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Evaluation of the pre- and post-treatment levels of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF- α) in patients with temporomandibular disorders

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Abstract

Context: Diagnosis of Temporomandibular disorders (TMDs) have been traditionally based on history, physical examination and imaging with their attendant limitations. Recent advocacy supports using inflammatory markers in blood and saliva as an objective means for early diagnosis, treatment monitoring, and targeted therapy development.
Materials and Methods: This study used a quasi-experimental design to recruit 25 participants using consecutive sampling. Pain scores were obtained from the participants using the Visual Analogue scale after which 5mls of venous blood was collected from antecubital veins. Also, 5mls of unstimulated whole saliva was collected. They were thereafter placed on appropriate treatment and reviewed after 2 weeks. Post-treatment VAS score was obtained, along with blood and saliva samples for the evaluation of the biomarkers. Data obtained were analyzed using the median and inter-quartile ranges, and Spearman's correlation. The Wilcoxon signed-rank test was used to compare the median pre and post-treatment levels of the biomarkers in both saliva and serum. The p-value was set at < 0.05.
Results: The salivary and the serum IL-1β median (Q3-Q1) level reduced from 963.5 (265.6)pg/ml to 514.6 (515.0)pg/ml ($p= 0.011$), and from 59.7 (125.1)pg/ml to 53.7 (102.7)pg/ml ($p = 0.657$) respectively. Also, the salivary and the serum TNF- α median level reduced from 616.9 (194.7)pg/ml to 571.6 (198.4)pg/ml ($p = 0.122$), and from 83.6 (79.9)pg/ml to 73.2 (80.9)pg/ml ($p = 0.638$) respectively.
Conclusion: The significant reduction in salivary IL-1β following treatment suggest that it may be a potential biomarker for assessing the effectiveness of therapeutic intervention in TMDs.

Keywords: Temporomandibular disorders, Biomarkers, Visual Analog Scale

Introduction

Temporomandibular disorders (TMDs) refer to a wide range of problems affecting the temporomandibular joint (TMJ), masticatory muscles, and surrounding bony and soft tissue components.[1] They are among the most prevalent maxillofacial disorders.[2] TMDs are characterised by pain in the TMJ and masticatory muscles, limited mouth opening, joint clicks, mandibular deviation, and headaches.[3,4,5] TMDs have a multifactorial etiology and are linked to a range of dental and medical factors. These include occlusion, posture, parafunctional habits, restorative and orthodontic procedures, emotional stress, psychosocial factors, age, and gender, as well as biochemical changes, structural abnormalities, muscle dysfunction, trauma, genetic mutations, hormonal changes, and systemic diseases.[3,6] Hypertension and insulin resistance have

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also been recognized as significant disease factors that are becoming more prevalent in the population and have an impact on the development of TMD.[6] Studies suggest that elevated blood pressure can impair central pain regulatory systems, which may contribute to painful TMD. Patients with TMD show increased sensitivity to aversive stimuli, indicating that painful TMD could partly stem from dysfunction in central pain modulation, with baseline arterial blood pressure playing a role.[7]

The involvement of elevated biochemical or inflammatory indicators in the development of TMDs has been recognized, allowing for the investigation of more sensitive and innovative biomarkers in this field.[8] Zwiri et al.[2] found that TNF-α, IL-8, IL-6, and IL-1 are the most prevalent biomarkers of TMDs. While earlier studies have primarily relied on synovial fluid to assess TMD biomarkers because it provides precise insights into the local environment of the TMJ and allows for the targeted detection of inflammatory markers specific to TMD,[9,10] obtaining an adequate volume from the small joint space demands specialized equipment and expert laboratory techniques, making it impractical for routine clinical use.[2,11] In contrast, blood and saliva offer more accessible and minimally or non-invasive alternatives.[12] As a result, there is increasing interest in using blood and saliva to examine these pro-inflammatory markers.[12-16]

Reports on the biomarkers associated with TMDs in our environment are lacking in literature, thus highlighting the novel nature of our study in our locality. As a result, this study aimed to evaluate and compare the pre- and post-treatment levels of IL-1β and TNF-α in both saliva and serum of patients with TMDs, and to evaluate how therapy influences the biomarker levels and their correlation with pain scores.

Materials and Method

Study design: This was a quasi-experimental study carried out among participants diagnosed with Temporomandibular disorders at the Oral Medicine Clinic, University of Benin Teaching Hospital, Benin City, Edo State.

Study population: These were participants aged >18 with complaints of pain in the TMJ and masticatory muscles with function.

Inclusion criteria: Participants aged 18 years and above who have been diagnosed with TMD according to the Diagnostic Criteria for TMDs (D/C TMDs)[3].

Exclusion criteria: Individuals who already had a TMD diagnosis and were receiving treatment, as well as those with comorbid conditions like chronic periodontitis (Basic Periodontal Examination, BPE>2), diabetes, uncontrolled hypertension, autoimmune diseases such as lichen planus or systemic lupus erythematosus, or any other debilitating illness were excluded.

Sample size: The sample size was calculated using the G*Power software version 3.1.9.7. The following parameters were used: $\alpha = 0.05$, Power $(1 - \beta) = 0.8$, Effect size, $f = 0.7$, Test Family: t-test. The minimum sample size computed was 19. Accounting for 10% attrition, the minimum sample size was $(0.10 * 19 + 19) = 20.9$. A total of 25 participants who met inclusion criteria were recruited during the study period (November 2021-October 2022).

Ethical clearance: Ethical approval was obtained from the University of Benin Teaching Hospital with PROTOCOL NUMBER ADM/E22A/VOL VII 14831234

Procedure:

1. Using the Visual Analogue Scale, pain scores were obtained from the participants.
2. Under aseptic precautions, 5mls venous blood was collected with a needle and syringe from each participant's antecubital vein into vacutainer tubes (plain bottle) and sent to the laboratory immediately (within 20 minutes).
3. Participants were asked to rinse their mouth with tap water to remove food debris. 5mls unstimulated whole saliva was collected from the participants via spitting into a plain bottle and sent to the laboratory immediately.
4. After obtaining the blood and saliva samples from the patients with TMD, they were placed on treatment appropriately. Participants diagnosed with arthrogenic TMDs like osteoarthritis, were placed on non-steroidal anti-inflammatory (NSAIDs) -Arthrotec (diclofenac and misoprostol)75mg 12 hourly and olfen gel (diclofenac gel) 8 hourly, for one week. Participants diagnosed with muscular TMDs like myofascial pain, were placed on

muscle relaxants- Ophesic (ophenadrine 35mg and paracetamol 450mg) 12 hourly and olfen gel, for 2 weeks. All the TMD patients were counseled on life style modifications, dietary modifications, and jaw relaxation techniques.

5. Participants were reviewed after 2 weeks; post-treatment pain scores were obtained, after which 5mls venous blood and 5mls unstimulated saliva were collected again for post-treatment evaluation of the biomarkers.

Laboratory analysis: Venous blood samples obtained from the participants were left to stand for 1 hour to clot. Samples were then centrifuged at 3000 revolutions/minute for 10 minutes; after which the serum was transferred with a clean Pasteur pipette into a 5ml plain tube and stored frozen at -20°C until analysis. The saliva samples were also stored frozen at -20°C. Upon analysis, ELISA kits from Elabscience Biotechnology Inc. were used for detecting and quantifying the cytokines from the various samples.[17]

Statistical analysis: Data obtained from the study were analyzed using the IBM Statistical Package for Social Science (SPSS) version 26.0. The Shapiro-Wilk test was used to test for normality of the data, and it showed that the data were not normally distributed. Hence the analysis was done using the median and inter-quartile range in place of the mean and standard deviation. The Wilcoxon signed-rank test was used to compare the pre-and post-treatment levels of the biomarkers in both saliva and serum. Correlation tests between the VAS scores and both the salivary and serum levels was done using Spearman's rank correlation coefficient. A p-value < 0.05 indicates statistical significance.

Results

Table 1: Demographic Characteristics of the Participants

Characteristic	Frequency(%) n = 25
Age(in years)(Median (IQR)	52.0(36.0- 66.0)
Gender	
Female	6 (24.0)
Male	19 (76.0)

IQR= Interquartile range

A total of 25 participants were recruited for this study. The demographic characteristics of the participants are presented in Table 1. The median age of the participants was 52 (30) years. Participants included 6 females (24.00%) and 19 males (76.00%).

Table 2: Changes in VAS in Response to Treatment

Treatment	N	Min	Max	Median	Q1	Q3	IQR
Pre-treatment	25	4	8	7	5	8	3
Post-treatment	25	0	7	4	2	4	2
Change1	25	-5	-1	-3	-4	-2	2

The result showed that the pre-treatment VAS score (Table 2) ranged from 4 - 8 with a median of 7 (3) while the post-treatment VAS score ranged from 0 - 7 with a median of 4 (2). The median reduction in the VAS score due to treatment was 3 (2). The Wilcoxon signed-rank test showed that the reduction in the VAS score was statistically significant ($p<0.001$). The negative (-) indicates a reduction in the VAS score. IQR-Interquartile range which is the difference between Q3 and Q1.

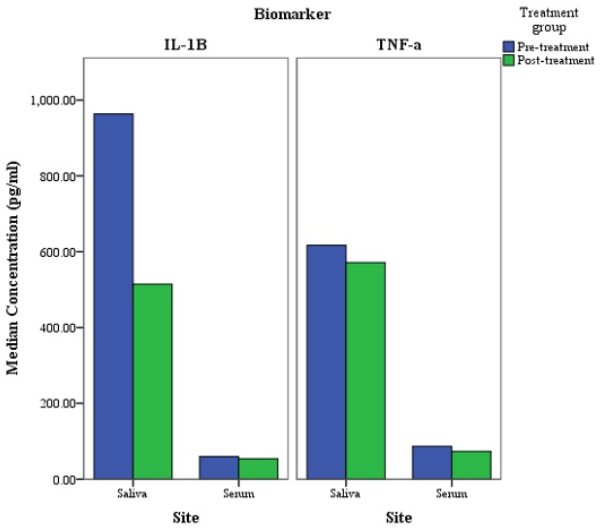

Figure 1: Pre-and Post-treatment Serum and Salivary Levels of IL-1β and TNF-α in Patients with TMDs.

Figure 1 showed that the salivary IL-1β median (Q3-Q1) level reduced from 963.5 (265.6) pg/ml to 514.6 (515.0) pg/ml. The reduction was statistically significant ($p= 0.011$), while the serum IL-1β median level reduced from 59.7 (125.1) pg/ml to 53.7 (102.7) pg/ml, this however, was not statistically significant ($p = 0.657$). Also, the salivary TNF- α median level reduced from 616.9 (194.7) pg/ml to 571.6 (198.4) pg/ml and the

reduction was not statistically significant (p = 0.122), while the serum TNF- α median level concentration reduced from 83.6 (79.9) pg/ml to 73.2 (80.9) pg/ml (p = 0.638).

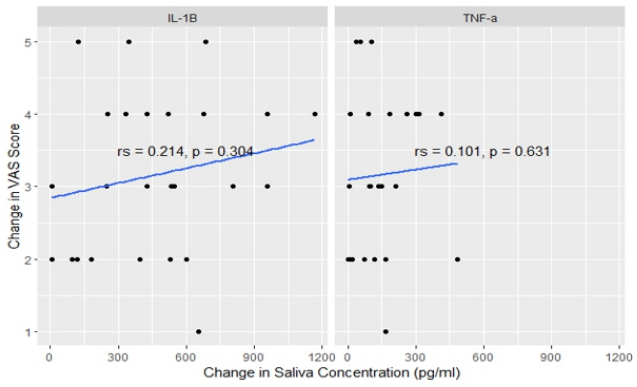

Figure 2: The relationship between the changes in VAS and changes in Salivary levels of IL-1β and TNF-α (due to treatment). (rs: Spearman's rank correlation coefficient).

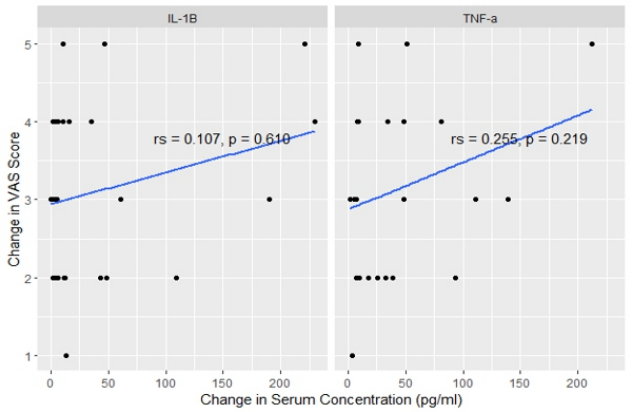

Figure 3: The relationship between the changes in VAS and changes in serum levels of IL-1β and TNF-α (due to treatment).

Spearman correlation analysis between the VAS scores and both the salivary and serum levels of IL-1β and TNF-α showed (Figure 2 and 3) a generally weak positive correlation between the variables, which was not statistically significant. Salivary IL-1β and TNF-α and VAS (rs=0.214 and 0.101) (p = 0.304 and 0.631); and serum IL-1β and TNF-α and VAS (rs=0.107 and 0.255) (p =0.610 and 0.219)

Discussion

A total of 25 participants were treated and. This study observed a statistically significant reduction in the salivary levels of IL-β following treatment. A reduction, though not statistically significant was also observed in the serum levels of IL-β, salivary and serum levels of TNF- α following treatment. There is a paucity of in vivo studies investigating the effect of therapy on proinflammatory cytokines in TMDs.[18] A study by Hashemi et al[19], however, observed a significant reduction in synovial and serum levels of IL-1β and TNF-α following ozone therapy. Low level laser therapy was associated with decrease in blood IL-6 and C-reactive protein in patients with TMD pain.[20] A systematic review 12 reported a reduction in the elevated levels of IL-1β and TNF-α in synovial fluid following treatment with hyaluronic acid, platelet-rich plasma, and low-level laser therapy with improved symptoms. Pharmacological agents such as glucocorticoids and tramadol have been shown to significantly lower IL-1β, TNF-α, and PGE2 levels.[21] Salivary and serum IL-1β, TNF-α and other biomarkers like IL-8, cortisol, and oxidative stress markers have been reported to demonstrate potential in monitoring disease progression, and evaluating treatment response.[20,22-24] Longer medical intervention may lead to a more significant reduction of IL-1β and TNF-α levels in the serum and saliva of the patients. It can thus be inferred that the institution of therapeutic intervention has a role to play in the reduction of IL-1β and TNF-α levels in the serum and saliva of the patients giving credence to their role in the pathophysiology of TMDs.

The treatment outcome showed a significant reduction in VAS scores among TMD patients at a 2-week-review after commencement of treatment. This indicates a positive response to treatment, which aligns with previous studies reporting improvements in VAS scores in response to diverse therapeutic interventions. Van Grottel et al.,[25] reported an improvement in VAS scores after treatment. In another study, Li Wenyan[26], observed a reduction in VAS scores in TMDs following electric shock wave therapy. Similarly, Panhoca et al.,[27] also reported a reduction in VAS scores in TMDs following laser and ultrasound therapy.

This study observed a weak positive correlation between the biomarkers (IL-1β and TNF-α) and

VAS scores in both serum and saliva, which was statistically insignificant. This implies that an increase in the biomarkers appears to be associated with an increase in the VAS score indicating a clinical significance despite the statistical finding. Conversely, a decrease in the biomarker levels (due to treatment) could be associated with a decrease in pain levels (VAS score). Not many reports exist in literatures on the correlation between VAS scores and biomarkers. A study by Zwiri et al.,[20] found no significant correlation between pain intensity and biomarkers levels except for IL6 at baseline and VAS directly after treatment. Elevated levels of IL-1β in TMDs have been reported to correlate with greater pain sensitivity.[28] In another study by Ulmner et al.,[10] which compared the cytokine concentration in synovial fluid and synovial tissue, it was observed that synovial tissue cytokines (IL-1β, TNFα, and IL-10) strongly correlated with symptoms (synovitis, pain etc.) related to TMD. This study was limited by a small sample size, hence, a future study with larger sample size is recommended.

Conclusion

The reduction in the salivary and serum levels of IL-1β and TNF-α following therapeutic intervention gives credence to their role in the pathogenesis of TMDs. This can serve as a basis for further investigations on the role of these markers in TMDs as an objective means of diagnosis and treatment monitoring, and the development of targeted therapy against these inflammatory markers.

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Declaration of conflict: None

Authors contributions: Both authors contributed meaningful to the work from conceptualization, to data collection and analysis.

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