Endocrinology & Metabolic Syndrome

Olsen-Bergem et al., Endocrinol Metab Synd 2014, 3:4
DOI: 10.4172/2161-1017.1000145

Research Article Open Access

Temporomandibular Joint Pain is Negatively Correlated to TNF Alpha and Osteoprotegrin Content in Synovial Fluid in Patients with Juvenile Idiopathic Arthritis

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Abstract

Objective: Temporomandibular joint (TMJ) involvement occurs in up to 80% of children with juvenile idiopathic arthritis (JIA). Little is known with regard to the complexity of the protein profile in synovial fluid (SF) from JIA arthritis during growth as compared to both JIA and rheumatoid arthritis (RA) of adults.

Design: Synovial fluid was collected from 54 joints/30 patients with TMJ arthritis (JIA 35 joints/20 patients, JIA adults 9 joints/5 patients, RA 10 joints/5 patients). Three cytokines and seven bone markers were quantified using Luminex multiplex assays and compared to demographic and clinical data of function and pain.

Results: Pain (spontaneous and upon palpation) and duration of pain were all negatively correlated with the TMJ SF content of tumor necrosis factor (TNF)-α. The level of Adrenocorticotropic hormone (ACTH) was negatively correlated to TMJ pain upon palpation and post-treatment pain and function. The concentration of ACTH was significantly lower in SF in JIA (1.4 ± 2.8 pg/ml) compared to adults with JIA (4.7 ± 12.2 pg/ml) and significantly higher compared to adults with RA (0.8 ± 1.5 pg/ml). Osteoprotegerin (OPG) was negatively correlated to spontaneous pain.

Conclusions: Our results indicate that the local concentrations of TNF-α, ACTH and OPG in TMJ fluid may not contribute to TMJ pain and tissue destruction in JIA/RA patients.

Keywords: Juvenile idiopathic arthritis; Temporomandibular joint; Rheumatoid arthritis; Synovial fluid

Introduction

Juvenile idiopathic arthritis (JIA) is a broad term that describes a clinically heterogeneous group of inflammatory joint diseases of unknown cause, with onset before 16 years of age [1], first described by Still in 1987 [2]. Temporomandibular joint (TMJ) involvement occurs in up to 80% of children with JIA [3], but is asymptomatic in 70% of these children [4].

Rheumatoid arthritis (RA) is a chronic, inflammatory condition that mainly affects joints; it is characterised by pain, erosion, disability, and reduced survival [5]. TMJ symptoms may occur in 65% of patients with RA [6].

	JIA	JIA adults	RA
Patients n=30	20	5	5
Female/male	16/4	5/0	4/1
Mean age (yr)	11 (6-17)	40 (31-53)	60 (29-82)
Rheumatoid disease duration (years)	4.7 (0.3-15)	32.9 (24-37)	17.4 (2-43)
TMJ disease duration (years)	1.8 (0.1-14)	18.9 (12-28)	5.4 (1.5-10)
MIO	26.5 (12-40)	30.4 (22-36)	29.8 (19-38)
Pain VAS (0-10, no pain-pain cannot be worse)	4.7 (0-9.7)	5.8 (2.3-9.1)	5.5 (3.6-9.3)
Function VAS (0-10, normal-cannot get worse)	4.2 (0.3-9.8)	5.9 (4.3-7.8)	6 (1.3-9)
Tenderness on palpation TMJ numerical scale (0-10, no pain-pain cannot be worse)	1.5 (0-6)	4.2 (0-8.5)	5.5 (0-9)
Joint samples total n=54	35	9	10
Bilateral samples n=24	15	4	5

Average numbers with range in brackets.

Table 1: Demographic data and characteristics for JIA (juvenile idiopathic arthritis), JIA adults (JIA with progression into adulthood) and RA (rheumatoid arthritis) patients. Of the 30 patients, four had unilateral TMJ arthritis, and in two more patients there was insufficient synovial fluid to perform the sample analysis.

Synovial fluid (SF) contains hyaluronic acid and interstitial fluid filtered from blood plasma. SF lubricates the articulating surfaces of the joint, absorbs shocks and transports nourishment and waste to and from the surrounding cartilage.

Arthritis of the TMJ may be characterised by pro-inflammatory cytokines, such as IL-1β, IL-2, IL-6 and tumour necrosis factor alpha (TNFα) [7], but little is known about the SF content of bone markers.

The purpose of this study was to characterize the concentration of cytokines and bone markers from SF and to compare these with clinical findings in children with JIA, adults with a persistent JIA, and adults with RA.

Materials and Methods

Subjects

SF was taken from TMJs in altogether 30 patients and 54 joints (24 bilateral TMJs and 6 unilateral TMJs). Twenty of the patients (35 TMJs) were children with juvenile idiopathic arthritis (JIA), five patients (9 TMJs) were adults with a previous JIA (AJIA) persisting into adulthood, and five patients (10 TMJs) had RA. Clinical findings have previously been published for the JIA patients [8]. Clinical recordings were noted

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Received October 14, 2014; **Accepted** November 20, 2014; **Published** November 24, 2014

Citation: Olsen-Bergem H, Bjørnland T, Reseland JE (2014) Temporomandibular Joint Pain is Negatively Correlated to TNF Alpha and Osteoprotegrin Content in Synovial Fluid in Patients with Juvenile Idiopathic Arthritis. Endocrinol Metab Synd 3: 145. doi:10.4172/2161-1017.1000145

	JIA	n=	JIA Adult	n=	RA	n=	Adults	n=	ALL	n=
Total protein	258 (570)	35	38 (36)	9	64 (107)	10	52 (80)	19	186 (470)	54
IL-1	1.1 (4.9)	34	0.7 (1.8)	9	0.2 (0.4)	8	0.4 (1.3)	17	0.8 (4.0)	51
IL-6	232.3 (462)	20	46.0	1	715.6 (114.1)	3	438.7 (874.5)	4	273.6 (551.0)	24
TNF	1.7 (4.7)	34	0.3 (0.2)	9	2.5 (5.7)	8	1.3 (4.0)	17	1.5 (4.5)	51
OPG	81.9 (236.1)	35	81.9 (78.3)	9	201.4 (374.4)	9	137.4 (270.4)	18	98.9 (245.1)	53
OC	1266.4 (3786.8)	30	437.1 (367.5)	7	930 (1745.2)	6	658.7 (1233.2)	13	1069.6 (3224.6)	43
Leptin	29.0 (26.4)	35	103.3 (109)	9	85.1 (140.8)	10	92.2 (119.7)	19	50.0 (77.2)	54
OPN	4108 (17081)	29	4914 (4663)	8	8767 (21011)	9	6535 (14880)	17	4863 (16039)	46
PTH	0.8 (1.3)	35	3.7 (9.6)	9	2.9 (6.8)	8	3.1 (7.8)	17	1.5 (4.6)	52
ACTH	1.4 *(2.8)	35	4.7*(12.2)	9	0.8*(1.5)	9	2.5*(8.2)	18	1.8 (5.2)	53
Adiponectin	129702 (170183)	35	60506 (98533)	9	179347 (228962)	8	121222 (175335)	17	124726 (169320)	52
Insulin	191.8 (102.2)	6	515.4 (663)	2	17 (12.7)	2	266.2 (479)	4	221.6 (289.4)	10

n= represents number of detectable samples on the Lumin-X Elisa. All data are presented in pg/ml, except total protein (µg/ml). * =Significant difference p<0.05.

Table 2: Protein levels in samples from patients with juvenile idiopathic arthritis (JIA), adults with continued juvenile arthritis (JIA Adult) and rheumatoid arthritis (RA), JIA Adults with RA (Adults). For all samples, mean concentrations are presented.

before SF collection and at two follow-ups (3 and 8 months). Patient characteristics are shown in Table 1. The Regional Medical Ethical Committee, East, Norway (S06269a), approved this prospective clinical study of arthrocentesis of the TMJ in patients with JIA, AJIA and RA.

Sample collection

Samples were collected from the TMJ using a push and pull technique previously described by Alstergren and Kopp [9]. Ultrasound guided sampling was performed in an operation field after previous disinfection of a 3 cm wide area of skin around the penetration site. Disinfection consisted of washing five times with chlorhexidine-ethanol 5mg/ml solution (Klorhexidinsprit 5mg/ml, Fresenius Kabi, Norway) according to the recommendations of Oslo University Hospital. All samples were immediately frozen at -80°C.

Protein analysis

The total protein content of the 54 samples was quantified using the BCA protein assay (Pierce Biotechnology, USA). Multi-analytic profiling on the level of specific proteins in SF was performed on the Luminex 200 system (Luminex, Austin, TX, USA) using xMAP technology. Acquired fluorescence data were analysed by the xPONENT 3.1 software (Luminex). The levels of the bone markers IL-1β, IL-6, ACTH, adiponectin, insulin, leptin, osteoprotegerin (OPG), osteocalcin (OC), osteopontin (OPN), parathyroid hormone (PTH) and tumour necrosis factor-α (TNFα) were measured using Human Bone Panel 1B (Millipore, Billerica, MA, USA). The levels of IL-1β, Il-6 and TNFα were also measured using the HSCYTMAG-60SK-3plex human high sensitive cytokine kit (Millipore).

Statistics

The data were analysed with Spearman rank order correlation test, and comparisons of the different groups with regard to the immunological data were performed using both *t*-test (Shapiro-Wilk) and Mann-Whitney rank sum test. One-way ANOVA was performed and, if the results were statistically significant, we tested for multiple comparisons using Dunn's test. Statistical analyses were performed using SigmaPlot 12.0 for Windows (Systat software Inc. (SSI), San Jose, CA, USA). A significance level of 5 % was used throughout the study.

Results

Cytokine and bone marker levels in synovial fluid

Total protein concentration in SF of the TMJ in children with JIA was slightly elevated (258 ± 570 µg/ml,) compared with adults with RA and JIA (52 ± 80 µg/ml) (Table 2), but differences were not statistically significant.

Levels of IL-1β, IL-6, TNFα, OPG, OC, leptin, OPN, PTH, ACTH, adiponectin and insulin for children and adults with JIA, and adults with RA are presented in Table 2. ACTH levels were significantly lower in SF from children compared with all adults (p=0.03), and compared with adults with JIA (p=0.03), but were significantly higher than in adults with RA (p=0.02). IL-6, OPG, leptin, OPN, PTH, and insulin were lower in SF from children than in adults, while IL-1β, TNFα, adiponectin and OC were higher, but not statistically significantly so. Concentrations of IL-6 in adults (n=4 of 19 samples) and insulin in both children (n=6 of 35) and adults (n=4 of 19 samples) above detection levels occurred in only a few SF samples. The SF levels of ACTH, PTH and leptin in adults with JIA were higher than in adults with RA, although not significantly so.

Correlation between protein content in SF and patient characteristics

Total protein concentrations in SF correlated negatively with age, duration of TMJ symptoms, visual analogue scale (VAS) score on palpation of TMJ and VAS score of pain and function at first and second follow-up, whereas a positive correlation was found between total protein concentration and cytokine content, except for IL-1β, OPN, PTH and insulin (Table 3).

The only factors significantly correlated with a JIA diagnosis (children and adults) were the TMJ SF concentrations of ACTH and insulin; both were positively correlated. Age and duration of the rheumatic disease were positively correlated with leptin, whereas age was also correlated with the ACTH level.

Duration of TMJ problems was negatively correlated with TNFα, and pain at baseline (VAS) was negatively correlated with TNFα and OPG. No significant correlation was found in this study, between problems with TMJ function (mouth opening, movement of the jaw, yawning and chewing) at baseline and SF concentrations of the proteins studied. Pain on palpation of the TMJ was negatively correlated with total protein concentration, TNFα and ACTH.

ACTH, the one factor significantly different between groups of children and adults, was positively correlated with concentrations of adiponectin and insulin, and negatively correlated with age and pain in the TMJ, and both pain and function at first and second follow-up. There was a statistically significant positive correlation between ACTH and improvement of function (VAS) from baseline to first follow-up (p=0.02), but this was not significant with regard to pain (VAS).

	Tot prot	IL-1b	IL-6	TNF	OPG	OC	Leptin	OPN	PTH	ACTH	Insulin
IL-6	0.487*										
TNF	0.351*	0.337**	0.461*								
OPG	0.542***	0.388**	0.590**	0.461***							
OC	0.531***	0.530***			0.516***						
Leptin	0.458***	0.386**			0.676***	0.422**					
OPN				0.521***	0.801***	0.424**	0.576***				
PTH		0.574***		0.272*	0.418**		0.455***				
ACTH	0.293*	0.775***		0.333*	0.496***	0.438**	0.463***		0.669***		
Adiponectin	0.798***	0.304*	0.304*	0.462***	0.716***		0.618***	0.462**		0.367**	
Insulin		0.640*								0.778**	
JIA+										0.269*	0.696*
Age	-0.258*						0.327*			-0.280*	
Disease duration							0.332*				
TMJ duration	-0.298*			-0.291*							
Pain				-0.301*	-0.269*						
Pain 1st follow-up	-0.372**	-0.314*								-0.426**	
Pain 2nd follow-up	-0.285*			-0.300*						-0.366**	
Function 1st follow-up	-0.290*									-0.270*	
Function 2nd follow-up	-0.313*									-0.302*	
TMJ pain palpation	-0.302*			-0.274*						-0.308*	

*p=0.01-0.05, **p<0.01, ***p<0.001 (Spearman rank order sum). Negative numbers indicate negative correlation.

Table 3: Spearman rank sum correlation coefficient between proteins in SF and clinical characteristics.

	Tot prot	IL-1b	IL-6	TNF	OPG	OC	Leptin	OPN	PTH	ACTH	Insulin
IL-6	0.495*			0.493*	0.503*						
TNF	0.525**		0.493*								
OPG	0.607***		0.503*	0.527***							
OC	0.519***	0.521**		0.366*	0.516***						
Leptin	0.526***				0.599***						
OPN	0.339*			0.610***	0.806***		0.430**				
PTH		0.518***			0.382*		0.359*				
ACTH	0.357*	0.729***			0.404**	0.472**	0.408**		0.672***		
Adiponectin	0.873***			0.534***	0.676***		0.504***	0.420**			
Insulin									0.786*	0.964***	
JIA+										0.333*	0.696*
Age				-0.321*			0.321*			-0.363*	
Disease duration							0.315*				
TMJ duration	-0.378*										
Pain					-0.316*						
Pain 1st follow-up	-0.551***									-0.511**	
Pain 2nd follow-up	-0.371*									-0.343*	
Function 1st follow-up	-0.336*									-0.270*	
Function 2nd follow-up	-0.342*									-0.302*	
TMJ pain palpation	-0.391*									-0.308*	

*p=0.01-0.05, **p<0.01, ***p<0.001

Table 4: Spearman rank sum correlation coefficient between protein in SF and clinical characteristics in girls.

In data from girls only (Table 4), there was no significant correlation between IL-1b and pain. TNFα was negatively correlated with age, and no negative correlation between TNFα and clinical parameters was detected. Girls had positive correlations between IL-6 and TNFα, and between IL-6 and OPG.

Discussion

To the best of our knowledge, no previous studies have measured both proteins and bone markers of TMJ SF in JIA, but bone markers have been studied in osteoarthritis [10,11] and internal derangement [12] in TMJ SF. With regard to the immunological tests, a specific bone panel has been chosen (Human Bone Panel 1B), containing cytokines and bone parameters, detecting changes in bone and cartilage. This has previously not been reported.

Studies of TMJ SF have mostly focussed on osteoarthritis closed lock and internal derangement and on demonstrating associations with pro-inflammatory cytokines, such as IL-1β, IL-2, IL-6, IL-8, IL-12 and TNFα. The TNFα level in TMJ SF was lower in this study than levels previously reported in serum from patients with JIA [13].

In our study, leptin correlated with age and duration of the general diseases, but not with the duration of TMJ disease. Leptin was higher in RA than in JIA, and even higher in JIA adults. None of the patients had high BMI and no diabetes was diagnosed. The mean level of leptin was not gender specific in this study. In the knee, findings have indicated a positive correlation between levels of leptin and pain [14], as well as negative correlations between leptin and the joint destruction in osteoarthritis [15]. Elevated levels of leptin and adiponectin have been

associated with RA [16], and with erosive, progressive destruction of the joint [17]. Studies with larger groups of adults with prolonged JIA are needed to explore further the role of leptin in the progression of rheumatoid disease.

Little is known about the relationship between bone markers and pro- and anti-inflammatory cytokines in joint arthritis. ACTH levels in this study were lower in children with JIA than in adults with JIA and RA. Both high and low levels of ACTH have previously been reported in JIA [18,19]. ACTH levels correlated with IL-1, and with bone markers OPG, leptin, PTH, insulin and adiponectin, but adiponectin was not significantly correlated with ACTH when boys were excluded from analysis.

In this study, significant negative correlations between pain and TNFα and between pain and OPG were found. This is in contrast with the increasing evidence that TNFα plays a critical role in inflammatory pain, neuropathic pain [20] and cancer pain [21]. It also contrasts with recent studies focusing on OPG as a biomarker in pain syndromes [22]. Further studies are needed to evaluate the possible effect on these findings of the clinical effect of anti-TNF therapy in JIA.

A concern to the push-pull technique [9] is the fact that some of the washing solution persists in the joint and may cause transient symptoms and functional problems. We did not observe any post-treatment complications in our study. In our study we included adults with rheumatoid arthritis as a control for growth factors, however, age and gender matched control group with healthy TMJs might have improved the results of this study. This was not performed due to ethical considerations.

This study has focused on cytokines and bone markers mainly seen in children and adolescents with active TMJ arthritis in JIA. Kristensen et al. [23] have recently published a study with regard to some cytokines in healthy adults, but little, if anything, is known of the cytokines and bone markers in TMJ SF in children and adolescents without disease. Considering both ethics and study design, studies are needed focusing on the cytokine level during normal growth, not influenced by joint disease.

Acknowledgment

This study was supported by the Institute of Clinical Dentistry, Faculty of Dentistry, University of Oslo, Norway. Write2Publish undertook English language correction.

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