

Can nano-fat injection in patients with temporomandibular disorder alters salivary matrix metalloproteinases 2 and 9?



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Abstract Temporomandibular disorder (TMD) is a musculoskeletal condition that affects temporomandibular joint, causing pain and dysfunction in the jaw area, jeopardizing the ability to eat and even sleep comfortably. A key factor in the pathogenesis of TMD is the enhanced breakdown of extracellular matrix components, such as collagen and elastin. This breakdown is mediated by enzymes known as matrix metalloproteinases (MMPs), specifically MMP2 and MMP9. Elevated expression of MMP2 and/ or MMP9 may be involved in the pathophysiology of TMD. Methodology: Stimulated saliva samples were obtained from Thirty-two TMD patients. MMPs in collected samples were analyzed by ELISA. Results: Three months after nano-fat injection, Means of MMP2 and MMP9 were significantly decreased compared to that before procedure (139.59 versus 128.46 ng/ml, $P= 0.003$; and 545.37 versus 469.09 ng/ml, $P= 0.001$ respectively). Conclusion: this study provides evidence for a significant reduction in salivary MMP2 and MMP9 levels among TMD patients following nano-fat injection therapy. These findings suggest that nano-fat injections may have therapeutic potential for managing TMD by modulating ECM remodeling processes through their effects on these enzymes.

Keywords: autologous fat, TMD, salivary proteins.

1. Introduction

Temporomandibular disorder (TMD) is a musculoskeletal condition that affects the temporomandibular joint, causing pain and dysfunction in the jaw area. This disorder can have a significant impact on patients' quality of life, affecting their ability to eat, speak, and even sleep comfortably. A study by Wajnaa published in 2005 inspected various etiological factors for this disorder. The results found that stress is responsible for over 40% of TMD symptoms (Wajnaa, 2005; Al-Aswad and Hidan, 2019).

Among many treatment modalities such as splints, laser and intra articular injections, one potential treatment option for TMD is nano-fat injection therapy, which involves the injection of autologous fat particles into the upper joint space to improve symptoms (Ameena and Farah, 2017; Khassaki and J.N, 2019).

The purpose of this paper is to discuss the application role of nano-fat injection in improving TMD symptoms.

One of the key factors involved in the pathogenesis of TMD is enhanced breakdown of extracellular matrix components, such as collagen and elastin. This breakdown is mediated by enzymes known as matrix metalloproteinases (MMPs), specifically MMP2 and MMP9 (Flavia *et al.*, 2020).

MMP2 and MMP9 are members of the gelatinase subfamily of MMPs, which play important roles in tissue remodeling processes by degrading various components of the extracellular matrix. However, dysregulation or excessive activity of these enzymes has been implicated in several pathological conditions including TMD. High levels of salivary MMP2 and MMP9 have been observed in patients with TMD, suggesting their potential involvement in disease progression (Asparuh Nikolov & Nikola Popovski, 2021).

The study aimed to investigate changes in salivary metalloproteinases after autologous fat grafting in to the upper joint space of patients complaining of internal derangement. Nano-fat injection involves harvesting fat cells from one part of the body through liposuction techniques, processing them into small particles using mechanical disruption methods, and injecting them into another part for therapeutic purposes such as tissue regeneration or repair (Zhu *et al.*, 2019).

Previous studies have explored the association between TMD and salivary MMP2 and MMP9 levels with mixed results. Some studies have reported increased levels of these enzymes in saliva samples collected from TMD patients compared to healthy control (Muskan Kukreja *et al.*, 2015; Anderson *et al.*, 2017). These findings suggest that elevated expression or activity of salivary MMP2 and/or MMP9 may be involved in the pathophysiology of TMD.



However, other studies have found no significant differences in salivary MMP2 and MMP9 levels between TMD patients and controls (Shchors *et al.*, 2013). These discrepancies may be attributed to variations in sample size, patient selection criteria, or differences in methodologies employed for MMP analysis (Jones *et al.*, 2022).

The existing literature highlights the need for further research to investigate the effects of nano-fat injection on salivary MMP2 and MMP9 levels in TMD patients. Understanding the impact of this therapeutic intervention on these enzymes can provide valuable insights into its mechanisms of action and potential benefits for TMD management.

2. Materials and Methods

Thirty-two patients (a total of 56 joints) with age range 20-40 (± 2) years, complaining of signs and symptoms of TMD (Wilkes' classification of TMJ internal derangement involved Stage III, IV and Stage V) were included as participants in this study. Stimulated Saliva samples (using sugar free gum) were collected from each participant before nano-fat injection using standardized protocols (Costa *et al.*, 2021). These samples were analyzed for salivary MMP2 and MMP9 levels using enzyme-linked immunosorbent assay (ELISA) techniques.

Autologous fat samples were obtained from the abdomen through a 3-4 mm infra-umbilical incision from 32 healthy patients who had not undergone liposuction, were not smokers, and/or did not have any metabolic diseases. All patients informed about the risks involved in the operation and gave their consent. The procedure performed under general anesthesia to ensure a pain free harvesting and blood free field. The preparation of nano fat followed the protocol of (Tonnard *et al.*, 2013).

A second saliva sample was obtained from each participant three months after nano-fat injection to assess any changes or trends observed in salivary MMP2 and MMP9 levels following the therapeutic intervention.

3. Results

3.1. Before nano-fat injection

The analysis of saliva samples collected from internal derangement patients before nano-fat injection revealed elevated levels of both salivary MMP2 and MMP9 compared to baseline values reported in healthy individuals. Mean values before measured to be (139.59 ± 22.7) for MMP2 and (545.37 ± 133.7) for MMP9 versus (128.46 ± 25.6) and (469.09 ± 94.2) respectively.

3.2. After nano-fat injection

Three months after nano-fat injection, a significant reduction was observed in salivary MMP2 and MMP9 levels among TMD patients. The decrease observed after treatment suggests that nano-fat injections may have a modulatory effect on these enzymes, potentially reducing their activity or expression within the temporomandibular joint environment.

Means of MMP2 and MMP9 were significantly decreased after procedure compared to that before procedure (139.59 versus 128.46 ng/ml, $P = 0.003$; and 545.37 versus 469.09 ng/ml, $P = 0.001$ respectively) as shown in Figure 1 and Table 1.

In patients aged ≥ 30 years, means of MMP2 and MMP9 were significantly decreased ($P < 0.05$) after procedure compared to that before procedure; while in patients aged < 30 years, no significant change in means of MMP2 and MMP9 ($P \geq 0.05$) after procedure compared to that before procedure as shown in Table 2.

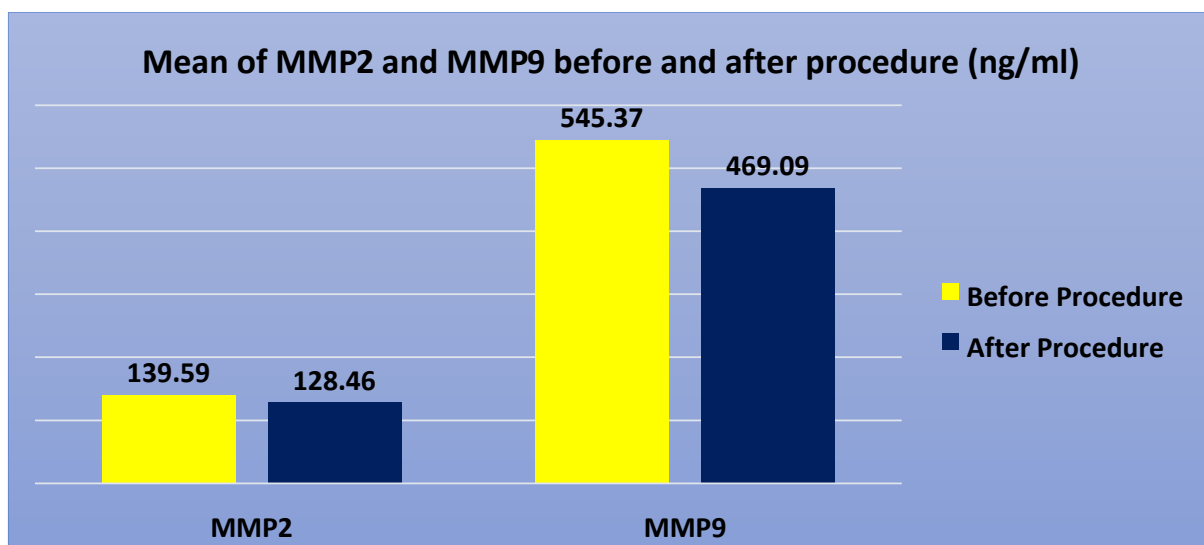


Figure 1 Mean of MMP2 and MMP9 before and after nanofat injection.

Table 1 Comparison in certain biological markers before and after procedure.

Variable	Time		Paired t-test	P - Value
	Before procedure Mean \pm SD	After procedure Mean \pm SD		
MMP2 (ng/ml)	139.59 \pm 22.7	128.46 \pm 25.6	3.168	0.003
MMP9 (ng/ml)	545.37 \pm 133.7	469.09 \pm 94.2	3.582	0.001

Table 2 Comparison in certain biological markers before and after procedure according age.

Variable	Time		Paired t-test	P - Value
	Before procedure Mean \pm SD	After procedure Mean \pm SD		
MMP2 (ng/ml) according age				
< 30 Years	148.41 \pm 19.1	140.16 \pm 30.2	1.394	0.191
\geq 30 Years	134.3 \pm 23.5	121.45 \pm 20.1	2.902	0.009
MMP9 (ng/ml) according age				
< 30 Years	519.66 \pm 97.4	478.66 \pm 91.0	1.994	0.071
\geq 30 Years	560.8 \pm 151.7	463.35 \pm 97.9	3.122	0.006

4. Discussion

In inflammatory TMJ diseases, various mediators—particularly cytokines—may be responsible for rearrangement of the extracellular matrix in joint issues, altering normal cell reactions and allowing enzymatic degradation of the matrix. Collagenases and matrix metalloproteinases (MMPs), zinc-containing proteins with enzymatic activity, likely play roles in this process (Tian and Zhang *et al.*, 2020).

Macromolecular degradation of the matrix determines physical and biological deterioration of the tissues and promotes the disease, because the degradation fragments, proteoglycans, and collagen released into the synovial fluid generate inflammatory pain, with further release of MMPs.

The findings of this study indicate a significant reduction in salivary MMP2 and MMP9 levels following nano-fat injection in TMD patients.

This reduction suggests a potential therapeutic effect of nano-fat injections, which may contribute to the management of TMD by targeting the dysregulated extracellular matrix remodeling processes implicated in disease pathogenesis. In the context of the temporomandibular joint (TMJ), a study investigated the effects of nano-fat injection on TMJ diseases.

Serhan *et al.*, (2015) Although this study does not directly mention the role of MMP2 and MMP9, it is known that MMP2 and MMP9 are involved in the pathogenesis of TMJ diseases. The study suggests that nano-proresolving medicines (NPRM) can be used to treat TMJ diseases, and these NPRMs have been shown to reduce nanotoxicity and promote wound healing (Holmström *et al.*, 2019).

The mechanisms by which nano-fat injections influence salivary protein expression are not fully understood. However, it has been proposed that the regenerative properties of adipose-derived stem cells present within the injected fat particles may play a role in modulating MMP activity and expression. These cells have been shown to secrete various growth factors and cytokines that can regulate tissue remodeling processes and potentially downregulate MMP production or activity (Holmström *et al.*, 2019).

This study is not without limitations. The small sample size used in this investigation limits its global impact on larger populations of TMD patients. Additionally, the lack of a control group hinders our ability to make direct comparisons between pre-and post-nano-fat injection results.

5. Conclusions

In conclusion, this study provides evidence for a significant reduction in salivary MMP2 and MMP9 levels among TMD patients following nano-fat injection therapy. These findings suggest that nano-fat injections may have therapeutic potential for managing TMD by modulating ECM remodeling processes through their effects on these enzymes.

Further research with larger sample sizes and appropriate control groups is warranted to confirm and expand upon these observations, as well as to elucidate the underlying mechanisms involved. Nonetheless, the current findings offer promising insights into the role of nano-fat injections as a potential treatment option for individuals suffering from temporomandibular disorder.

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Ethical considerations

All of our research subjects were informed about any potential risks or complications that might be included before or after the procedure, they provided their written consents according to ethical research committee at dental college of Baghdad university. I confirm that I have obtained all consent required by the applicable law to publish any personal details or images of patients, research subjects, or other individuals used. I agree to provide Multidisciplinary Science Journal with copies of the consent or evidence that such consent has been obtained if requested. (ethical clearance reference number 435).

Conflict of Interest

The authors declare no conflicts of interest.

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