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Synovial tissue cytokine profile in disc displacement of the temporomandibular joint

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Abstract

Background: Symptomatic disc displacement (DD) of the temporomandibular joint (TMJ) may cause pain and limited mouth opening. The aetiopathogenesis is obscure and probably complex, which makes the diagnostic classification crude and mainly based on clinical criteria rather than disease mechanisms, and tissue characteristics.

Objectives: The study aim was to characterise and quantify synovial tissue in DD, where specific cytokine patterns might serve as potential biomarkers.

Methods: An observational cohort study was performed harvesting synovial tissue from 63 patients: 44 with DD without reduction (DDwoR) and 19 with DD with reduction (DDwR). DDwoR was subdivided depending on type of onset (sudden, n = 17; delayed, n = 27), and DDwR served as the control group. Proteins were extracted from tissue samples and investigated in a multi-analytic profiling system.

Results: DDwoR patients had significantly higher concentrations in 12 out of 28 analysed cytokines compared to DDwR. In the same statistical model, significantly lower concentrations of interferon gamma-induced protein (IP) 10, osteoprotegerin (OPG) and RANTES were detected in DDwoR patients. Women showed significantly higher concentrations of epidermal growth factor and interleukin (IL) 1ra compared to men. DDwoR with sudden onset had significant higher concentrations of bone morphogenetic protein 4, eotaxin and IL-8 compared to DDwoR with delayed onset.

Conclusions: Characterising the biomarker panel for TMJ conditions may serve as suggestible targets for disease classification and novel treatment options. The significantly lower concentrations of IP-10, OPG and RANTES could be proposed as putative markers for the separation of the studied conditions to other TMJ diseases.

KEYWORDS

temporomandibular joint disorders, chemokines, cytokine(s), growth hormones, joint disease, protein expression

1 | INTRODUCTION

Disc displacement (DD) of the temporomandibular joint (TMJ) is common affecting 15 to 31% of investigated populations.[1-3] Few individuals with DD suffer to such an extent that they need treatment, implying that in most cases, a displaced disc needs a cofactor, catalyst or interactor to give rise to symptomatic arthralgia, arthritis and/or limited mouth opening. However, patients with TMJ complaints often show DD.[2]

Treatment of symptomatic DD is primarily conservative, such as orthotic splint, medication and physiotherapy.[4,5] Surgical intervention might be considered when non-invasive therapeutic modalities fail. DD with reduction (DDwR) describes a displaced disc that reduces to a normal position during mouth opening and displaces again on closing.[6] DD without reduction (DDwoR) refers to a displaced disc that does not reduce during mouth opening.[6] It has been recognised earlier that DDwR has a much lower grade of inflammation and less joint degeneration compared to DDwoR.[7,8] DDwoR may present with two different types of onset, either sudden without previous symptoms from the joint or delayed, preceded by a longer period of clicking and intermittent locking before developing into DDwoR.[7,9] It can be hypothesised that these two onsets may represent different entities with different tissue characteristics.

Biomarkers are used to diagnose diseases but also to screen disease activity and treatment response.[10] TMJ synovial fluid has been investigated in TMJ patient cohorts and healthy controls.[11,12] The focus has mainly been cytokines with pro-inflammatory or anti-inflammatory properties. Synovial fluid may contain both locally and distantly produced cytokines, and thereby indirectly reflect TMJ synovial tissue activity. Although sparsely investigated in the TMJ, synovial tissue might therefore be a valid complement.[10,13] To date, no broad investigations of TMJ synovial tissue cytokine profile have been performed. Immunohistochemical studies exist but do not allow quantification of cytokine concentrations.[14-17]

The aim of the present study was to characterise and quantify synovial tissue cytokines and relate the result to the diagnoses DDwR and DDwoR. A secondary aim was to investigate possible differences between the subgroup divisions of DDwoR, sudden onset (SO) and delayed onset (DO). Characterising a biomarker panel for particular conditions may serve as future putative targets for novel treatment options.

2 | MATERIAL AND METHODS

2.1 | Study design

This prospective cohort observational study was conducted at the Department of Craniofacial Diseases, Karolinska University Hospital, Stockholm, Sweden. The Regional Ethics Review Board authorised the study (registration number: 2014/622-31/1). Eligible patients were those referred because of symptomatic DDwR or DDwoR during the period December 2014 to January 2017. Written informed consent was collected prior to inclusion. Patients with DDwR were used as controls because of anticipated low-grade inflammation, as ethical considerations framed us from including TMJ asymptomatic patients.[7,8] The study was designed and the article written in accordance with the STROBE statement.

2.2 | Study population

A power calculation, based on earlier findings of differences in synovial fluid IL-6 concentration between patients with DDwR or DDwoR, was made.[8] A power of 80% and $P = .05$ was reached with 23 patients in the DDwR group and 46 in the DDwoR group. A calculated dropout rate of 10% gave 25 and 51 patients, respectively.

Diagnoses were set according to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) (Schiffman et al. 2014). Criteria for surgery were as follows: DDwR or DDwoR, non-invasive therapy tried for at least 3-6 months, visual analogue scale (VAS) value of ≥ 4 for TMJ functional pain or TMJ disability, and DDwoR patients had to have a maximum interincisal opening (MIO) of ≤ 35 mm. A subdivision of the diagnosis DDwoR was made. Patients with no TMJ symptoms before initiation of DDwoR were categorised as having a sudden onset (DDwoR-SO). A history of long-term clicking and/or intermittent locking before developing DDwoR were considered as having delayed onset (DDwoR-DO). Exclusion criteria were prior open joint surgery, patient unable to give informed consent and age under 18 years.

2.3 | Clinical examination

A standardised case record form was used for collecting patient-specific data. Surgeons at the department (M.U, A.N-A, C.K-W, B.L), calibrated in patient classification and clinical examination, performed patient inclusion and data gathering. Patients were asked for present illnesses, medication, ongoing tinnitus/ear fullness affected side and duration of present TMJ symptoms. Prior jaw trauma (yes/no) was registered if the trauma occurred before developing TMJ symptoms. MIO, lateral excursion, protrusion, palpation tenderness of the masticatory muscles and the TMJ were recorded according to DC/TMD.[6] TMJ symptoms of pain, disability and psychosocial impact were graded on a 0-10 graded VAS by the patients.[18] Wilkes classification was preceded by an initial calibration exercise thereafter performed individually by two of the researchers (M.U and B.L).[7] In case of divergent values, consensus was achieved after discussion.

2.4 | Surgical procedure and collection of tissue samples

Surgical interventions were performed under general anaesthesia, and the national guidelines for TMJ surgery were followed.[19] Patients with DDwoR had arthroscopic lysis and lavage, and discectomy was

undertaken on patients with DDwR. No prophylactic antibiotic was given. Two synovial tissue biopsies were harvested from the superior part of the posterior bilaminar zone in close proximity to the disc attachment. During arthroscopy, synovial biopsies were taken under direct visualisation using the triangulation technique in order to verify the localisation of the sample.[20] Biopsy forceps (Karl Storz SE & Co) were used resulting in tissue samples of approximately 4 mm^2. The samples for protein extraction were immediately placed in RNAlater (Thermo Fisher Scientific) and refrigerated for 24 hours, prior to its removal and sample storage at −80°C. The second biopsy was fixed in 4% paraformaldehyde and paraffin-embedded, prior to sectioning and staining with haematoxylin and eosin (H&E, Histolab Products AB, Gothenburg, Sweden) for routine histology examination.

2.5 | Analysis of synovial tissue

Synovial tissue was weighed and washed twice in ice-cold phosphate-buffered saline (PBS) without calcium (Thermo Fisher Scientific) prior to grinding in liquid nitrogen to disrupt the tissue piece. Proteins were extracted in ice-cold cell lysis buffer with 1mM NP-40 (Thermo Fisher Scientific), containing phenylmethanesulphonyl fluoride (PMSF) (Sigma-Aldrich) prepared in 0.3 mol/L dimethylsulphoxide (DMSO) and protease inhibitor cocktail (Sigma-Aldrich).[21] 50 μL cell lysis buffer per 10 mg of tissue was used and the tissues ground on ice until only white fibrous connective tissue remained. The mixtures were centrifuged at 20 000 *g* at 4°C for 10 minutes, and the supernatant stored at −80°C until analysis.

The total protein concentration was determined with the Qubit Fluorometer (Thermo Fisher Scientific) using the Qubit Protein Assay Kit (Thermo Fisher Scientific). Specific proteins were detected with multi-analytic profiling using a Luminex 200 system (Luminex) with xMAP technology. Resulting data were analysed by the xPONENT 3.1 software (Luminex). Two immunoassays, Human Cytokine/Chemokine Magnetic Bead Panel (HCYTOMAG-60K [Merck Millipore]) and Human Magnetic Luminex Assay 20-plex (LXSAHM-20 [R&D Systems]), were used to determine protein levels of bone morphogenetic protein (BMP) 2, BMP 4, BMP 9, epidermal growth factor (EGF), eotaxin, fibroblast growth factor (FGF) 2, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage (GM) CSF, interleukin (IL) 1β, IL-1 receptor antagonist (IL-1ra), IL-6, IL-7, IL-8, IL-10, interferon gamma-induced protein (IP) 10, IL-17, monocyte chemoattractant protein (MCP) 1, macrophage inflammatory protein (MIP) 1α, MIP-1β, osteoprotegerin (OPG), platelet-derived growth factor (PDGF) AA, PDGF-AB/BB, RANTES, transforming growth factor (TGF) α, tumour necrosis factor (TNF) α, TNF-β, triggering receptor expressed on myeloid cells (TREM) 1 and vascular endothelial growth factor (VEGF). The investigated cytokines were chosen based on earlier studies on TMJ synovial fluid and tissue as well as studies on other joints.[8,10-12]

2.6 | Statistical analyses

Data were analysed with Stata version 15 (StataCorp) and IBM SPSS version 25.0 (IBM Corp). Specific protein concentrations were normalised to the total protein concentration, that is normalised ratio, and used in the statistical analysis, as well as the specified protein concentrations separately. Descriptive statistics were calculated as mean ± SD for all continuous data and as number, and percentage for bivariate data. Data on patient characteristics were analysed with Student's *t* test for continuous data and Fisher's exact test for categorical data. Protein concentrations were not normally distributed why median, using quantile regression, was analysed. The model to predict the median protein concentration included the diagnoses (DDwoR/DDwR or DDwoR-DO/DDwoR-SO) in the unadjusted analyses. The adjusted analyses also included sex and previous trauma as dichotomous variables, and age in years and duration of symptoms in months as continuous variables. In the adjusted model, missing data regarding TMJ trauma (n = 1) and duration (n = 3) were replaced by the most common value for trauma and the median value for duration. The *P*-values were based on 20 bootstrap samples, and a *P*-value of ≤ .05 was regarded as significant.

3 | RESULTS

3.1 | Patient demographics

Patients eligible for inclusion in the study and reasons for not participating or being excluded are displayed in Figure 1. In all but four patients, biopsies from the synovial tissue were possible to harvest. When the study closed, 63 patients had completed the protocol. Patient cohort characteristics are shown in Table 1. Symptom duration was significantly longer in patients with DDwR compared to DDwoR ($P = .008$), and as expected, DDwoR had significantly smaller MIO ($P = .000$). DDwoR had significantly higher subjective TMJ functional pain score ($P = .019$) and higher Wilkes criteria ($P = .000$), the latter reflected in the operative features (Figure 2A,B). Subgroup analyses performed on DDwoR-DO and DDwoR-SO, revealed that pre-operative trauma affected significantly more patients in the SO group ($P = .024$). None of the other patient variables were significantly different.

3.2 | Synovial tissue analysis

Histologically, DDwoR tissues presented with mild inflammation, whereas little or no inflammation was observed with DDwR tissues (Figure 2C,D). Nevertheless, patients with DDwR reported on TMJ pain, which might be explained by more pronounced inflammation in the joint capsule, masticatory muscles or similar, when no or small signs of inflammation in the synovium were present. Furthermore, in similarity to previous studies degenerative tissue changes were apparent in DDwoR tissues.[18]

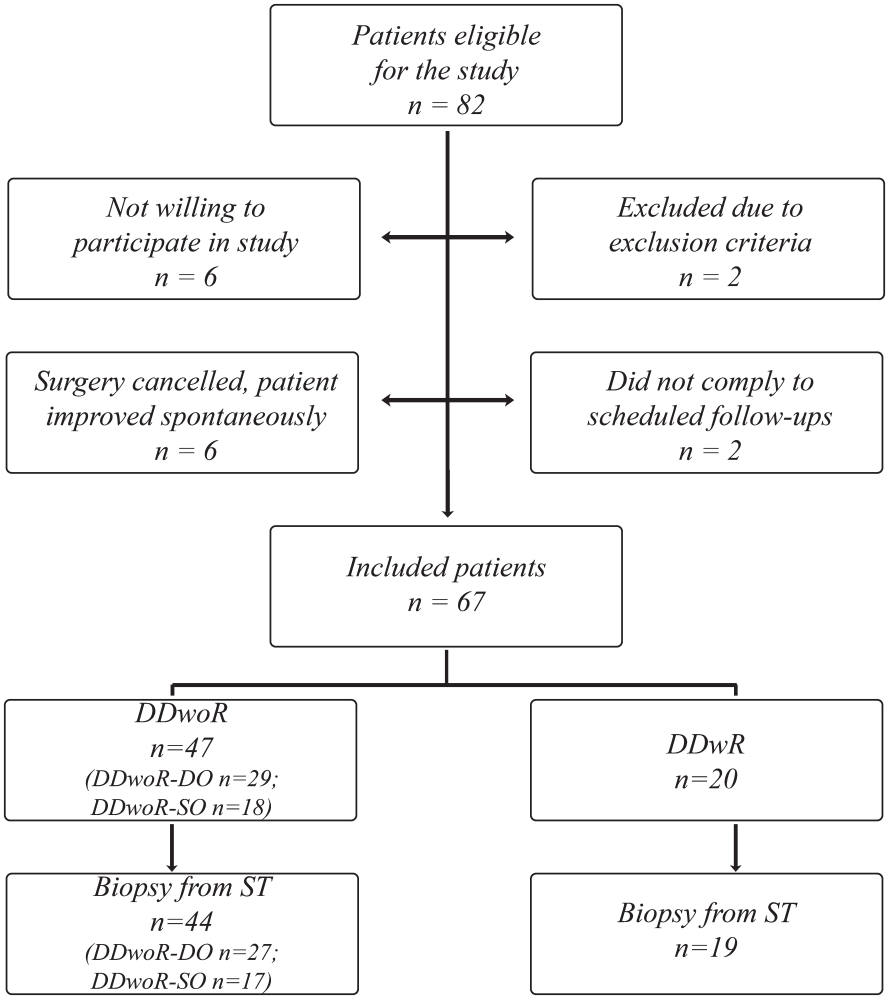

FIGURE 1 Flow chart illustrating patients eligible for inclusion and reasons for not participating. DDwoR, disc displacement without reduction; DDwR, disc displacement with reduction; DO, delayed onset; n, number; SO, sudden onset; ST, synovial tissue

In the multi-plex protein analysis of the tissue preparations, some cytokine concentrations were identified to be below the lowest standard (<3.2 pg/mL). These were set at 3.2 pg/mL, whereas values above the highest standard (10 000 pg/mL) were set at 10 000 pg/mL. Samples with cytokine measurements outside of the precision and recovery of the assays had no values indicated and were consequently regarded as missing values. The number of tissue samples with detectable levels of the specific protein concentration in each group is indicated in Table 2. The statistical outcome was comparable when separately using normalised ratio or specified protein concentration.

In patients with DDwR, the median total protein concentration was 11.80 mg/mL (min 8.19, max 19.60, SD 2.43) and in DDwoR 15.65 mg/mL (min 3.47, max 44.86, SD 9.49). The difference in total protein concentration between the two diagnoses was not significant in a multivariate quantile regression analysis, but the variable trauma was correlated with a higher total protein concentration (coef. 2.73; $P = .021$; 95% CI 0.44, 5.03). Sex, age and duration of TMJ symptoms were included in the analysis but showed no significance.

All analysed cytokines and comparison of measured concentrations between DDwoR and DDwR are shown in Table 2. All detected proteins were then analysed in a multivariate regression model (Table 3), where possible confounders of sex, age, duration of TMJ symptoms and previous TMJ trauma were included. In the adjusted model, DDwoR showed significantly higher concentrations of BMP-2, BMP-4, EGF, eotaxin, G-CSF, IL-1β, IL-7, IL-8, IL-10, MIP-1β, TNF-α and TNF-β, and significantly lower concentrations of IP-10, OPG and RANTES. The variables age and TMJ trauma had no significant correlation with any of the cytokines.

An unadjusted subgroup analysis of DDwoR-DO and DDwoR-SO was made and DO was set as the reference. A significant concentration difference was found regarding some protein concentrations: BMP-2 (coef. 253.9; $P = .016$; 95% CI 49.7, 458.1), BMP-4 (coef. 342.2; $P = .026$; 95% CI 43.4, 641.0), TNF-α (coef. 4.6; $P = .000$; 95% CI 2.2, 7.0) and TNF-β (coef. 2.7; $P = .047$; 95% CI 0.0, 5.4). When performing an adjusted analysis with the variables sex, age, duration of TMJ symptoms and previous trauma, DDwoR-SO had significantly higher concentrations of BMP-4 (coef. 401.2; $P = .004$; 95% CI 133.5, 668.8), eotaxin (coef. 137.2; $P = .032$; 95% CI 12.7, 261.7) and IL-8 (coef. 14.4; $P = .014$; 95% CI 3.1, 25.7) compared to DDwoR-DO. Trauma was found to have significant relationship with lower concentration of PDGF-AA (coef. −131.1; $P = .015$; 95% CI −235.6, −26.5). No significant differences were shown for the variables sex, age, or TMJ symptom duration.

TABLE 1 Pre-operative registration of demographic data, anamnestic information, objective and subjective measurements of included patients

Classification	DDwoR total	DDwoR-SO	DDwoR-DO	DDwR	Total
Demographic data					
Number of patients	44	17	27	19	63
Sex, W/M	37/7	13/4	24/3	14/5	51/12
Age (y), mean (SD)	43.0 (16.0)	46.1 (13.0)	41.0 (17.6)	37.3 (12.0)	41.3 (15.1)
Patient history					
Duration[a] (mo), mean (SD)	21.1 (24.8)	15.9 (8.3)	24.3 (30.4)	44.7 (41.2)	27.8 (31.8)
Tinnitus/ear fullness, n (%)	12 (27)	4 (24)	8 (30)	5 (26)	17 (27)
TMJ trauma, n (%)	10 (23)	7 (41)	3 (11)	7 (37)	17 (27)
Medical history, n (%)					
Healthy	18 (41)	8 (47)	10 (37)	10 (53)	28 (44)
Psychiatric disorder	14 (32)	3 (18)	11 (41)	3 (16)	17 (27)
Neuropsychiatric disorder	1 (2)	0 (0)	1 (4)	1 (5)	2 (3)
Autoimmune disease	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Metabolic disease	6 (14)	3 (18)	3 (11)	2 (11)	8 (13)
Other disease	19 (43)	7 (41)	12 (44)	6 (32)	25 (40)
Objective measures					
GJH, n (%)	9 (21)	3 (18)	6 (22)	6 (32)	15 (24)
MIO, mm (SD)	29.2 (4.8)	28.6 (5.6)	29.6 (4.2)	43.6 (9.9)	33.6 (9.4)
Wilkes (1-5), mean (SD)	3.9 (0.6)	4.0 (0.7)	3.9 (0.5)	2.5 (0.9)	3.5 (0.9)
Subjective measures mean VAS 0-10 (SD)					
TMJ pain	5.7 (2.4)	6.0 (2.8)	5.5 (2.1)	4.1 (2.4)	5.2 (2.5)
TMJ disability	6.3 (1.7)	6.7 (1.8)	6.0 (1.6)	6.2 (2.0)	6.2 (1.8)
TMJ psychosocial[b]	3.6 (2.7)	4.4 (2.6)	3.2 (2.7)	5.0 (3.3)	4.0 (2.9)
Global pain	2.7 (2.8)	3.4 (3.4)	2.3 (2.4)	3.0 (3.2)	2.8 (2.9)

Abbreviations: DDwoR, disc displacement without reduction; DDwR, disc displacement with reduction; DO, delayed onset; GJH, general joint hypermobility; M, men; MIO, maximum interincisal opening; mo, months; SD, standard deviation; SO, sudden onset; TMJ, temporomandibular joint; VAS, visual analogue scale; W, women.

[a]Duration refers to duration of TMJ symptoms.

[b]TMJ psychosocial refers to the psychosocial influence of TMJ problems.

4 | DISCUSSION

A cohort of patients diagnosed with DDwoR or DDwR has been compared to evaluate differences in synovial tissue concentration for a number of cytokines, in order to describe potential discrepancies between the two diagnoses. TMJ synovial tissue has not been investigated in this fashion earlier, and the broad panel of cytokines examined also distinguishes this study from earlier synovial fluid and tissue analyses, and potentially highlights putative biomarkers.

Patient characteristics revealed that the diagnosis groups were homogenous across most variables albeit with some exceptions. Patients with DDwoR had significantly higher TMJ pain score and Wilkes classification but shorter duration of TMJ symptoms. The latter might be explained in that more intense pain potentially induces patients to seek health care earlier. Overall, total protein content was significantly higher amongst DDwoR, which was not unexpected considering this group exhibited more pronounced TMJ pathology, both clinically and radiographically, and it was reflected in Wilkes classification.[7]

Concentrations of 15 out of 28 cytokines were significantly different comparing DDwoR to DDwR patient groups. BMP-2, BMP-4, EGF, eotaxin, G-CSF, IL-1β, IL-7, IL-8, IL-10, MIP-1β, TNF-α and TNF-β were all significantly elevated in DDwoR patients compared to DDwR. These multi-functional cytokines may stimulate bone and cartilage formation as a result of osteoblast activation, whilst others promote osteoclast activity, block chondrocyte function and contribute to tissue damage.[22-24] Thus, the observed cytokine variations correspond to the tissue degeneration and/or remodelling discerned between these patient groups. The majority of elevated cytokines were pro-inflammatory, with chemotactic activity recruiting neutrophils, granulocytes and lymphoid cells to sites of inflammation.[22-28] In particular, IL-7, EGF and G-CSF initiate tissue responses, to recruit and regulate hematopoietic stem cells from bone marrow to differentiate into inflammatory cells.[29-31] Furthermore, these cytokines

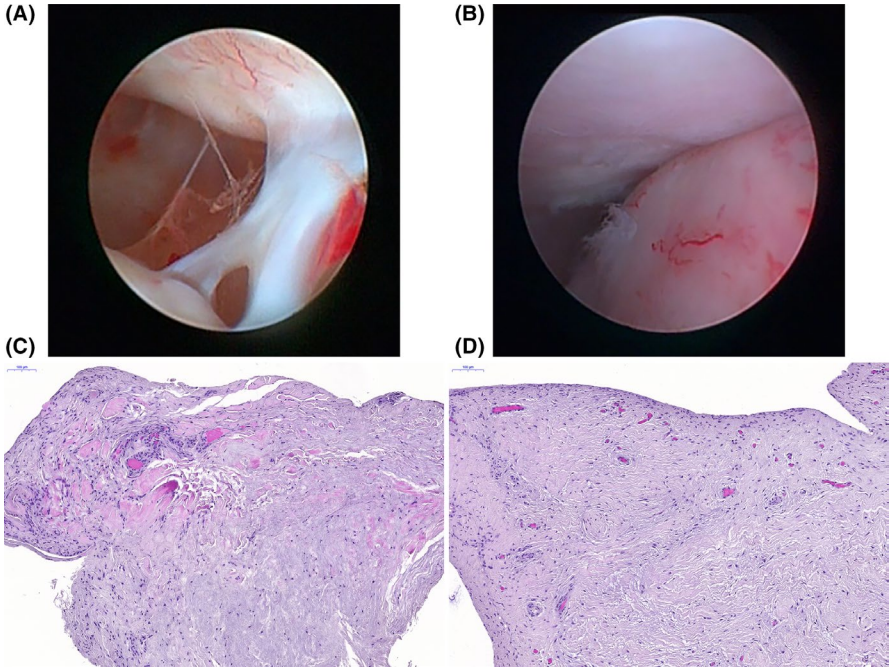

FIGURE 2 Histologically stained synovial tissues (scale bar 100 μm) and intra-articular photographs from the superior joint compartment. A, Patient with DDwoR showing a fibrotic adhesion between the articular tubercle and the disc. Synovial creeping covering part of the articular tubercle illustrating inflammation. B, DDwR with mild capillary hyperaemia affecting the posterior bilaminar zone and a minor area with signs of cartilage breakdown. C, Representative H&E-stained image of DDwoR tissue. D, H&E-stained representative DDwR image

may increase endothelial permeability, promoting chemotaxis of inflammatory cells and stimulation of angiogenesis.[22,23,26,27,32] Interestingly, IL-1β, IL-8, IL-10 and TNF-α are suggested to be triggered by lipopolysaccharides and infections,[22,28,32,33] and it has been suggested that an infectious genesis could apply to TMJ disease,[34,35] whilst others have not been able to confirm this.[36,37] DDwoR patients had significantly higher subjective pain scores, which corresponded to the increased occurrence of pro-inflammatory cytokines, including IL-1β associated with hyperalgesia, compared to DDwR patients.[22]

An interesting finding was the significantly lower concentrations of IP-10 and RANTES observed amongst DDwoR patients compared to DDwR. These two cytokines have been found in higher concentrations in chronic inflammatory diseases, like rheumatoid arthritis, correlated to enhanced bone and chondral resorption, leucocyte recruitment, angiogenesis and fibrosis.[10,22,27] These earlier findings indicate that IP-10 and RANTES should have had higher concentrations in DDwoR compared to DDwR, which obviously is not the case in this investigation. The concentration of OPG, known as a bone-protecting protein, was also significantly lower in DDwoR, which might indicate an interaction between OPG and IP-10 and RANTES in DDwoR bone turnover homoeostasis.[38] The finding of lower concentrations of IP-10, OPG and RANTES in DDwoR might perhaps suggest a different disease activity compared to both DDwR and to chronic inflammatory arthritis (CIA), as higher levels could have been anticipated.[10,27,38] Bilateral affection of TMJ is common in CIA, which is rarely the case in DDwoR. In the current study, only two cases were bilaterally affected why meaningful statistical evaluation was not possible. However, a daring suggestion might be that bilateral cases could advocate the clinician to further investigate different aetiologies, like CIA. OPG, IP-10 and RANTES might eventually serve as biomarkers to aid diagnosis and guide to alternative treatment options before scheduling surgery. All cytokines shown to have significantly divergent concentrations might be useful biomarkers in the complex cytokine inflammatory response to TMJ disorder and warrants further confirmatory investigations.

Both EGF and IL-1ra presented with significantly higher concentrations amongst women. Previously, it has been recognised that EGF has a sexual dimorphism, with higher levels in women's urine and kidneys.[30] Considering the strong female predominance amongst DD patients, the significance of these findings should be addressed in future studies. The presence of OPG also strongly correlated with an extended duration of symptoms, indicating that osteoclast inhibition may increase with time.[38]

DDwoR-SO was shown to have significantly higher concentrations of BMP-4, eotaxin and IL-8 compared to DDwoR-DO. BMP-4 promotes bone and chondral repair whilst eotaxin is upregulated during bone inflammation increasing osteoclast activity.[23,24] IL-8, known as a potent chemokine, stimulates phagocytosis and potentially may be the regulator of inflammatory-driven bone turnover, which requires increased numbers of scavenger cells.[28] Furthermore, healing may be impaired in DDwoR patients since PDGF-AA concentration showed negative correlation with previous trauma.[39] However, the limited cohort of only 44 patients must moderate these bold suggestions of cytokine context in DDwoR.

Synovial fluid analyses have suggested differences in cytokine concentrations in healthy controls compared to patients with DD.[12] Harvesting of intra-articular tissue is considered an invasive procedure and ethical considerations refrained us from including healthy controls. Patients with DDwR were used as substitutes to healthy controls because of earlier reports on low-grade inflammation.[7,8] From this perspective, there are limitations in interpretation of the cytokines' concentration. The current selection of analysed cytokines was based on previous investigations, but

TABLE 2 Concentration of cytokines in DDwoR and DDwR and an unadjusted quantile regression analysis comparing median cytokine concentration related to diagnosis

Protein	DDwoR/DDwR[a]	DDwoR/DDwR (pg/mL)		Quantile regression[b]			
		Min-max	Median (SD)	Coef.	*P*	95% CI	
BMP 2	44	67.1-1659.4	399.9 (349.2)	−331.9	.000	−487.5	−176.3
	19	66.4-169.2	70.8 (31.5)				
BMP 4	44	70.8-2550.2	540.8 (531.5)	−439.8	.000	−610.1	−269.5
	19	101.3-243.1	105.0 (31.7)				
BMP 9	5	0.6-312.0	120.6 (154.0)	−117.4	.510	−492.1	257.3
	10	0.3-568.4	2.8 (178.8)				
EGF	44	9.5-160.0	56.8 (36.8)	−30.4	.014	−54.3	−6.4
	19	7.1-137.4	26.8 (36.6)				
Eotaxin	44	14.0-586.0	113.5 (142.9)	−86.5	.000	−130.7	−42.3
	19	4.6-76.8	30.2 (19.3)				
FGF2	44	1157.6-37281.5	10616.3 (7292.3)	1430.9	.441	−2257.0	5118.9
	19	5666.4-22374.1	12296.9 (3615.5)				
G-CSF	44	5.6-1675.5	273.7 (396.9)	−229.8	.001	−361.6	−98.0
	19	1.0-2011.7	43.9 (452.4)				
IL-1β	40	0.1-20.4	1.7 (4.3)	−1.3	.004	−2.2	−0.4
	18	0.1-3.1	0.5 (0.7)				
IL-1ra	44	6.9-208.7	51.9 (49.9)	−16.7	.456	−61.3	27.9
	19	1.4-614.0	36.4 (138.5)				
IL-6	11	1.0-83.9	10.3 (24.2)	−5.8	.541	−25.4	13.8
	7	1.4-7.8	4.5 (2.7)				
IL-7	44	1.8-283.0	44.6 (68.0)	−31.0	.000	−43.8	−18.2
	19	4.2-134.4	15.3 (28.1)				
IL-8	44	0.9-85.9	26.2 (20.5)	−20.6	.000	−30.0	−11.1
	19	2.8-35.6	5.9 (7.0)				
IL-10	43	0.2-41.3	8.1 (9.1)	−4.7	.000	−7.2	−2.2
	19	0.2-10.8	2.5 (2.5)				
IP-10	44	9.6-5806.2	91.6 (877.7)	172.9	.000	88.6	257.3
	19	62.6-1338.7	268.9 (335.0)				
MCP-1	44	10.5-1608.6	331.4 (380.9)	−105.0	.472	−395.2	185.2
	19	127.2-4979.9	243.7 (1289.1)				
MIP-1α	16	2.5-104.9	13.4 (31.5)	−10.4	.212	−27.2	6.4
	10	0.8-14.9	3.1 (5.2)				
MIP-1β	17	3.2-127.0	20.8 (32.3)	−11.8	.159	−28.5	4.9
	14	1.8-40.3	8.4 (9.6)				
OPG	44	150.9-61073.2	2997.6 (10370.6)	2551.1	0.661	−9035.8	14137.9
	19	1278.0-202633.2	5900.0 (46094.3)				
PDGF-AA	44	23.9-2429.6	138.4 (416.2)	170.1	.021	26.0	314.2
	19	108.2-822.0	313.3 (221.6)				
PDGF-AB/BB	44	20.6-20159.9	663.4 (3129.1)	1566.0	.190	−795.3	3927.3
	19	482.1-21388.6	2264.3 (5070.6)				
RANTES	44	372.3-34905.2	3683.4 (8172.4)	6677.7	.021	1036.7	12318.6
	19	4312.9-30000.0	10627.5 (6029.7)				
TNF-α	44	0.8-21.4	4.5 (4.9)	−2.5	.000	−3.5	−1.4
	19	1.2-7.0	2.1 (1.4)				

(Continues)

TABLE 2 (Continued)

Protein	DDwoR/DDwR[a]	DDwoR/DDwR (pg/mL)		Quantile regression[b]			
		Min-max	Median (SD)	Coef.	*P*	95% CI	
TNF-β	34	0.5-22.0	2.7 (4.1)	−2.0	.018	−3.7	−0.4
	18	0.3-5.6	0.7 (1.4)				
TREM-1	35	13.6-5264.0	360.6 (981.6)	−193.0	.247	−523.4	137.4
	19	36.3-3299.9	167.6 (727.9)				
VEGF	44	9.6-983.8	60.0 (192.2)	−6.3	.858	−77.0	64.3
	19	9.6-1398.4	53.7 (382.6)				
GM-CSF[c]	7	0.8-8.1	1.4 (2.7)				
	6	0.5-5.1	1.9 (1.6)				
IL-17[c]	4	3.7-7.7	5.3 (1.8)				
	0	-	-				
TGF-α[c]	3	0.2-4.2	0.4 (2.3)				
	4	1.2-72.2	2.3 (35.1)				

Abbreviations: CI, confidence interval; DDwoR, disc displacement without reduction; DDwR, disc displacement with reduction; mL, millilitre; pg, picogram; SD, standard deviation.

[a]Number of synovial tissue samples with detected levels of the specified protein concentration.

[b]The quantile regression was modelled from DDwoR, and the coefficient thereby shows if DDwR has a lower (negative coef.) or higher (positive coef.) median concentration.

[c]Quantile regression was not possible because there were too few samples with detectable protein concentrations.

TABLE 3 Quantile regression of cytokine concentrations in DDwoR and DDwR, adjusted for the variables sex, age, duration of TMJ symptoms and previous trauma

Variables	DDwoR vs DDwR[a]			Sex[b]			Duration		
Protein	Coef.	95% CI		Coef.	95% CI		Coef.	95% CI	
BMP2	−326.0**	−470.8	−181.3	−8.3	−114.5	97.8	−0.0	−1.2	1.2
BMP4	−439.9*	−780.2	−99.5	−0.5	−235.3	234.3	0.0	−4.9	4.9
EGF	−20.9*	−40.1	−1.7	23.2*	2.1	44.3	0.1	−0.3	0.4
Eotaxin	−83.8**	−120.0	−47.6	−14.7	−73.1	43.8	−0.0	−0.7	0.6
G-CSF	−226.5**	−331.7	−121.2	−26.5	−208.6	155.6	0.2	−5.2	5.6
IL-1β	−1.3**	−2.0	−0.7	0.2	−0.5	0.9	0.0	−0.0	0.0
IL-1ra	−13.6	−38.1	10.9	30.8*	3.4	58.3	−0.0	−0.3	0.2
IL-7	−30.8**	−50.4	−11.1	8.5	−27.9	45.0	−0.0	−0.2	0.1
IL-8	−19.1**	−27.9	−10.4	4.6	−3.2	12.5	−0.0	−0.1	0.0
IL-10	−4.7*	−7.8	−1.6	0.0	−4.5	4.5	−0.0	−0.1	0.1
IP-10	178.9**	24.7	333.1	4.9	−96.2	106.1	−0.1	−2.4	2.2
MIP-1β	−14.1*	−24.1	−4.1	11.8	−2.4	26.0	−0.0	−0.2	0.1
OPG	2230.1*	38.3	4421.9	119.6	−2931.4	3170.6	139.0*	29.1	248.9
RANTES	6548.3**	2876.0	10620.6	2190.2	−1948.2	6328.5	−38.1	−126.7	50.4
TNF-α	−2.1**	−3.4	−0.7	0.8	−1.2	2.8	−0.0	−0.0	0.0
TNF-β	−1.9*	−3.5	−0.2	−1.3	−4.5	1.9	−0.0	−0.0	0.0

Note: Only cytokines and variables with significant results are included in the table.

Abbreviations: CI, confidence interval; DDwoR, disc displacement without reduction; DDwR, disc displacement with reduction.

[a]The quantile regression was modelled from DDwoR, and the coefficient thereby shows if DDwR has a lower (negative coef.) or higher (positive coef.) median concentration.

[b]Male gender was modelled as the reference.

*$P < .05$.

**$P < .005$.

several cytokines, such as IFN-γ and TGF-β, were not included, further limiting the study. Additional studies with analysis of a broader panel of cytokines, together with *in vitro* studies on cytokine interaction, are required to elucidate the specific mechanisms of inflammation in DD and to deepen the understanding of the pathogenesis.

The generalisability of the study cohort results is considered fairly good considering the well-defined inclusion criteria and patient characteristics in combination with power-based study-population size. However, one cannot neglect that possible geographical or cultural differences may influence the data. In addition, less pronounced individual differences may remain undetected in this limited cohort.

5 | CONCLUSION

Significant differences in concentrations of synovial tissue extracted cytokines were found when comparing patients with DDwoR to patients with DDwR. IP-10, OPG and RANTES showed significantly higher concentrations in DDwR patients, and BMP-2, BMP-4, EGF, eotaxin, G-CSF, IL-1β, IL-7, IL-8, IL-10, MIP-1β, TNF-α and TNF-β had significantly higher concentrations in DDwoR patients. EGF and IL-1ra concentrations were significantly higher in women compared to men. DDwoR-SO patients had concentrations of BMP-4, eotaxin and IL-8 that were significantly higher than in DDwoR-DO. The current findings may lead to further insights in TMJ disease and form basis for research on biomarkers and novel medical treatments.

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CONFLICT OF INTEREST

No conflict of interest is declared.

AUTHOR CONTRIBUTION

M. Ulmner contributed to conception, design, data acquisition, analysis and interpretation; drafted; and critically revised the manuscript. R. Sugars contributed to conception, design, data acquisition, analysis and interpretation; drafted; and critically revised the manuscript. A. Naimi-Akbar contributed to data analysis and interpretation, and critically revised the manuscript. S. Suslu contributed to data acquisition and critically revised the manuscript. J.E. Reseland contributed to data acquisition and analysis, and critically revised the manuscript; C. Kruger-Weiner contributed to conception and design, and critically revised the manuscript; B. Lund contributed to conception, design, data analysis and interpretation; drafted; and critically revised the manuscript. All authors gave their final approval and agree to be accountable for all aspects of the work.

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