributes to regeneration of periodontal tissue. Therefore, we evaluated the association between serum IGF-related variables and periodontitis in the Study of Health in Pomerania.

Principal findings: Low serum IGFBP-3 levels were associated with higher levels of periodontal disease. Serum IGF-I and IGF-I/IGFBP-3 ratio were not associated with periodontitis.

Practical implications: If confirmed in further studies, an association between IGF-axis and periodontitis may open the horizon for a better understanding of biological mechanisms related to periodontal disease progression.

Appendix

Publications

Harb A*, Holtfreter B, Friedrich N, Wallaschofski H, Nauck M, Albers M, Meisel P, Biffar R, Kocher T
Association between the insulin-like growth factor axis in serum and periodontitis in the Study of Health in Pomerania: an exploratory study (Originalartikel)
JOURNAL OF CLINICAL PERIODONTOLOGY. 2012; 39(10):931-939. (Published)

Harb A*, Holtfreter B, Friedrich N, Wallaschofski H, Nauck M, Kocher T
Evaluation of the Periodontal Status in Acromegalic Patients: A Comparative Study (Originalartikel) ISRN Dentistry. 2012; (Published)

Harb A, Holtfreter B, Friedrich N, Wallaschofski H, Nauck M, Albers M, Meisel P, Biffar R, Kocher T
Association between Insulin-Like Growth Factor I (IGF-I) – related variables and Periodontitis. (Poster) Europerio 7; 2012 Jun 06; Wien. In: JOURNAL OF CLINICAL PERIODONTOLOGY. 39(Supplement s13):1-429. (completed)

Meisel P*, Harb A, Kocher T
Orale Leukoplakien: Erhöhtes Risiko bei Diabetes (Originalartikel)
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Buchwald S*, Kocher T, Biffar R, Harb A, Holtfreter B, Meisel P
Tooth loss and periodontitis by socio-economic status and inflammation in a longitudinal population-based study (Originalartikel)
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