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RESEARCH

Skin inflammatory cytokine gene expression in chronic whiplash-associated disorder: a cross-sectional study

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Abstract

Purpose Neurological findings are present in some patients with whiplash-associated disorder (WAD) Grade II, including reduced intra-epidermal nerve fibre density (IENFD). Reasons for reduced IENFD in WAD are unclear. This exploratory study assessed (i) skin inflammatory cytokine expression in people with chronic WAD II, and (ii) associations between skin inflammatory cytokine gene expression, IENFD, and clinical factors.

Methods Skin mRNA expression of genes encoding inflammatory cytokines at the finger and ankle in chronic WAD II (N = 21) was compared with age- and sex-matched controls (N = 21). Realtime quantitative polymerase chain reaction analysis (RT-qPCR) was performed blinded to study group. Genes examined encoded tumour necrosis factor (TNF), interleukin (IL)-6, IL-8, IL-1β and IL-10, expressed relative to a house-keeping control gene (*HPRT1*). Clinical outcomes were pain, disability, and psychological measures. Gene expression was compared between groups using Wilcoxon rank-sum tests and associations between gene expression, IENFD and clinical outcomes evaluated using Spearman correlations. Benjamini–Hochberg correction was performed (5% FDR).

Results At the finger, *TNF* mRNA expression was ~ twofold higher in chronic WAD than controls (median [IQR] *TNF*/*HPRT1*: 3.47 [4.16] vs 1.80 [2.33], P = 0.006). There were no group differences in expression of genes encoding other cytokines, nor group differences at the ankle. No significant correlations were found between gene expression and IENFD, pain, disability and psychological measures at the finger or ankle.

Conclusions These novel findings suggest an association between local skin inflammatory processes and chronic WAD at the finger, however specific links between skin cytokine expression, reduced IENFD or clinical outcomes remain unclear.

Keywords Whiplash injuries · Chronic pain · Neck pain · Pain · Cytokines · Polymerase chain reaction

Introduction

Mechanisms underpinning ongoing pain in chronic whiplash-associated disorder (WAD) are not well understood [1]. We recently observed small nerve fibre pathology in the skin (reduced intra-epidermal nerve fibre density [IENFD] [2]) in people with chronic WAD Grade II (i.e., no fracture/dislocation or frank neurological deficit). The reason for small fibre pathology in this population is unclear. Although WAD II does not meet current diagnostic criteria for neuropathic pain (i.e., pain in neuroanatomically plausible distribution [possible], associated sensory dysfunction in that distribution [probable], corresponding diagnosis of lesion/pathology affecting sensorimotor nervous system [definite] [3]), there is evidence of functional and structural neurological involvement in some patients [4].

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Inflammatory processes could be linked to reduced IENFD. In people with diagnosed small fibre neuropathy, increased expression of genes encoding interleukin (IL)−6, IL-8, IL-1β and tumour necrosis factor (TNF) was found in affected skin, with mRNA levels of genes encoding IL-2, IL-10 and transforming growth factor-β1 being increased in venous whole blood [5]. Increased expression of genes encoding IL-6 and IL-10 in whole skin [6], and IL-6 and IL-8 in cultured dermal fibroblasts [7], was found in patients with peripheral neuropathy diagnoses cf. healthy controls. However, prior studies in fibromyalgia (a condition not meeting criteria for neuropathic pain [3], like WAD II) yielded conflicting findings regarding presence/absence of increased skin cytokine gene expression [8, 9]. Whether skin cytokine gene expression in WAD II is more akin to small fibre neuropathy or fibromyalgia is unknown. Further, whether there are causal links between nerve fibre degeneration and (local and systemic) inflammation remains to be determined [7].

Inflammatory mechanisms are increasingly implicated in chronic pain, including musculoskeletal conditions such as neck and back pain [10, 11]. In people with chronic WAD, systemic inflammation was associated with hyperalgesia [12], consistent with inflammatory-mediated sensitisation of nociceptors. Given potential links between (i) skin cytokine expression and small fibre neuropathies, (ii) inflammation and chronic WAD, and (iii) chronic WAD and reduced IENFD, examining local skin inflammatory processes in chronic WAD II will inform our understanding of possible mechanisms associated with reduced IENFD in this condition.

This exploratory study compared skin expression of genes encoding specific inflammatory cytokines (IL-6, IL-8, IL-1β, IL-10, TNF; implicated in prior studies [5, 7]) in people with chronic WAD II to controls. A secondary aim was to investigate associations between skin inflammatory cytokine gene expression and IENFD, pain and psychological factors.

Method

Study design

Our cross-sectional study compared skin cytokine gene expression at the finger and ankle in people with chronic WAD II to age- and sex-matched controls. We hypothesised cytokine gene expression would be higher and associated with reduced IENFD and more severe clinical symptoms in people with chronic WAD. This study used data and skin biopsy samples (snap frozen segments) from a previously published study examining IENFD in chronic WAD [2]. Gene expression data/analyses have not previously been reported.

Ethics statement

Ethical approval was granted by The University of Queensland and Griffith University human research ethics committees. All participants provided written informed consent. The study was performed in accordance with the Declaration of Helsinki.

Participants

Individuals with chronic neck pain after a whiplash injury (>3 months duration) were recruited between 2016–2019. Potential participants were interviewed by phone to determine eligibility (WAD II [no fracture/dislocation or neurological deficit upon bedside neurological examination]) and to screen for exclusion criteria. Individuals were excluded if they had a history of (i) neuropathy, diabetes, fibromyalgia or other condition associated with small fibre pathology (e.g., HIV), (ii) conditions affecting upper/lower limbs (e.g., carpal tunnel syndrome), (iii) specific pathology contributing to neck pain (e.g., inflammatory conditions such as ankylosing spondylitis, rheumatoid arthritis), (iv) cervical spine surgery, (v) allergy to lidocaine, (vi) anticoagulant medication use or (vii) current pregnancy. A control group comprised healthy volunteers with no history of neck injury or significant neck pain (lasting>1 week), age- and sex-matched 1:1 with chronic WAD patients (±5 years age).

Participants attended one appointment at a university research laboratory. They completed questionnaires, underwent bedside clinical neurological examination (to confirm no frank neurological deficit) by a physiotherapist, quantitative sensory testing at the finger by a physiotherapist (reported elsewhere [2]), and skin biopsies performed by a general practitioner.

Clinical questionnaires

Demographic (age, sex) and clinical details (pain duration [years], neck pain intensity [0–10 numerical rating scale, 0 is no pain and 10 is the worst pain imaginable], distribution of pain/paraesthesia [body chart]) were collected, in addition to a series of questionnaires. For chronic WAD participants, we used data for the following outcome measures: Neck Disability Index [13] (0–100, higher scores reflect worse disability), Depression, Anxiety and Stress Scale (DASS-21) [14] (three subscales 0–28, higher scores reflect worse symptoms), Impact of Events Scale-Revised [15] (0–88, higher scores reflect worse post-traumatic stress symptoms), and Self-complete Leeds Assessment of

Neuropathic Symptoms and Signs [16] (0–24, higher scores suggestive of neuropathic pain). Control group participants completed the DASS-21.

Skin biopsies

Three-millimetre skin punch biopsies were performed under sterile conditions at the ventrolateral aspect of the proximal phalanx of the index finger and at the contralateral leg (10 cm superior to the lateral malleolus). Subcutaneous lidocaine injection was used for local anaesthesia (1%, no adrenaline, 0.2–0.4 mL). The finger was selected as it is an established site for evaluation of IENFD [17] and altered sensory function has been observed at this site in chronic WAD [18]. The ankle was selected as a distal site outside of cervical spine dermatomes. The main site of pain (neck) was not selected as a biopsy site to facilitate higher participation rates, as it was anticipated that people with chronic WAD would be reluctant to undergo biopsies in the painful neck region. Biopsies were 1–2 mm deep to include the epidermal-dermal junction. Each biopsy was cut in half. One half was used for histological evaluation of IENFD (reported elsewhere [2]) and the other half was collected under sterile and RNase-free conditions to limit RNA degradation (RNaseZap, Thermo Fisher Scientific, Waltham MA), placed in a sterile 1.5 mL microcentrifuge tube, snap frozen and stored at −80 °C. Snap freezing was performed using liquid nitrogen and was typically completed within 1 min of biopsy collection. For transportation from clinic to laboratory, the samples remained in liquid nitrogen until storage at −80 °C. For subsequent transportation between laboratories, the samples were transferred in a vessel containing dry ice.

Gene expression analyses

Realtime quantitative polymerase chain reaction analysis (RT-qPCR) was performed blinded to study group using the snap frozen half of the skin samples from the finger and ankle. Genes encoding inflammatory cytokines implicated in prior studies of small fibre neuropathy were selected for evaluation, namely TNF, IL-1β, IL-6, IL-8, IL-10 [5–8]. We limited the panel to these cytokines with prior evidence of elevated expression [5–8] to reduce the risk of Type I error, while still spanning a range of cytokines with pro- and anti-inflammatory functions [19–23]. Briefly, total RNA from patient samples (finger, ankle) was extracted using liquid nitrogen crushing followed by RNA purification kits (Zymo Research, Irvine CA), as per manufacturer's instructions. Genomic DNA was removed using an off-column DNAse digestion (Thermo Fisher Scientific, Waltham MA). RNA was reverse transcribed to cDNA using Superscript III (Invitrogen, Waltham MA) and oligo dT. Levels of specific mRNAs were quantified by qPCR in duplicates using SyBR Green-PCR mix (Thermo Fisher Scientific, Waltham MA) in the Applied Biosystems Viia 7 RT-PCR system (Thermo Fisher Scientific, Waltham MA). Appropriate negative controls with no Superscript III were included for all experiments. Data for average duplicates were expressed relative to the housekeeping gene hypoxanthine phosphoribosyltransferase 1 (*HPRT1*, human) using the ΔCt method [24]. We find that *HPRT1*, a commonly used housekeeping control gene, is not regulated by inflammatory stimuli in various cell types, including primary human keratinocytes and keratinocyte cell lines [25]. Thus, it was used as the housekeeping control gene for this study. Primers used for RT-qPCR are listed in Supplementary Table S1.

Statistical analyses

Data were analysed using STATA 16 (StataCorp, College Station, TX). Distribution of continuous variables was assessed visually using histograms. Demographic, questionnaire and cytokine gene expression data were compared between chronic WAD and control groups using independent sample t-tests or Wilcoxon rank-sum tests, as appropriate to distribution. Effect sizes were calculated for significant group differences using Cohen's d (or r for non-normally distributed data), interpreted as small (d = 0.2, r = 0.1), medium (d = 0.5, r = 0.3) or large (d = 0.8, r = 0.5) [26]. We ran a sensitivity analysis comparing *TNF* gene expression between groups excluding an outlier to determine robustness of the effect. We also ran an exploratory analysis subgrouping the chronic WAD group by presence/absence of upper limb pain or paraesthesia. In the chronic WAD group, associations between cytokine gene expression and IENFD were evaluated using Spearman correlations, as were associations between cytokine gene expression and key clinical measures (pain, disability, stress, post-traumatic stress symptoms). We limited our exploratory correlation analyses to these measures as we hypothesised more 'severe' WAD or higher levels of stress may be associated with inflammatory activity. Significance was set at $P < 0.05$ and Benjamini–Hochberg corrections were used to adjust for multiple comparisons (i.e., across the five cytokines for assessment of group differences and for Spearman correlations).

As a secondary exploratory study arising from our prior study on IENFD in chronic WAD [2], the sample size of the present study was determined by that of the prior study. That is, no a priori sample size calculation was performed for this analysis.

Results

The original study comprised 24 people in each group [2], however technical issues affected sample processing for three people in the chronic WAD group. As such, 21 patients with chronic WAD and 21 matched controls were included in the present study.

Demographic and clinical questionnaires

Demographic and clinical summary data for the chronic WAD and control groups are presented in Table 1.

Gene expression analyses

Quantitative PCRs were successful for all 21 chronic WAD participants at the finger and ankle. For one finger sample and two ankle samples in the control group, quantitative PCRs were unsuccessful due to poor RNA yields (<5 ng/μL), so reliable data could not be captured for these samples.

Cytokine gene expression in chronic WAD vs controls

Summary data for gene expression of each cytokine for the chronic WAD and control groups can be seen in Table 2 and in Figs. 1 and 2. Gene expression is quantified as expression relative to *HPRT1* housekeeping control gene. At the finger, gene expression of *TNF* was approximately twofold higher in chronic WAD than controls (median [IQR] *TNF*/*HPRT1*: 3.47 [4.16] vs. 1.80 [2.33], r=0.43 [95%CI 0.14, 0.65], P=0.006 [P.adj=0.027]). To consider if this finding was driven by an outlier in the chronic WAD group, we re-ran the analysis with the outlier removed and the difference remained significant (median [IQR] *TNF*/*HPRT1*: 3.41 [4.08] vs. 1.80 [2.33], r=0.41 [95%CI 0.11, 0.64], P=0.010 [P.adj=0.048]). There were no other significant group differences in gene expression of the other cytokines examined, nor group differences in any cytokine gene expression at the ankle. Further exploratory analyses subgrouping the chronic WAD group based on upper limb pain/paraesthesia are presented in Supplementary Table S2.

Correlations between cytokine gene expression and IENFD

Table 3 and Supplementary Figures S1 and S2 present Spearman correlations between cytokine gene expression and IENFD at the finger and ankle. No significant correlations were found.

Correlations between cytokine gene expression and clinical outcomes

Table 4 and Supplementary Figures S3 and S4 present Spearman correlations between cytokine gene expression at the finger and ankle and key clinical measures (pain, disability, stress, post-traumatic stress). No significant correlations were found following adjustment for multiple comparisons.

Table 1 Demographic and clinical characteristics of chronic WAD and control groups. Compared with controls, the chronic WAD group had lower IENFD and higher depression, anxiety and stress symptoms

Characteristic	Whiplash (N=21) Mean (SD)/Median [IQR]	Controls (N=21) Mean (SD)/Median [IQR]	Test statistic	P-value
Age, years	48.1 (11.5)	47.4 (11.9)	t=0.36	0.72
Sex, n (%)	13 (62%) F, 8 (32%) M	13 F, 8 M	–	–
Duration of pain, years	5.0 [18.3]	–	–	–
IENFD finger, fibres/mm	4.33 [3.73]	8.36 [4.23]	z=−3.06	0.002
IENFD ankle, fibres/mm	7.41 [5.33]	9.11 [6.34]	z=−1.54	0.13
NDI, 0–100	35.2 (17.2)	–	–	–
NRS, pain intensity 0–10	5.48 (2.43)	–	–	–
DASS D, subscale	4.0 [12.0]	2.0 [4.0]	z=2.87	0.004
DASS A, subscale	4.0 [10.0]	0.0 [2.0]	z=2.49	0.012
DASS S, subscale	14.0 [12.0]	6.0 [8.0]	z=3.59	<0.001
IESR, total	14.5 [26.0]	–	–	–
SLANSS, total	6.0 [10.0]	–	–	–
Pain in upper limb*, n (%)	9 (43%)	–	–	–
Occasional upper limb paraesthesia**, n (%)	10 (48%)	–	–	–

NB: IESR is missing for one patient in Whiplash Group. DASS: Depression, Anxiety, Stress Scale, with depression (D), anxiety (A) and stress (S) subscales; IENFD: intraepidermal nerve fibre density; IESR: Impact of Event Scale Revised; NDI: Neck Disability Index; NRS: Numerical rating scale; SLANSS: Self-complete Leeds Assessment of Neuropathic Symptoms and Signs

*In addition to neck pain

**No patients had frank neurological deficit on clinical neurological examination (i.e., loss of muscle strength, light touch sensation, tendon reflexes) as per study eligibility criteria

Table 2 Skin mRNA expression of genes encoding inflammatory cytokines at finger and ankle sites (quantified relative to the hypoxanthine phosphoribosyltransferase 1 housekeeping gene), with Wilcoxon rank-sum test results, with unadjusted and adjusted P-values

Cytokine (Gene)	Whiplash Median [IQR]	Controls Median [IQR]	Test statistic z	P-value Unadjusted	P-value Adjusted
Finger					
IL-1β (*IL1B*)	0.63 [0.61]	0.29 [0.84]	1.44	0.15	0.39
IL-6 (*IL6*)	0.08 [0.28]	0.08 [0.31]	−0.29	0.78	0.78
TNF (*TNF*)	3.47 [4.16]	1.80 [2.33]	2.74	**0.006**	**0.028**
IL-8 (*CXCL8*)	1.94 [2.71]	1.86 [3.25]	−0.52	0.61	0.77
IL-10 (*IL10*)	0.17 [0.21]	0.29 [0.48]	−1.20	0.24	0.39
Ankle					
IL-1β (*IL1B*)	0.27 [0.87]	0.60 [0.73]	−0.90	0.38	0.47
IL-6 (*IL6*)	0.07 [0.30]	0.30 [0.78]	−1.30	0.20	0.33
TNF (*TNF*)	3.84 [3.07]	3.80 [4.26]	−0.26	0.81	0.81
IL-8 (*CXCL8*)	3.01 [4.26]	6.02 [6.75]	−1.86	0.07	0.33
IL-10 (*IL10*)	0.77 [0.99]	1.01 [2.64]	−1.77	0.08	0.20

Bold denotes P-value significant after correction for multiple comparisons. N = 21 and N = 20 samples analysed in the whiplash and control groups, respectively. Data also presented in Figs. 1 and 2

IL: interleukin, TNF: tumour necrosis factor

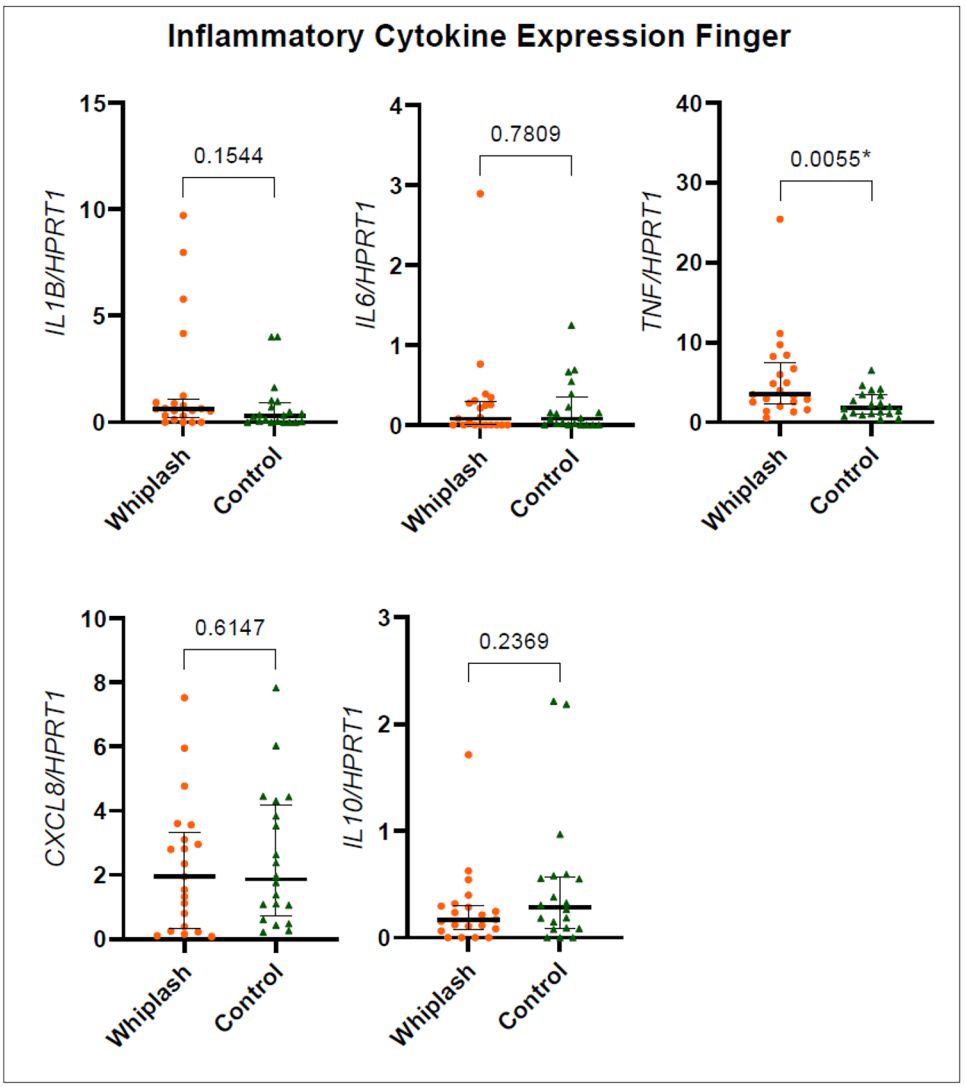

Fig. 1 Skin inflammatory cytokine gene expression at the finger in chronic whiplash-associated disorder Grade II and control groups (quantified relative to the HPRT1 housekeeping control gene). N = 21 and N = 20 samples analysed in the whiplash and control groups, respectively. CXCL8 – interleukin 8; HPRT1 – hypoxanthine phosphoribosyltransferase 1; IL – interleukin; TNF – tumour necrosis factor. Unadjusted P-values are for Wilcoxon rank-sum tests. *Denotes significance following correction for multiple comparisons

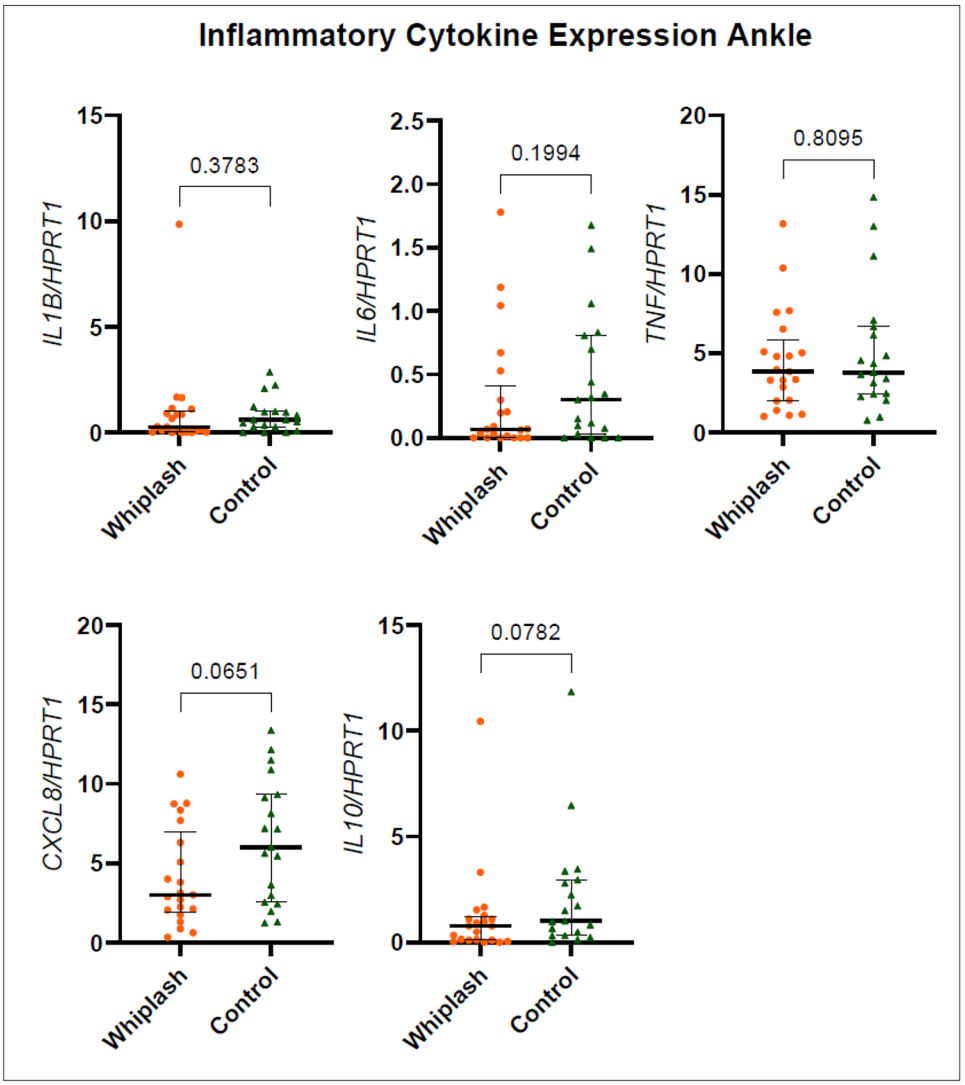

Fig. 2 Skin inflammatory cytokine gene expression at the ankle in chronic whiplash-associated disorder Grade II and control groups (quantified relative to the *HPRT1* housekeeping control gene). N = 21 and N = 19 samples analysed in the whiplash and control groups, respectively. CXCL8 – interleukin 8; HPRT1 – hypoxanthine phosphoribosyltransferase 1; IL – interleukin; TNF – tumour necrosis factor. Unadjusted P-values are for Wilcoxon rank-sum tests

Table 3 Spearman correlations for inflammatory cytokine gene expression vs intraepidermal nerve fibre density (IENFD) at each site in Whiplash Group (N = 21). Rho (unadjusted P-value) [adjusted P-value]

Finger		Ankle	
Cytokine (Gene)	IENFD Rho (P) [adj-P]	Cytokine (Gene)	IENFD Rho (P) [adj-P]
IL-1β (*IL1B*)	−0.03 (0.89) [1.00]	IL-1β (*IL1B*)	−0.15 (0.51) [1.00]
IL-6 (*IL6*)	−0.21 (0.36) [1.00]	IL-6 (*IL6*)	0.02 (0.94) [1.00]
TNF (*TNF*)	−0.13 (0.58) [1.00]	TNF (*TNF*)	0.07 (0.77) [1.00]
IL-8 (*CXCL8*)	−0.03 (0.89) [0.89]	IL-8 (*CXCL8*)	0.02 (0.95) [0.95]
IL-10 (*IL10*)	−0.10 (0.68) [1.00]	IL-10 (*IL10*)	−0.25 (0.28) [1.00]

Discussion

Our findings reveal higher *TNF* gene expression in finger skin in people with chronic WAD II compared with controls (medium-large effect). For the other inflammatory cytokines investigated, there were no group differences at the finger site. At the ankle, there were no group differences in skin gene expression for any cytokines. For our secondary exploratory aim, there were no associations between skin cytokine gene expression and IENFD, or self-reported clinical outcomes at the finger or ankle site, including pain, disability and stress measures. This investigation builds upon our earlier data demonstrating an association between reduced IENFD in the finger and chronic WAD II [2]. The mechanisms underpinning this association are not clear. It is possible that reduced IENFD (in a cervical spine dermatome) could reflect mild injury to peripheral nerves in whiplash trauma in some patients, sufficient for intraneural

Table 4 Spearman correlations for expression of genes encoding inflammatory cytokines vs clinical measures, for finger and ankle sites in Whiplash Group (N = 21)

	Finger				
	IL-1β	**IL-6**	**TNF**	**IL-8**	**IL-10**
NDI	−0.13 (0.56) [0.93]	−0.05 (0.82) [0.82]	0.16 (0.50) [1.00]	0.12 (0.61) [0.76]	0.29 (0.20) [1.00]
NRS	−0.17 (0.47) [0.59]	−0.20 (0.37) [0.62]	−0.23 (0.34) [0.85]	−0.16 (0.50) [0.50]	0.29 (0.21) [1.00]
DASS S	−0.08 (0.72) [1.00]	0.03 (0.89) [1.00]	0.18 (0.45) [1.00]	0.03 (0.91) [0.91]	0.14 (0.54) [1.00]
IESR	−0.22 (0.36) [1.00]	−0.07 (0.77) [0.96]	−0.01 (0.97) [0.97]	−0.08 (0.75) [1.00]	0.09 (0.72) [1.00]
	Ankle				
	IL-1β	**IL-6**	**TNF**	**IL-8**	**IL-10**
NDI	−0.09 (0.71) [1.00]	0.04 (0.86) [1.00]	−0.24 (0.30) [1.00]	−0.13 (0.59) [1.00]	−0.01 (0.97) [0.97]
NRS	−0.27 (0.24) [0.40]	−0.01 (0.97) [0.97]	−0.29 (0.21) [0.53]	−0.35 (0.12) [0.60]	−0.22 (0.35) [0.44]
DASS S	−0.06 (0.78) [0.98]	0.33 (0.15) [0.75]	0.05 (0.84) [0.84]	0.17 (0.46) [0.77]	0.25 (0.27) [0.68]
IESR	0.08 (0.72) [0.72]	0.55 (0.01) [0.06]	−0.38 (0.09) [0.23]	−0.24 (0.32) [0.53]	0.09 (0.70) [0.88]

Rho (unadjusted P-value) [adjusted P-value]. No correlations were significant following correction for multiple comparisons

NB: IESR is missing for one patient in Whiplash Group. DASS S – Depression, Anxiety, Stress Scale subscale; IENFD – intraepidermal nerve fibre density; IESR – Impact of Event Scale Revised; NDI – Neck Disability Index; NRS – Numerical rating scale pain intensity

inflammation but not frank neuropathy [27–29]. Another possible explanation is that reduced IENFD is systemic, non-specific and related to ongoing stress, potentially via immune-mediated pathways [2]. This would be consistent with presence of reduced IENFD across numerous non-neuropathic pain conditions [30], given the association between chronic pain and stress [31], and impact of stress upon systemic and skin immune function [32, 33]. The current analysis provides insight on these speculated mechanisms by characterising associations between skin immune measures (cytokine gene expression) and chronic WAD II, IENFD, and clinical outcomes including stress.

Our findings suggest a possible association between skin inflammatory processes at the finger and chronic WAD II. At a group level, patients with chronic WAD had higher finger *TNF* gene expression and lower IENFD than controls. However, there was no correlation between finger IENFD and *TNF* (or any other cytokine) gene expression. This could mean that patients with chronic WAD have lower IENFD and increased local inflammation at this site through non-related mechanisms. Another consideration is the non-significant correlations could reflect the relatively small sample size of this exploratory analysis, which is not well powered to detect correlations. The difference between groups in *TNF* mRNA expression at the finger but not the ankle may reflect cervical spine dermatome vs distal locations. Further, we decided not to perform skin biopsies at the main site of pain (the neck), as we were concerned that people with chronic WAD would be reluctant to undergo this procedure in the painful region. While there are no data comparing IENFD in painful vs non-painful sites in WAD or other musculoskeletal conditions [30], studies in people with chronic post-surgical pain (post-herniorrhaphy [34, 35], post-thoracotomy [36]) report reduced IENFD at painful vs contralateral non-painful sites. Whether such a difference in IENFD is present in chronic WAD, and whether skin inflammatory cytokine expression varies between painful and non-painful locations, remain to be determined.

We are not aware of any other studies assessing skin inflammatory cytokine expression in people with chronic WAD. In lieu of other data in WAD for direct comparison and the exploratory nature of this study, we compare our findings to prior data in peripheral neuropathy and fibromyalgia. In patients with peripheral neuropathy diagnoses, increased skin expression of mRNAs encoding IL-6, IL-8, and IL-10 was reported in lower limb sites compared with healthy controls [5–7]. While these studies did not directly assess correlations between IENFD and cytokine gene expression, patients with length-dependent small fibre neuropathy (i.e., reduced IENFD in the distal leg cf. proximal leg) demonstrated increased expression of genes encoding TNF, IL-6, IL-1β and IL-8 in distal skin compared to proximal skin [5]. In fibromyalgia, a non-neuropathic condition like WAD II, results are less consistent. One study

[8] reported skin expression of genes encoding IL-1β, IL-6 and TNF at the shoulder in fibromyalgia (N = 53) compared with no detected expression in healthy controls (N = 10). In contrast, another [9] reported no group differences in lower limb skin cytokine gene expression in fibromyalgia (N = 25) compared with controls (N = 35) and people with depression (N = 10). These contrary findings may be a consequence of methodological differences (semi-quantitative reverse transcription PCR [8] cf. more sensitive RT-qPCR [9]). One study [9] reported no association between cytokine gene expression and IENFD, whereas this relationship was not evaluated by the other [8]. Taken together, while there is some evidence for higher skin cytokine gene expression in chronic pain conditions compared with controls, associations between cytokines and IENFD remain uncertain, with emerging data indicating no association between lower IENFD and higher cytokine gene expression in WAD and fibromyalgia [9].

Clinically, it is proposed that in chronic pain conditions associated with reduced IENFD such as small fibre neuropathy, fibromyalgia and chronic WAD, remaining nociceptors are sensitised by inflammatory mediators [5, 7, 9]. This could contribute to pain, hyperalgesia or allodynia observed in patients with WAD [37], particularly via TNF and IL-6 [7, 38]; elevated synovial fluid TNF levels were associated with mechanical hyperalgesia and poor post-operative outcome in temporal mandibular joint pain [39]. In our study, after correction for multiple comparisons, skin inflammatory cytokine gene expression was not associated with pain, disability, or stress measures at the finger or ankle sites. This is consistent with prior studies also reporting no relationship between skin cytokine gene expression and clinical outcomes in small fibre neuropathy (pain, disability, neuropathic pain symptoms, depression) [5] and fibromyalgia (pain) [9]. Cumulatively, these data suggest there is no association between clinical outcomes and skin inflammatory cytokine gene expression in chronic pain conditions. However, the sites of the skin biopsies were distal from the main pain site in chronic WAD (i.e., the neck), which may have affected our findings.

A limitation of our study is the small sample size, particularly for correlation analyses (for context, N = 26 is required per group for an independent sample t test to detect a large effect [d = 0.8] at 80% power and P = 0.05 [two-tailed] [40]; N = 21 has 80% power to detect a moderate correlation coefficient [r = 0.55] at P = 0.05 [41]). This was a consequence of being a secondary, exploratory investigation using tissue from a previous study [2]. It also meant that statistical methods with capacity to adjust for co-variates (e.g., linear regression) were not suitable, so we were unable to account for potential confounding factors in bivariate analyses (e.g., age, sex). Further, we examined five key inflammatory cytokines implicated by prior data [5–8], however there are other inflammatory mediators relevant to inform possible links between IENFD, chronic WAD and skin inflammation. Only mRNA expression was examined, so it is unclear if higher *TNF* expression in the finger was accompanied by increased local protein levels. Although unlikely given the quick processing time, the use of subcutaneous lidocaine in the skin biopsy procedures may have impacted mRNA levels of genes encoding inflammatory cytokines, given its known anti-inflammatory effects [42]. We screened our participants by self-report for medical conditions associated with reduced IENFD (e.g., peripheral neuropathy, diabetes, fibromyalgia etc.); a medical assessment with diagnostic screening for known contributors to small fibre neuropathy (e.g., metabolic, nutritional, autoimmune and toxicological panels) would provide greater certainty that other medical conditions did not account for reduced IENFD in the chronic WAD group.

Future studies should use larger samples to evaluate a broader range of markers (e.g., larger panel of inflammatory mediators and/or RNA sequencing) to characterise molecular profiles of reduced IENFD in chronic pain conditions. Longitudinal studies would provide insight on temporal relationships between cytokine gene expression, IENFD and clinical outcomes. Further, cellular sources of cytokine gene expression remain to be determined.

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Data Availability De-identified study data are available from the corresponding author upon reasonable request. Data will be shared with qualified researchers for non-commercial purposes and subject to relevant ethical approvals.

Declarations

Competing interests The authors declare no competing interests.

References

1. Farrell S et al (2019) Cervical spine findings on MRI in people with neck pain compared with pain free controls: a systematic review and meta-analysis. J Magn Reson Imaging 49(6):1638–1654
2. Farrell SF, Sterling M, Irving-Rodgers H, Schmid AB (2020) Small fibre pathology in chronic whiplash-associated disorder: a cross-sectional study. Eur J Pain 24(6):1045–1057
3. Finnerup N et al (2016) Neuropathic pain: an updated grading system for research and clinical practice. Pain 157(8):1599–1606
4. Fundaun J et al (2022) Nerve pathology and neuropathic pain after whiplash injury: a systematic review and meta-analysis. Pain 163(7):e789–e811
5. Üçeyler N et al (2010) Elevated proinflammatory cytokine expression in affected skin in small fiber neuropathy. Neurology 74:1806–1813
6. Üçeyler N, Riediger N, Kafke W, Sommer C (2015) Differential gene expression of cytokines and neurotrophic factors in nerve and skin of patients with peripheral neuropathies. J Neurol 262(1):203–212
7. Kreß L et al (2021) Differential impact of keratinocytes and fibroblasts on nociceptor degeneration and sensitization in small fiber neuropathy. Pain 162(4):1262–1272
8. Salemi S et al (2003) Detection of interleukin 1beta (IL-1beta), IL-6, and tumor necrosis factor-alpha in skin of patients with fibromyalgia. J Rheumatol 30(1):146–150
9. Üçeyler N et al (2014) Skin cytokine expression in patients with fibromyalgia syndrome is not different from controls. BMC Neurol 14:185
10. Farrell SF et al (2023) Genetic impact of blood C-reactive protein levels on chronic spinal & widespread pain. Eur Spine J 32:2078–2085
11. Farrell SF et al (2024) C-Reactive Protein (CRP) is associated with chronic pain independently of biopsychosocial factors. J Pain 25:476–496
12. Sterling M, Elliott JM, Cabot PJ (2013) The course of serum inflammatory biomarkers following whiplash injury and their relationship to sensory and muscle measures: a longitudinal cohort study. PLoS One 8(10):e77903
13. Vernon H, Mior S (1991) The neck disability index: a study of reliability and validity. J Manipulative Physiol Ther 14(7):409–415
14. Lovibond SH, Lovibond PF (1995) Manual for the Depression Anxiety & Stress Scales, 2nd edn. Psychology Foundation, Sydney
15. Weiss DS (2007) The impact of events scale: revised. Cross-cultural assessment of psychological trauma and PTSD. Springer, United States, pp 219–238
16. Bennett M, Smith B, Torrence N, Potter J (2005) The S-LANSS score for identifying pain of predominately neuropathic origin: validataion for use in clinical and postal research. J Pain 6(3):149–158
17. Schmid AB, Bland JDP, Bhat MA, Bennett DLH (2014) The relationship of nerve fibre pathology to sensory function in entrapment neuropathy. Brain 136:1857–1867
18. Chien A, Sterling M (2010) Sensory hypoaesthesia is a feature of chronic whiplash but not chronic idiopathic neck pain. Man Ther 15:48–53
19. Hunter CA, Jones SA (2015) IL-6 as a keystone cytokine in health and disease. Nat Immunol 16(5):448–457
20. Matsushima K, Yang D, Oppenheim JJ (2022) Interleukin-8: an evolving chemokine. Cytokine 153:155828
21. Ren K, Torres R (2009) Role of interleukin-1β during pain and inflammation. Brain Res Rev 60(1):57–64
22. Steen EH et al (2020) The role of the anti-inflammatory cytokine Interleukin-10 in tissue fibrosis. Adv Wound Care (New Rochelle) 9(4):184–198
23. van Loo G, Bertrand MJM (2023) Death by TNF: a road to inflammation. Nat Rev Immunol 23(5):289–303
24. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25(4):402–8
25. Ramnath D et al (2015) TLR3 drives IRF6-dependent IL-23p19 expression and p19/EBI3 heterodimer formation in keratinocytes. Immunol Cell Biol 93(9):771–9
26. Fritz C, Morris P (2011) Effect size estimates: current use, calculations, and interpretation. J Exp Psychol Gen 141(1):2–18
27. Greening J, Anantharaman K, Young R, Dilley A (2018) Evidence for increased magnetic resonance imaging signal intensity and morphological changes in the brachial plexus and median nerves of patients with chronic arm and neck pain following whiplash injury. J Orthop Sports Phys Ther 48(7):523–532
28. Kitamura T, Takagi K, Yamaga M, Morisawa K (1995) Brachial plexus stretching injuries: microcirculation of the brachial plexus. J Shoulder Elbow Surg 4(2):118–123
29. Schmid AB, Coppieters M, Ruitenberg M, McLachlan E (2013) Local and remote immune-mediated inflammation after mild peripheral nerve compression in rats. J Neuropathol Exp Neurol 72(7):662–680
30. Ranathunga N et al (2025) Small nerve fibre pathology in non-neuropathic chronic pain conditions: a systematic review and meta-analysis. Pain. https://doi.org/10.1097/j.pain.0000000000003826
31. Abdallah CG, Geha P (2017) Chronic pain and chronic stress: two sides of the same coin? Chronic stress (Thousand Oaks, Calif.) 1:10.1177/2470547017704763
32. Hansel A, Hong S, Camara R, von Kanel R (2010) Inflammation as a psychophysiological biomarker in chronic psychosocial stress. Neurosci Biobehav Rev 35:115–121
33. Hunter H, Momen S, Kleyn C (2015) The impact of psychosocial stress on healthy skin. Clin Exp Dermatol 40:540–546
34. Bischoff JM et al (2014) A capsaicin (8%) patch in the treatment of severe persistent inguinal postherniorrhaphy pain: a randomized, double-blind, placebo-controlled trial. PLoS One 9(10):e109144
35. Bischoff JM et al (2013) Lidocaine Patch (5%) in Treatment of Persistent Inguinal Postherniorrhaphy Pain: A Randomized, Double-blind, Placebo-controlled, Crossover Trial. Anesthesiology 119(6):1444–1452

36. Springer JS et al (2017) Functional and structural assessment of patients with and without persistent pain after thoracotomy. Eur J Pain 21(2):238–249
37. Farrell S et al (2024) Trajectories of cold but not mechanical sensitivity correspond with disability trajectories following whiplash injury. PAIN
38. Sommer C, Kress M (2004) Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. Neurosci Lett 361(1–3):184–187
39. Shafer DM, Assael L, White LB, Rossomando EF (1994) Tumor necrosis factor-alpha as a biochemical marker of pain and outcome in temporomandibular joints with internal derangements. J Oral Maxillofac Surg 52(8):786–791 (**discussion 791–2**)
40. Faul F, Erdfelder E, Lang AG, Buchner A (2007) G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 39(2):175–191
41. Algina J, Olejnik S (2003) Sample size tables for correlation analysis with applications in partial correlation and multiple regression analysis. Multivar Behav Res 38(3):309–323
42. Wang J et al (2024) The mechanism of perioperative intravenous lidocaine in regulating the inflammatory response: A review. Medicine Baltimore 103(36):e39574