International Journal of Pediatric Otorhinolaryngology 80 (2016) 74–77

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Decreased salivary matrix metalloproteinase-8 reflecting a defensive potential in juvenile parotitis

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ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form 12 November 2015
Accepted 13 November 2015
Available online 26 November 2015

Keywords:
Matrix metalloproteinase
Parotitis
MMP-9
MMP-8

ABSTRACT

Objective: Matrix metalloproteinases MMP-2 and MMP-9 have been associated with juvenile parotitis. However, the role of MMP-8 has not been addressed previously. This work focuses on salivary MMP-8 and -9 levels in juvenile parotitis.
Methods: During a five-year period at Helsinki University Hospital, a tertiary care hospital, 41 patients aged 17 or under, were identified as having parotitis; from 36 of these patients, saliva samples were collected for MMP-8 IFMA (time-resolved immunofluorometric assay) analyses. Control saliva samples were collected from 34 age- and gender-matched children admitted for an elective surgery who had no history of parotitis. For comparison, salivary levels of MMP-9, tissue inhibitor of matrix metalloproteinase (TIMP-1), MMP-8/TIMP-1 ratio, human neutrophil elastase (HNE), and myeloperoxidase (MPO) were analyzed by ELISA. Additionally, salivary MMP-8 levels were compared to historical saliva samples from 18 adult gingivitis patients as well as to 10 healthy adult controls.
Results: The median (25%, 75% percentile) MMP-8 concentration in saliva of parotitis patients was significantly lower than MMP-8 concentration in saliva of their controls [50.4 ng/ml (37.5, 72.9) vs. 148.5 ng/ml (101.2, 178.5) $p < 0.0001$] and lower than in patients with gingivitis [347.9 ng/ml (242.6, 383.2) $p < 0.0001$] or healthy adult controls [257.2 ng/ml (164.9, 320.7) $p < 0.0001$]. The MMP-8/TIMP-1 ratio was lower than in controls [0.13 (0.05–0.02) vs. 0.3 (0.17–0.46) $p < 0.0001$]. The median MMP-9 concentration in saliva of parotitis patients was significantly higher than in controls [143.9 ng/m (68.8–189.0) vs. 34.9 ng/ml (16.3–87.6) $p < 0.0001$]. Neither HNE, MPO, nor TIMP-1 alone separated the patients from the control groups.
Conclusions: MMP-9 was up-regulated in juvenile parotitis saliva, suggesting that MMP-9 may play a destructive role in juvenile parotitis, as others have suggested. The present novel findings reveal a decreased salivary MMP-8 concentration, suggesting that MMP-8 may reflect in juvenile parotitis down-regulated or anti-inflammatory immune characteristics.

1. Introduction

In the pediatric population parotitis is traditionally associated with mumps. However, due to effective vaccinations, mumps is currently rare in western countries [1]. Nowadays, the etiology of pediatricparotitis remains unknown in the majority of cases [2]. Other viruses such as Epstein-Barr virus, parainfluenza virus, adenovirus, enterovirus, human herpes virus 6, enterovirus, and parvovirus can cause acute mumps-like symptoms [3]. Bacterial etiology in parotitis is often associated with a secondary cause such as poor dental hygiene or obstruction in salivary flow, which can result from congenital malformations in the salivary duct system, strictures, or siololithiasis. These conditions are more common among adults than among children, however [4]. Complications of bacterial parotitis are rare, but can be severe in nature including abscess formation and spread of infection [5].

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http://dx.doi.org/10.1016/j.ijporl.2015.11.012
0165-5876/

Earlier we discovered that in pediatric populations parotitis recurs in about 50% of the cases [2,6]. Recurrent juvenile parotitis is an infrequent condition of unknown etiology [4,6]. Even though the overall condition of the patient remains good, recurrent episodes of parotid swelling and pain can be life-disturbing. Treatment modalities include anti-inflammatory drugs, hydration, and antibiotics. Most likely, however, no microbe etiology is involved in recurrent symptoms [2,4,7], making the role of antibiotics controversial.

The genetically distinct but structurally related MMPs are zinc-dependent metalloendopeptidases**,** which can be classified based on their primary structures and substrate specificities into several groups: collagenases (MMP-1, -8, -13), gelatinases (MMP-2,-9),stromelysins (MMP-3,-10,-11,-19), matrilysins (MMP-7,-26), membrane-type MMPs (MT-MMPs) (MMP-14,-15,-16,-17,-24,-25) and other MMPs [8,9]. MMPs can degrade almost all extracellular matrix components as well as modulate the immune system [8–10]. The decisive substrate cleavages of MMPs can direct their function to be, as surrogates, either tissue destructive or defensive and anti-inflammatory [8,11–18]. MMP-2 and -9 have been associated with parotitis [19,20], but the role of MMP-8 has undergone, as yet, no study in juvenile parotitis. This is a study of salivary MMP-8 in this parotitis. For comparison, we also studied MMP-9 and -13, tissue inhibitors of matrix metalloproteinase 1 (TIMP-1), human neutrophil elastase (HNE), and myeloperoxidase (MPO).

2. Materials and methods

2.1. Patients and controls

During 2005–2010 at the Department of Otorhinolaryngology and Head and Neck Surgery, Helsinki University Hospital, a tertiary care hospital, 41 (male, 22) pediatric patients aged 17 or under were identified prospectively having parotitis as described earlier [2]. The inclusion criteria were acute symptoms, and assigning of a diagnosis of parotitis. The length and characteristics of symptoms, clinical findings, and laboratory parameters were recorded. All children had been vaccinated against mumps. Clinical examination of oral health status revealed no caries or periodontal or oral mucosal manifestations [21,22].

Control subjects were 34 age- and gender-matched children admitted for elective surgery who were healthy at the moment and had no history of parotitis. Clinical examination of the oral health status of these control patients revealed no caries and no periodontal, or oral mucosal manifestations. For comparison in MMP-8 studies, we also used historical saliva samples from 18 systematically healthy adult gingivitis patients as described earlier [21,22], and saliva samples from 10 voluntary systemically and orally healthy dental nurses [23].

The study was approved by the ethics committee of Helsinki University Hospital. A written informed consent form was obtained from every patient and/or guardian and from all the control subjects.

2.2. Samples

A saliva sample stimulated by parafilm chewing was attempted in the acute phase of the diseases. However, due to lack of co-operation, and reduced salivary flow in acute infection, an adequate amount of saliva was obtainable only from 36 patients. Saliva samples of the study patients as well as from all the controls were stored at −20 °C until analyzed.

2.3. Time-resolved immunofluorometric assay (IFMA)

Salivary MMP-8 concentration was determined by a time-resolved immunofluorometric assay (Medix Biochemica, Kauniainen, Finland) [21,22,24].

2.4. ELISA

Levels of MMP-9, -13, TIMP-1, MPO and HNE were analyzed with an enzyme-linked immunoabsorbent assay (ELISA) as described [21,22,24,25].

2.5. Statistics

Data analysis was by GraphPad Prism version 4.0 (GraphPad Inc, San Diego, CA, USA). Data of each two groups were compared by Mann–Whitney test. The results are presented as medians (25%, 75% percentile). Clinical characteristics of the patients are expressed by mean/median (range). Pearsons's bivariate 2-tailed test served for correlations. A p-value less than 0.05 was considered statistically significant.

3. Results

Table 1 summarizes the clinical characteristics of the 36parotitis patients reported in detail earlier [2]. No children had any concomitant illnesses, and their overall condition was excellent or good; all of them practiced good dental hygiene. Mean duration of symptoms was 2.3 days (range 0.5–7). Body temperature ranged from 35.5 to 39.2 °C, mean 37.6 °C. Blood leukocyte count was elevated among 10 and C-reactive protein among 24 patients. Of eight bacterial cultures, one was positive for *Haemophilus influenzae*, and others showed normal oral flora. Children receiving antibiotics numbered 12, and 14 were treated symptomatically with NSAIDs only; 11 had recurrent symptoms.

The median (25%, 75% percentile) MMP-8 concentration in the saliva of the 36 parotitis patients was significantly lower than its concentration in the saliva of the 34 age- and gender-matched control patients [50.3 ng/ml (37.4–72.9) vs. 148.5 ng/ml (101.2–178.5) $p < 0.0001$] (Fig. 1).

We also compared the median (25%, 75% percentile) MMP-8 concentration in the saliva of parotitis patients to concentrations in the 18 gingivitis patients, as well as, in the 10 healthy adult controls. It was significantly lower in parotitis patients [50.4 ng/ml (37.4–72.9)] than in gingivitis patients [347.9 ng/ml (242.6–383.2) $p < 0.0001$)] or in the saliva of the adult controls [257.2 ng/ml (164.9–320.7) $p < 0.0001$] (Figs. 2 and 3).

The median TIMP-1 (ng/ml) concentration in the saliva of parotitis patients and of their controls showed no significant difference ($p > 0.05$) (Table 2). The MMP-8/TIMP-1 ratio was significantly lower among parotitis patients than among their controls ($p = 0.0001$) (Table 2).

Table 1
Clinical characteristics of 36 juvenile parotitis patients, 22 male.

	Age (years)	Duration of symptoms (days)	Temperature (°C)	Blood Leucocyte count (E9/l)	C-reactive protein (mg/ml)
Range	2.6–17	0.5–7	35.5–39.2	4.2–20.8	5.0–170
Mean	8.3	2.3	37.6	11.1	31.4
Median	7.7	2.0	37.2	10.7	13.0