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Infantile Chronic Recurrent Parotitis (ICRP): Analysis of Changes in the Expression of Parotid Salivary Proteins Associated with the Disease

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1. Introduction

Infantile chronic recurrent parotitis (ICRP) affects life quality from early childhood to adolescence.

ICRP is an inflammatory disease usually associated to a non-obstructive sialectasis of parotid glands. It is characterized by recurrent episodes of inflammatory volume increase of one or both glands. Occasionally, the episode is accompanied by fever and with the patient feeling unwell. During alternate interepisode periods of normality, lasting from weeks to months, the glands are clinically asymptomatic (Baurmash, 2004; Chitre & Premchandra, 1997). The first episode occurs at ages between 3-months and 13-years old. More frequently, however, the disease appears between 2 and 6 years-old. The number of recurrences varies from one subject to another but an episode every 3 to 4 months is a representative frequency. The highest occurrence frequency is at the first year of school. Then the frequency decays and follows an unpredictable pattern that varies from patient to patient until adolescence. The episodes are manifested by a varying degree of general ill-feeling with moderate fever. Sometimes, this ill condition may oblige to hospitalize the patient for as long as a week (Landaeta et al., 2003). The episodes of gland swelling are associated with upper respiratory conditions, such as a cold, amigdalitis, pneumonia and bronchitis (Landaeta et al., 2003; Vinagre et al., 2003). The incidence rate by sex appears to be identical. Frequently, ICRP occurs unilaterally and among bilateral patients one of the glands is more affected. The increase in parotid volume is quite painful and marked skin redness occurs in the corresponding area. Salivary flow in the affected gland decreases significantly and the corresponding salivary secretion becomes viscous, whitish and mucopurulent (Baurmash, 2004; Chitre & Premchandra, 1997; Vinagre et al., 2003). The inflammatory increase in gland volume may last for 24-hours, 1-2 weeks or, sometimes, several months. After an apparent regression to normality, the gland becomes quiescent until a new inflammatory episode. After puberty symptoms diminish and may fully disappear spontaneously although
sometimes the disease may last until adulthood (Baurmash, 2004; Chitre & Premchandra, 1997; Vinagre et al., 2003; Concheiro et al., 2000).

2. The etiology of ICRP is unknown

Several causing factors have been invoked, namely, congenital duct malformations, genetic factors, allergies, local manifestations of autoimmune diseases, immunodeficiencies, maxillary dysfunction due to tooth misalignment, etc. (Baurmash, 2004; Chitre & Premchandra, 1997; Bernkopf et al., 2008). None of these factors has been unequivocally demonstrated as a causing agent and to date the etiopathogenesis of the disease remains an enigma. In attempts to elucidate the origin of this clinical condition, a number of microbiological analyses of parotid secretion have been carried out. By direct analysis of parotid saliva from ICRP patients, parotitis virus and several respiratory virus have been discarded as eventual causing agents (Landaeta et al., 2003). Bacteriological analyses by Concheiro et al. (2000) have shown the presence of a mixed flora of Gram-positive cocci. Giglo et al. (1997) have shown that 80% of patients display *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus viridans* y/o *Moraxella catarrhalis*. Despite the relevance of these insights into the disease, several characteristics of the disease challenge its infectious origin. Among them we can remark that the glandular process is mainly of inflammatory nature, that the general health condition of the patient is just moderately affected, the unilateralness of the condition among patients carrying the above-mentioned flora and, finally, the occurrence of a number of cases in which cultures for bacterial microorganisms give negative results. In the specialized literature expressions like “At the present there is a tendency to consider a multifactorial origin of the disease” have become quite usual. According to this undemonstrated view, sialectasis would be the ICRP-triggering factor. Thus, due to partial malformations of the duct system, iterated ascending infections would result in symptomatic episodes and reinforced the duct alterations (Baurmash, 2004; Chitre & Premchandra, 1997; Rostion et al., 2004; Nahlieli et al., 2004; Nazar et al., 2001). In our view, much more data are still necessary to put forward a consistent hypothesis on the origin of this disease.

3. Sialography and ultrasonography confirm diagnosis of ICRP and allow assessing the degree of glandular involvement

Diagnosis of ICRP is based on the clinical history and is confirmed by both complementary imaging procedures. Sialography demands the introduction of iodinated contrast media via the Stenon’s duct in order to opacify the gland ducts and put in evidence the duct dilatations as a typical radiographic flowering pump appearance (Fig. 1). According to images of glandular filling, sialectasis may be punctate, globular, cavitary, destructive or combinations between them (Chitre & Premchandra, 1997; Ramirez et al., 1996). These images would be a reflection of the inflammatory condition affecting the glandular tissue. Histopathological analysis of glandular tissue dissected from ICRP patients shows lymphocyte infiltration tending to organize lymphoid follicles around dilated interlobular ducts (sialectasia) (Chitre & Premchandra, 1997; Nahlieli et al., 2004; Shimizu et al., 1998). Besides confirming diagnosis, sialography is also useful to assess the progression of the gland condition. Thus, sialographic changes tend to decrease or even disappear when the
In a different study on 17 patients a correlation between sialographic findings and duration of the disease has been postulated (Ramírez et al., 1996). After multiple recurrences the gland may experience a permanent destruction. Those altered glands, as evidenced by sialography, and those patients would experience more frequent and more prolonged episodes than patients displaying less accumulated glandular damage (Baurmash, 2004). Surprisingly, altered sialography is usually bilateral although the clinical condition may be unilateral. There is no clear explanation for the fact that only one of the glands undergoes clinical inflammation (Baurmash, 2004; Concheiro et al., 2000; Chitre & Premchandra, 1997;
Nazar et al., 2001). This evidence suggests that a subclinical glandular alteration may occur previous to the clinical condition associated to the increase in gland volume.

In the past few years, the use of ultrasonography as support for ICRP diagnosis has increased. A main advantage of ultrasonography over sialography is its lower invasiveness. Ultrasonographic images of ICRP glands show a diffuse volume increase of the gland, a decreased gland ecogenicity and numerous nodular images on the parenchyma, which may represent sialectasis and lymphocyte infiltrates (Encina et al., 1996; Isaacs et al., 2002; Mandel & Bijoor, 2006; Miziara et al., 2005; Nahlieli et al., 2004; Nazar et al., 2001; Sitheeque et al., 2007; Shacham et al., 2009; Shimizu et al., 1998). Most of the clinical reports indicate a correlation between both imaging studies (Encina et al., 1996; Mandel & Bijoor, 2006; Nazar et al., 2001; Shimizu et al., 1998). However, in some cases sialography has not been compatible with ICRP diagnosis but ultrasonography has shown bilateral glandular involvement (Isaacs et al., 2002). Some authors suggest that ultrasonography would be more sensitive for detecting glandular involvement and therefore advantageous for diagnostic purposes and for monitoring the disease (Shimizu et al., 1998).

4. ICRP treatment is a controversial and confounding issue

Because the etiology of ICRP is unknown and because its clinical manifestations are unpredictable, there is no agreement on the therapeutic management of the disease. As observed in the specialized literature, patients receive care from multiple medical specialists who prescribe different therapies for the same pathological entity. Sometimes, with the aim of increasing the salivary flow, several physical maneuvers are recommended: local heat, gland massages, gum chewing and consumption of acidic sweets (Nahlieli et al., 2004; Nazar et al., 2001). Usually, these maneuvers are recommended for preventing recurrences although gland swelling is abrupt and volume increase occurs within a few hours. In other studies, recurrences are treated with non-steroidal antiinflammatories (Rostion et al., 2004; Nazar et al., 2001), which may be combined with sialogogues, antihistaminics, lisozyme and antibiotics (Concheiro et al., 2000). Based on positive experience with mucolytics in the treatment of a variety of pathologies, such as Sjögren’s syndrome and xeroftalmia, at the Surgery Department of the Hospital Roberto del Río the ICRP patients are treated with 4 mg of bromhexina every 8 hours during recurrences (Rostion et al., 2004). By contrast, also based on own experience that following sialography the symptoms of ICRP fully disappear in an important number of patients, and assuming that the contrast medium would have antiseptic activity, at the Child Maxillofacial Unit of the Hospital San Juan de Dios the treatment consists in regular intraglandular washings with an iodinated antiseptic via the excretory duct of the parotid gland (Fig. 2). Such a treatment is repeated until the gland displays a sialographic image that is consistent with normality. In case the patient is ill-feeling the treatment is combined with amoxicillin therapy (Landaeta et al., 2003). In a recent pilot study, the authors treat the jaw malfunctioning due to misoclusion in order to prevent ICRP recurrences. Those authors observed a decrease in the symptoms of 9 out of 13 cases although three of the patients were refractory to the treatment (Bernkopf et al., 2008). Another study recommends treating ICRP patients during the active stage of the condition by an inspection of the Stenon’s duct with a sialendoscope and a simultaneous washing of the gland with saline solution under general anesthesia. Once the duct has been washed, the
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Treatment is completed by an intraglandular injection of 100 mg hydrocortisone and intravenous amoxicillin-clavulanic acid (Issacs, 2002; Shacham et al., 2009). In cases of severe non-reversible glandular damage derived from prolonged acute episodes, ligation of the excretory duct of the gland to provoke its atrophy has been recommended (Baurmash, 2004; Nahlieli et al., 2004). As a last resource to deal with severe ICRP conditions, which is recommended when the other treatments have proven unsuccessful, partial or total parotidectomy is performed despite the complexity derived from the facial nerve proximity (Baurmash, 2004; Nahlieli et al., 2004). There are reports in the literature describing bilateral parotidectomy in a 11-year old boy affected by an extreme condition (Moody et al., 2000).

Fig. 2. Intraglandular washing via the excretory duct of the parotid gland in ICRP patient.

Despite the pathological process in ICRP is basically of inflammatory nature, as shown by histopathological assessment, most of the reports show that the therapeutical trials include the use of antibiotics. The most frequently used antibacterial drugs are amoxicillin or amoxicillyn combined with clavulanic acid (oral or systemic) or first generation cefalosporines (Baurmash, 2004; Concheiro et al., 2000; Chitre & Premchandra, 1997; Nahlieli et al., 2004; Nazar et al., 2001). Clearly, these therapeutetic schemes are oriented to deal with the general ill-condition and so to prevent its worsening. However, it is necessary to bear in mind the risk of microbial resistance derived from the iterative use of drugs to treat a disease whose origin may be other than bacterial. Thus, in some studies topic penicillin or topic chloramphenicol is used for gland lavages during acute episodes. Such a wide spectrum of treatments is a clear reflection of the lack of a minimum consensus on ICRP therapy. Although most of the reports indicate a variable degree of success, none of them claims a full prevention of the recurrences. Furthermore, the general tendency of current therapies seems to be focused on alleviating symptoms, shortening the time of the acute
episodes and extending the quiescent periods, preventing a worsening of the general ill-
feeling and avoiding as much as possible a damage of the glandular parenchyma due to
inflammatory recurrences (Baurmash, 2004; Concheiro et al., 2000; Rostion et al., 2004;
Nahlieli et al., 2004; Nazar et al., 2001).

5. Parotid gland as an organ with endocrine functions

Besides its well known role in saliva production and so in the lubrication and protection of
the soft tissues that are bathed in it, mounting evidence point to this gland as responsible of
some endocrine and metabolic functions. Thus, some studies suggest that this gland plays
an active role in nitrite and nitrate metabolism and that its experimental destruction in
laboratory animals leads to an unbalance between those molecular species (Xia et al., 2003a).
Human parotid saliva presents higher levels of nitrate compared with urine and blood
serum. Patients with Sjögren’s syndrome (an inflammatory autoimmune exocrinopathy)
show a decreased level of nitrate in saliva and a highly increased level of this chemical in
urine (Xia et al., 2003b). Experimental bilateral parotidectomy in laboratory animals results
in a reduction of dentin apposition in developing molars. Parotin, a protein that has been
isolated from porcine parotid glands (Tieche et al., 2005), has been shown to produce
hypocalcemia and leucocytosis, to affect bone, cartilage and connective tissue homeostasis
and to increase dentinary mineralization (Leonora et al., 2002). Recent evidence suggests
that parotin is a proline-rich protein that is biologically active in producing displacement of
dentiniferous fluid (Zhang et al., 2005). In consequence, the effect that the partial or total loss of
this organ may have for the human organism, particularly among growing children, is
mostly unknown. On that perspective, the control of the accumulative damage of this organ
may be more relevant than previously suspected.

6. Contraindication of parotid biopsy among ICRP patients has limited the
study of this disease

Parotid biopsy is restricted to some pathologies with a proper cost/benefit ratio. Restriction
is explained by the close proximity of facial nerve branches passing through the gland. In
this pathology, minor salivary glands remain unaffected. Accordingly, studies involving
tissular, cellular or molecular assessments of the parotid gland are extremist rare. Most of
the studies on ICRP consist either in clinical assessment, imaging and hematological studies
or, alternatively, deal with the outputs of various treatment strategies (Concheiro et al.,
2000; Rostion et al., 2004; Miziara et al. 2005; Nahlieli et al., 2004; Nazar et al., 2001;
Sitheeque et al., 2007; Shacham et al., 2009). In this context, the analysis of the fluid secreted
by the affected parotid gland would constitute a valuable noninvasive tool for the indirect
assessment of the physiopathological condition of the gland. Although several studies on
ICRP coincide in concluding that parotid saliva is qualitatively altered (Baursham, 2004;
Chitre & Premchandra, 1997; Landaeta et al. 2003; Nazar et al., 2001), studies on the
chemical properties of this fluid are rare. Sialochemical analysis has been performed in
regard to other medical conditions affecting this organ, such as Sjögren’s syndrome and
therapeutical irradiation of the maxillofacial territory, a condition resulting in chronic
inflammation of salivary glands (Asatsuma et al., 2004; Hanemaaijer et al., 1998; Kaufman &
Lamster, 2002; Streckfus & Bigler, 2002; Vuotila et al., 2002). Taback in 1978 and Erikson in

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1996 reported that parotid saliva from ICRP patients have increased levels of albumin, lactoferrin, kallikrein and IgA, as compared to healthy controls (Ericson & Sjöbäck, 1996; Tabak et al., 1978). On this background, in addition to the observation of a variety of physical changes in saliva from ICRP patients, our research group started in 2005 to analyze chemical properties of parotid saliva from ICRP patients with the aim of identifying molecular alterations, which may be of value in the understanding of the etiology and/or physiopathology of this condition.

7. Occurrence of molecules associated with extracellular matrix turnover in the salivary secretion

Metalloproteinases (MMPs), a family of macromolecules involved in normal and pathological extracellular matrix remodelling, have been observed in saliva of patients with various inflammatory conditions. This observation has contributed to get insights into the pathological process affecting the gland (Asatsuma et al., 2004; Hanemaaijer et al., 1998; Vuotila et al., 2002, Wu et al., 1997). These calcium- and zinc-dependent endopeptidases, whose expression is regulated by growth factors, cytokines, hormones and extracellular matrix components (Sternlicht & Werb, 2001), are initially synthesized as zymogens that are proteolyzed at the extracellular space to become active MMPs (Nagase & Woessner, 1999). Gelatinases MMP-2 and MMP-9 have been involved in a variety of pathological conditions in adults, such as Sjögren’s syndrome, Diabetes mellitus and periodontitis (Asatsuma et al., 2004; Collin et al., 2000; Pozo et al., 2005). They have also been observed in inflammatory pathologies of the child, as in chronic suppurative otitis media and in chronic lung diseases (Ekekezie et al., 2004; Jang et al., 2006). Both gelatinases have been found in a variety of body fluids, namely, caphaloraquid fluid, tracheal secretion, middle ear secretion, gingival fluid, blood plasma and saliva (Ekekezie et al., 2004; Jang et al., 2006; Kettlun et al., 2003; Schulz et al., 2004; Valenzuela, 2000; Wu et al., 1997). Patients subjected to therapeutical irradiation of the maxillofacial territory have active and inactive forms of MMP-2 and MMP-9 in saliva and irradiation would increase the MMP-9 activity (Vuotila et al., 2002). Patients with Sjögren’s syndrome have an increased activity of MMP-9 in saliva as well as an altered MMP-9 / TIMP-1 (a tissular inhibidor of MMPs 1) ratio, as compared to healthy control subjects (Asatsuma et al., 2004; Hanemaaijer et al., 1998; Wu et al., 1997). More recent studies in biopsies of minor salivary glands of patients with Sjögren’s syndrome have shown alterations in tissular metalloproteinases that coincide with those observed in saliva (Pérez et al., 2005).

The occurrence of these enzymes in saliva has also been tested in relation to pathologies affecting organs and systems other than salivary glands. Levels of MMP-9 and TIMP-1 in saliva of patients with adult periodontitis are higher than in healthy control subjects (Ingman et al., 1996). In a study with patients displaying taste disorders the levels of MMP-9 in saliva were not different compared to the respective controls. However, this study also showed that MMP-9 does occur in saliva of healthy subjects (Watanabe et al., 2005). In a similar observation, Raitio et al. (2005) reported that non-smoker control subjects presented both latent and active forms of MMP-9 whereas the smokers exhibited higher concentrations of latent MMP-9. Concerning systemic conditions, saliva of healthy control subjects has lower levels of the active and latent forms of MMP-2, MMP-9, TIMP-1 and TIMP-2 than
saliva of VIH-positive patients (Mellanen et al., 1998). In another study, saliva of control non-pregnant women was found to display lower concentrations of MMP-9 compared to saliva of pregnant women with premature membrane rupture (Miller et al., 2006).

Considering that ICRP is an inflammatory alteration producing damage of the glandular tissue it would be reasonable to suspect that metalloproteases are present in parotid saliva. In a recent study dealing with molecular analysis of parotid saliva from 33 ICRP patients we reported changes in the salivary composition as compared to 33 healthy control subjects. In that study we scored the frequency of subjects having MMP-2 and MMP-9 activities in parotid saliva and found that only two healthy subjects presented these enzymes in saliva whereas 18 and 10 ICRP patients were positive for MMP-9 and MMP-2, respectively (Morales-Bozo et al., 2007). A later study showed a positive association between degree of sialographic damage of single parotid glands and the occurrence of those gelatinases in the corresponding parotid saliva. Zymographic analysis showed that saliva from 32 and 21 parotid glands displaying altered sialographies also exhibited MMP-9 and MMP-2, respectively. When this analysis was performed on glands with no sialographic damage, only 2 of the glands presented MMP-9 and none of them showed MMP-2 (Morales-Bozo et al., 2008). These were the first reports on the occurrence of metalloproteases in saliva of ICRP patients, thus suggesting their involvement in the mechanism of parotid damage.

In a recent study with 32 ICRP patients we assessed the presence of both the active and latent forms of those gelatinases in parotid saliva from ill and unaffected glands of the same patients (Table 1). We found latent MMP-9 in 15 healthy and in 46 affected glands and latent MMP-2 in 9 healthy and in 36 affected glands. We found the active form of MMP-2 only in 2 healthy glands and in 14 ill glands.

<table>
<thead>
<tr>
<th>Salivary Gland</th>
<th>ProMMP-9</th>
<th>ProMMP-2</th>
<th>MMP-2</th>
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<tbody>
<tr>
<td>Unaffected</td>
<td>-</td>
<td>+</td>
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<tr>
<td>ICRP</td>
<td>1</td>
<td>15</td>
<td>2</td>
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Table 1. Presence of salivary metalloproteinases MMP-2 and -9 in salivary glands of ICRP patients.

In this study, the affected glands presented higher levels of all the gelatinase forms compared with the healthy glands although differences were not statistically significant. However, in this study we observed a high molecular weight gelatinase (130 kDa) in saliva obtained from 61 parotid glands. Besides, saliva of 15 healthy glands showed 1.75 ± 2.3 ng of this gelatinase per ml of parotid saliva versus 9.6 ± 2.3 ng of the enzyme per ml de saliva obtained from 46 ill glands (p=0.418) (Pinochet, 2008).

Gelatinases interact with a wide variety of molecules to form complexes like MMP-2/TIMP, MMP-2/glycosaminoglycans, MMP-9 dimers, MMP-9/MMP-1, MMP-9/NGAL (Neutrophyl gelatinase-associated lipocalin), MMP-9/Haptoglobuline, MMP-9/ proteoglycans, etc. (51). These complexes may play important roles under physiological and pathological conditions (Malla et al., 2008). The molecular size of the gelatinase complex that we have observed in parotid saliva of ICRP patients suggests that it would correspond to a MMP-9/NGAL complex. The molecular weight of the active form of this complex is 125-130 kDa and is
comprised by MMP-9 covalently bonded with NGAL of human neutrophiles (Yan et al., 2001). Through this interaction, MMP-9 becomes protected from degradation, thus breaking the balance between synthesis and degradation (Yan et al., 2001; Devarajan et al., 2007). This complex has been involved in pathogenic mechanisms of a variety of conditions, such as cancer, osteoarthritis, rheumatoid arthritis and renal damage (Devarajan et al., 2007; Gupta et al., 2007; Katano et al., 2009; Zhang et al., 2009). NGAL or lipocalin 2 (25 kDa) is a protein that binds siderophores with a high affinity, thus taking part of the innate immune system against bacterial infections in tissues that are exposed to the external environment (respiratory, gastrointestinal and urinary tracts). Its expression rises up during infection or inflammation (Xu et al., 2009). In submandibular glands of 10 patients having chronic sialadenitis NGAL has been found to be overexpressed in inflammatory infiltrates and in duct cells, as compared to gland tissue from control subjects (Woo et al., 2007). In lip salivary gland of patients with amyloid polyneuropathy, both mRNA NGAL and a 130 kDa form of MMP-9 have been found to be overexpressed compared to lip biopsies from healthy controls (Sousa et al., 2005). To date there are no reports on the presence of either NGAL or complexes between MMP-9 and other molecules in parotid gland or in parotid saliva under normal or pathological conditions.

8. The search for ICRP-associated salivary molecules should be performed in parotid saliva

Excepting the report of Wu et al. (1997), all the studies dealing with the assessment of metalloprotease levels in saliva have been performed in whole saliva. Whole saliva is a complex mix of diverse salivary fluids, plus components of the gingival crevicular fluid, exfoliated cells from the oral mucose, microbial flora and food remnants. In this fluid, the occurrence of MMP-2, MMP-9, TIMP-1 and TIMP-2 has been reported under conditions of gingival normality as well as in periodontal disease (Pozo et al., 2005). Thus, it would be hard to define the origin of those molecules present in whole saliva. In order to elucidate the point, Mäkelä et al. (1994) analyzed parallel samples of parotid saliva, submandibular/sublingual saliva, whole saliva and gingival crevicular fluid, all of them taken from 17 healthy control subjects. Those authors observed that total saliva and the crevicular fluid displayed similar levels of MMP-9 whereas only trace levels of those molecules were observed in glandular saliva. Collin et al. (2000) reported that MMP-9 activity in whole saliva from edentulous subjects, that is, without gingival crevicular fluid, was significantly lower than in whole saliva from dentulous subjects. Both observations suggest that MMP-9 in whole saliva would be provided by the periodontus. Hystolochemical studies on slices of parotid and submandibular glands indicate that MMP-2 and MMP-9 would be mostly located in the duct system and absent from the acinar system (Ogbureke & Fisher, 2004; Nagel et al., 2004; Teymoortash et al., 2004). Similar studies on lip salivary glands have shown the presence of MMP-2 and MMP-9 in duct cells and at the basal region of acinar cells (Pérez et al., 2000, 2005). These observations would suggest that those molecules would not be released from the gland to the oral cavity under normal conditions. On these evidence it becomes necessary that the identification of parotid-produced molecules would require analysis of parotid secretion and that much care should be taken to collect this fluid free of contaminants from other salivary components. To this
end, we have designed an ad hoc device to collect with high efficiency and comfort very pure parotid saliva (Morales et al., 1998). In addition we have developed an objective method to assess the purity of samples of parotid saliva. Protein fractionation of salivary samples from individual subjects of different ages and genders (N = 190) using unidimensional gel electrophoresis in SDS-polyacrylamide gels has shown that submandibular / sublingual saliva present two polypeptide bands (19 and 18 kDa) that are not present in other types of saliva, excepting whole saliva. Those polypeptide markers should not be present in samples of parotid saliva (Morales et al., 1998; Morales-Bozo et al., 2006).

9. Proteomic analysis of saliva as a tool for investigating ICRP-associated molecules

Parotid saliva comprises a wide variety of protein families whose characterization has been the product of diverse experimental approaches. In the past few years, proteomics of this body fluid has provided a large amount of compositional data in normality (Denny et al., 2008; Hardt et al., 2005; Walz et al., 2006). Changes in the proteome of parotid saliva have also been reported in some pathological conditions. A recent study compares the proteome of parotid saliva of elderly patients displaying root caries with the one of healthy controls (Preza et al., 2009). In 2006, Ryu et al. identified biomarkers in parotid saliva among patients with Sjogren’s syndrome. That study comprised 41 patients and 21 control subjects and showed that the proteomic profiles of those patients displayed a marked increase in inflammatory proteins compared with the profiles of control subjects. Particularly, significant increases in β-2 microglobuline, lactoferrin, kappa light chain immunoglobulin, polymeric immunoglobulin receptor, lisozyme C and cystatin C, were observed, in parallel, with a significant decrease in acinar cell proteins, such as amylase, carbonic anhydrase VI and two putative proline-rich proteins (Ryu et al., 2006).

In previous studies from our laboratory we have shown changes in the polypeptide profiles of parotid saliva from ICRP patients as compared to the ones of healthy paired controls. Such alterations were represented by an increase in the frequency of individuals displaying polypeptides of 43, 37, 33, 29, 26, 16 and 10 kDa in parotid saliva produced by clinically affected glands (Morales-Bozo et al., 2007). In a complementary study by protein fingerprinting and MALDI-TOF mass spectrometry of the 26 kDa polypeptide, which is present with no exception in the saliva of all the enrolled ICRP patients, we have found that it corresponds to the C-region of the kappa light chain immunoglobulin, thus suggesting its participation in the pathological process (Werner, 2008). Currently, we are defining bidimensional electrophoretic profiles of parotid saliva from healthy control subjects in order to set a standard profile to contrast the varying profiles of ICRP patients.

10. The problem

There are no studies on the prevalence of ICRP either in Chile or the rest of the world. At the Hospital San Juan de Dios in Santiago, Chile, there are currently around 300 children affected by ICRP and under medical care. Bibliographic references show that this is a worldwide distributed disease. From 1991 to date, the following cases have been reported: 1991: 20 children in Sweden; 1996: 16 children in Chile and 17 children in Sweden; 1997: 56 children in
Chile; 1998: 14 children in Japan; 1999: 3 children in Germany; 2000: two children in Germany and 30 children in Spain; 2001: 16 children in Chile and 12 children in Switzerland; 2002: one child in Australia; 2003: 50 children in Chile; 2004: one child in Israel and 38 children in Chile; 2005: 26 children in Israel, 133 children in Finland, 23 in Austria, 53 in Australia and 5 in Brazil; 2006: 12 children in Taiwan and one child in USA; 2007: 26 children in Sri Lanka; 2008: 10 children in France and 33 children in Chile; recently in 2009: 13 children in Italy and 76 children in Israel (Bernkopf et al. 2008; Concheiro et al., 2000; Encina et al., 1996; Ericson et al., 1991, Fazekas et al., 2005; 1996; Giglio et al., 1997; Huisman et al., 2001; Isaacs et al., 2002; Kolho et al., 2005; Leerdam et al., 2005; Mandel & Bijoor, 2006; Menauer et al., 1999; Miziara et al., 2005; Nazar et al., 2001; Nahlieli et al., 2004; Quenin et al., 2008; Rostion et al., 2004; Shacham et al., 2009; Shimizu et al. 1998; Shkalim et al., 2004; Sitheeque et al., 2007; Usmüller, 1999; Vinagre et al., 2003; Wang et al., 2006; Wittekindt et al., 2000).

ICRP is a complex disease given the lack of knowledge about its etiology and pathogenic mechanism and because of the lack of medical treatment leading to a definite cure. On the other hand, systemic effects derived from a prolonged parotid dysfunction, especially among growing infants, are also unknown. On our own experience, most of the patients are initially diagnosed for Viral Parotitis. Once recurrences start to occur, the child patients deambulate through medical and dental care centers until they are derived to reference centers for this disease. Since current medical treatments are only palliative in nature, patients are treated for several years, from preschool age to adolescence or even young adulthood, thus affecting life quality and overusing medical resources. Diagnosis is confirmed by means of sialography, an invasive complementary examination involving the local injection of a contrast medium that may remain in the gland even for years. Possibilities of substituting sialography by ultrasonography are remote due to the lack of equipment and trained professionals in the public health services in Chile.

Although ICRP is not an infectious disease, it uses to be confounded with a bacterial parotitis because salivary secretion becomes thick and whitish and then patients are treated with systemic antibiotics. Thus, despite the diagnosis of ICRP, most of the therapeutical schemes include associated antibiotherapy with a subsequent risk of bacterial resistance (Burmash, 2004; Chitre & Premchandra, 1997; Concheiro et al., 2000; Nahlieli et al., 2004; Nazar et al., 2001). The scarce histological studies on ICRP suggest that it involves an inflammatory condition with lymphocytary infiltration (Ericson, 1995; Ussmüller & Donath, 1999). Because of the high risk of biopsyng parotid glands, this maneuver is contraindicated in ICRP. Thus, the compositional analysis of saliva secreted by this gland represents an advantageous noninvasive tool to get insights into the functioning of the affected gland tissue.

Pioneering sialochemical studies indicate that parotid saliva of ICRP patients has higher concentrations of albumin, lactoferrin, kallicrein and IgA, as compared to control subjects (Ericson & Sjöbäck, 1996; Tabak et al., 1978). Lactoferrin, kallicrein and IgA are proteins that are normally present in parotid saliva and play defensive roles. Albumin seems to be passively contributed by blood plasma due to inflammatory processes affecting permeability of the glandular epithelia. Most of these data derive from studies with low numbers of patients (N = 1 and N = 17) whose main aim was to search for specific proteins.
Studies from our laboratory have shown that regardless unilaterality or bilaterality of the clinical condition, saliva of both parotid glands of ICRP patients present a higher concentration of proteins compared to that of control subjects (Morales-Bozo et al., 2007). We have also shown that parotid saliva of ICRP patients display gelatinase activity, which is absent in parotid saliva of healthy control subjects (Morales-Bozo et al., 2007). MMP-2 and MMP-9 activities show a positive relation with the degree of damage of the parotid gland, as assessed by sialography (Morales-Bozo et al., 2008). Active and latent forms of MMP-9 and latent form of MMP-2 were observed. No relationship was observed between the clinical involvement of the gland and the levels of the active and latent forms of those metalloproteases. However, the clinically affected ICRP glands displayed a much higher concentration of a high molecular weight gelatinase (130 kDa) (Pinochet, 2008). The molecular size of this gelatinase suggests that it would correspond to a MMP-9/lipocalin 2 complex (Gupta et al., 2007; Malla et al., 2008). In other pathologies, a covalent association between both molecular entities results in an unbalance between synthesis and degradation of MMP-9, thus increasing the enzyme activity of the complex (Devarajan et al., 2007; Yan et al., 2001 52,53). Lipocalin 2 is secreted by neutrophiles in tissues that are exposed to the external environment, as it is the case of the oral cavity. This protein of 25 kDa acts by binding and inactivating bacterial siderophores: in the presence of MMP-9 it becomes bonded covalently to the enzyme thus preventing its degradation (Devarajan et al., 2007). These results suggest that polymorphonuclear neutrophiles may be involved in the pathogenesis of this disease.

With the aim of screening for a wide number of salivary proteins from a single subject at once, we have performed unidimensional electrophoresis analysis in parotid saliva from ICRP patients. Our studies have shown marked changes in parotid saliva of these patients compared with the one from control subjects. Analyses of the most frequent changes in definite polypeptides have shown an increase in a group of seven polypeptide bands compared to control electrophoretograms (Morales-Bozo et al., 2007). A 26 kDa band has been identified by protein fingerprinting and Maldi-TOF mass spectrometry as corresponding to the C-region of the human kappa light chain immunoglobulin according to three different databanks (Werner, 2007). To date, there are no reports on bidimensional electrophoresis profiles of parotid saliva either from healthy control children or ICRP patients. Thus, this experimental approach may additionally increase the spectrum of relevant salivary proteins in ICRP.

11. Conclusions

Altogether, the evidence derived from the molecular analysis of parotid saliva of ICRP patients highly suggests that this body fluid may be used advantageously to identify molecules that are related to pathogenesis of the disease, to monitor the ill-condition and to assess the efficacy of the treatment.

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13. References


Ekekezie, IL; Thibeault, DW.; Simon, SD.; Norberg, M.; Merrill, JD.; Ballard, RA.; Ballard, PL. & Truong, WE. (2004). Low levels of tissue inhibitors of metalloproteinases with a high matrix metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio are present in tracheal aspirate fluids of infants who develop chronic lung disease. *Pediatrics, Vol.113, Nº.6, (June 2004), pp. 1709-1714. ISSN 0031-4005*


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matrix metalloproteinase 3/tissue inhibitor of metalloproteinases 1 and matrix metalloproteinase 9/tissue inhibitor of metalloproteinases 1 ratios. *Arthritis and rheumatism*, Vol.52, Nº.9, (September 2005), pp. 2751-2760. ISSN 0004-3591

Pinochet, A. (2008). Determinación de la frecuencia y actividad enzimática de las formas activas y latentes de las metaloproteínas 2 y 9 en la saliva parotídea de pacientes afectados por Parotiditis Crónica Recurrente Infantil. Tesis para optar al Título de Cirujano Dentista, Facultad de Odontología, Universidad de Chile.


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