

structures. The main goal of TMD therapy is the reduction of clinical symptoms such as pain and limited lower jaw movement. Research has recently suggested that oxidative stress (OS) can play a part in the pathophysiological processes that occur in TMD. OS occurs as a result of an imbalance between oxidants and antioxidants in favour of oxidants, and it is believed to play a role in various adverse processes in an organism. Studies have found significant differences in the expression of OS biomarkers and antioxidants between TMD patients and healthy individuals. This study evaluates the change in salivary oxidative stress in patients with temporomandibular joint myalgia before and after occlusal soft splint therapy. The result indicates that there was a significant difference in clinical parameters of visual analog scale (VAS), Maximal comfortable mouth opening (MCO) and Perceived Stress Scale (PSS) with P value <0.001. Among all biochemical parameters, TAC (Total anti oxidative capacity) showed statistically significant changes in post operative findings. All the patients with temporomandibular joint myalgia experienced a decrease in their clinical symptoms and salivary oxidative stress markers, the salivary biochemical assay performed to evaluate the oxidative stress revealed improved antioxidant capacity, indicating a compensatory mechanism to lower oxidative stress.

KEYWORDS : Temporomandibular joint, oxidative stress, biomarkers, occlusal soft splint.

# INTRODUCTION

# Temporomandibular disorders (TMD):-

encompass the most prevalent painful musculoskeletal and neuromuscular orofacial conditions and have an impact on the jaw joints, masticatory muscles, and surrounding structures.

Due to their complex pathogenesis, which is still not fully understood, these disorders were still predominantly diagnosed through medical history and clinical examination. A viable method for diagnosing TMD was used to investigate symptoms, indicators, and behavioural risk factors utilising well-defined, evidence-based questionnaires and protocols. The three most frequent diagnoses for pain-related TMD were myofascial pain (MP), disc displacement (DD), and degenerative joint disease. The main goal of TMD therapy was to minimize clinical symptoms such discomfort and restricted lower jaw movement.

Recent studies had raised the possibility that oxidative stress (OS) contributed to the pathophysiological processes underlying TMD. Biomarkers had been investigated in TMD patients to clarify the processes of pain, to give a basis for early identification of pain and degeneration, and provide a potential target for therapeutic drugs to stop the development to more severe pain and dysfunction.

Oxidative stress is caused by an imbalance of oxidants and antioxidants in favour of oxidants, and it was thought to have a role in a variety of undesirable processes in an organism. The role of OS in a number of human disorders, particularly those relating to oral health, has been studied. The expression of antioxidants and OS indicators differed noticeably between TMD patients and healthy individuals, according to biochemical investigations. To help with the early detection, diagnosis, and monitoring of TMD patients, the examination of OS biomarkers was used as a supplementary approach. Many successful treatment methods with varied degrees of efficacy, including physical therapy, behavioural therapy, and the use of occlusal splints, were highlighted. In several trials, using a combination of noninvasive modalities yielded superior outcomes to using a single therapy.

Occlusal splints' function was not yet fully understood,

although some data seem to support the idea that they are a better alternative than no therapy at all. A possible placebo effect is one of the variables that may have contributed to treatment effectiveness, which was thought to be the result of a number of different factors.

Thus, this study involves the use of soft occlusal splint therapy for the management of temporomandibular joint myalgia by reducing symptoms of depression and improving pain-related disability. Saliva from TMJ myalgia patients was collected before and after occlusal soft splint therapy in order to determine the level of OS biomarkers (TAC - total antioxidative capacity, SOD - Superoxide dismutase, and UA -Uric acid). The efficacy of Soft splint reflected in a significant change in oxidative stresses in salivary sample which are tested biochemically under colorimetric and uricase method. Thereby the study proves the efficacy of occlusal soft splint used in temporomandibular joint myalgia patients with clinical and biochemical evidences.

# **OBJECTIVES:**

The objective of the study is to evaluate the change in salivary oxidative stress in patients with temporomandibular joint myalgia through the following parameters:

# Clinical parameters:

- Visual analogue scale (VAS),
- Maximal comfortable mouth opening (MCO),
- The level of perceived stress (Perceived Stress Scale
  [PSS])-(questionnaire)

# Salivary biochemical parameters:

- TAC (total antioxidative capacity)
- Superoxide dismutase (SOD),
- Uric acid (UA)

# MATERIALS AND METHODS:

The prospective and Comparative In Vivo Study was approved by the Institutional Review Committee and was carried out in Mahatma Gandhi Postgraduate Institute of Dental sciences (MGPGI) Puducherry. The patients with temporomandibular joint myalgia of age group from 25 to 50 years with unequal Ratio of men and women attending the Out Patient section of the Department of prosthodontics and Implantology at (MGPGI) Puducherry, seeking dental care fulfilling the inclusion criteria were selected.

# Sample size:

 $N\!=\,13$  GPower software (Version 3.1.9.4) was used for the estimation, effect size is 1.00, with alpha error at 0.05 and beta error at 0.90.

## Inclusion criteria:

- Patient with chronic pain and a diagnosis of only temporomandibularjointmyalgia.
- Patient with more than 30mm level in visual analog scale of pain.

## Exclusion criteria:

- Orofacial pain unrelated to TMD,
- Smoking,
- · Gingivitis, periodontitis and oral lesions,
- Chronic systemic diseases (e.g., diabetes, cardiovascular diseases, cancer, and autoimmune diseases),
- Uses of medications known to affect oxidative status are like antiviral, antineoplastic anti inflammatory drugs, etc.

General examination followed by intraoral and extraoral examinations were recorded in written case history format (annexure 1) along with necessary investigations. Before they agreed to participate in the study, the participants received thorough information and obtained their informed consent (annexure 2).

# Clinical Parameter: -

#### 1. Visual analogue scale (VAS)

The visual analog scale (VAS) is a validated, subjective measure for acute and chronic pain. Scores are recorded by making a handwritten mark on a 10-cm line that represents a continuum between "no pain" and "worst pain."

The temporomandibular joint and the masticatory muscles' spontaneous discomfort were assessed using a 100 mm horizontal VAS scale. The ranges on the scale are "worst agony imaginable" to "no discomfort" (0 mm) (100 mm).

The study only included participants with spontaneous pain measuring greater than 30 mm on the VAS.

#### 2. Maximal comfortable mouth opening (MCO)

The maximum mouth opening that a participant could make without feeling any more pain or discomfort was referred to as a pain-free opening. A temporomandibular joint disease may cause limited mouth opening during mandibular motions (TMD).

Maximal comfortable mouth opening was measured using digital vernier caliper before and after 3 months of occlusal splint therapy.

# 3. The level of perceived stress (Perceived Stress Scale [PSS])

The PSS is a well-known, self-administered psychological test for determining how stressful one thinks certain life situations entail. People who experience more stress had a higher risk of developing disease, which is connected with objective biological stress markers.

The PSS is a commonly used 10-item questionnaire that evaluates subjective sensations of stress over the past month. Response options form a 5-point Likert scale:

- 0 = Never,
- $l = \alpha lmost never,$
- 2 =sometimes,
- 3 = fairly often,
- 4 =very often.

Higher numbers indicated higher levels of stress, with possible scores ranging from 0 to 40.

#### Salivary samples

were collected before tooth brushing. Subjects were instructed to fast before saliva collection in the morning and not to eat or drink anything but water for at least 2 hours before sampling.

Five mL whole, unstimulated saliva samples were collected in graduated tubes (50 mL self-standing centrifuge tubes). The collections done after rinsing the mouth with water.

The saliva collection procedure will be performed before and after splint therapy. The splint therapy is given and patient's saliva is collected after 3 months also for evaluation.

## **Biochemical Parameter: -**

**TAC** (Total anti oxidative capacity) and **SOD** (superoxide dismutase) were measured using the commercial colorimetric reagent kits as **FRAP** (Ferric Reducing Antioxidant Power) Assay and uricase reagent respectively. Saliva uric acid (UA) levels were measured using the enzymatic Uricase method using commercially available reagents.

[The following biochemical parameters were evaluated through colorimetric and uricase methods performed by an expert biochemist from PCBS (Pondicherry center for biological science and educational trust) lab]

The **soft occlusal splint** was constructed for the upper arch of each patient from a 3 mm thick, soft polyvinyl sheet. The fabrication was done in a vacuum former, pressure molding device (drufomat – vacuum forming machine) with a thermally controlled infrared heater over the maxillary cast and occlusal contacts were neutralized.

The vacuum former's cast was perfectly and correctly accommodated by the rubber sheet. The sheet was taken off, and the edges of the splint were neatened with sturdy scissors. To achieve the desired shape, the palatal section of the splint was removed. For each patient, the splint is set so that the opposing teeth are covered by the splint surface at the same time.

After **3 months** of splint therapy follow up, all patients were studied again on both clinical and biochemical parameters to evaluate the changes after the soft occlusal splint therapy.

The collected data of pre operative and post operative clinical as well as biochemical parameter among the samples was analyzed statistically. The T-test was used to compare pre operative and post operative values and Chi-square test was used to determine the distribution of categorical score. The P values were fixed at <0.05 and all the analysis was done by SPSS version 20.

# RESULTS:

The result indicates that there was a significant difference in clinical parameters of visual analog scale (VAS), Maximal comfortable mouth opening (MCO) and Perceived Stress Scale (PSS) with P value <0.001. Among all biochemical parameters, TAC (Total anti oxidative capacity) showed statistically significant changes in post operative findings.

Although there was a change observed in the biochemical parameters post intervention as compared to the values pre intervention, however there were no significant difference observed in biochemical parameters with respect to SOD (superoxide dismutase) and UA (uric acid) from salivary samples.

All the patients with temporomandibular joint myalgia experienced a decrease in their clinical symptoms and

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salivary oxidative stress markers. The initial clinical symptoms of each patient with temporomandibular joint myalgia were found to be worse than expected on the visual analogue scale, the comfortable mouth opening scale, and the subjective stress scale. Even the salivary biochemical assay performed to evaluate the oxidative stress revealed improved antioxidant capacity, indicating a compensatory mechanism to lower oxidative stress.

# DISCUSSION:

The temporomandibular joint (TMJ) is an extremely complex joint system. Using well-defined, evidence-based questionnaires and protocols, which allow for few diagnostic errors and are therefore thought to be valid approaches for diagnosing Temporomandibular disorders, symptoms, signs, and behavioural risk factors were examined.

The major objective of temporomandibular disorders therapy was to lessen clinical symptoms including muscle pain and restricted lower jaw movement. Because of the difficulty in determining the aetiology and the possibility that the symptoms are secondary to other temporomandibular joint or masticatory muscle disorders, the initial treatment should be reversible. Physical therapy, behavioural therapy, and the use of occlusal splints were widely mentioned as successful treatment approaches with varying degrees of success.

When a splint is inserted, the jaws adjust to a new resting postural position. Occlusal splints that extend the occlusal vertical dimension beyond the freeway space result in an immediate adaptation to the new freeway space with an expanded vertical dimension. As a result, an occlusal splint allows a muscle to function more efficiently during contact, while remaining less active during postural functions. As a result, the vertical dimension increased from the occlusal contact, muscular effort decreases, resulting in muscle relaxation

In the current study, occlusal soft splint given for temporomandibular joint myalgia patient for a period of 3 months showed reduction in clinical symptoms and salivary oxidative stress markers. The patient initial clinical symptoms recorded with visual analogue scale, comfortable mouth opening and perceived stress scale were found out to be elevated from normal level among the entire temporomandibular myalgia patient. Even the salivary biochemical analysis done for evaluating the oxidative stress showed increased antioxidant capacity indicating a compensatory mechanism to suppress oxidative stress.

Visual analogue scale (VAS) scores for pain showed significant improvement throughout all study intervals. After three months of occlusal splint therapy, individuals with myofacial discomfort showed a reduction in VAS scores and the number of affected muscles.

Over the course of the study, the mouth opening significantly improved for every subject. The material's resilience may have contributed to the early improvement in mouth opening seen with the soft splint therapy by helping to distribute the strong functional occlusal forces and expedite the alleviation of muscular spasms. This adaptability may possibly be the reason why the discomfort in the masticatory muscles subsided so quickly.

The perceived stress scale among all 13 samples after the follow up showed reduced stress levels with proper education and counseling about modifying the stressful life style.

The soft splint therapy also reduced facial myalgia and TMJ clicking in some of the temporomandibular joint myalgia patient. These improvements can be linked to the uniform

intensity of contacts across all teeth, the posterior teeth's disocclusion, and the condylar's guiding throughout all movements. These circumstances result in the elevator and positioning muscles relaxing, which helps to lessen the aberrant muscle hyperactivity. When an occlusal splint is placed, the resting position is changed, and as a result, the occlusal vertical dimension grows beyond the open space. The improved resting position lowers muscle activity during postural duties, allowing muscles to work more effectively upon contact. The muscles and TMJ are relaxed as a result of the reduction in the amount of muscular effort needed as the vertical dimension is increased adequately.

The purpose of this study was to determine whether there were changes in the salivary oxidative status in individuals with TMD after occlusal soft splint therapy. As a result, there was found out to be an increased antioxidant activity following the soft splint therapy.

Reactive oxygen and nitrogen species (RONS) have been implicated in oxidative damage to biomolecules and the emergence of a number of diseases in the past. Recent data, however, seem to indicate that intracellular RONS play a significant role in intracellular signaling cascades.

Total antioxidant capacity (TAC) methods were used for being reliable, sensitive, and inexpensive. Moreover, these methods use durable reagents. It was determined that the elevated levels of TAC in individuals with temporomandibular joint myalgia were a compensatory response to a disrupted oxidant balance.

As uric acid (UA) can exhibit both antioxidant and oxidant properties, it is frequently referred to as a paradoxical biomarker. In our investigation, 70% of the patients had substantial increases in salivary UA at levels comparable to TAC, suggesting that UA may be a marker for antioxidant activity.

The literature shows that the activity of the superoxide dismutase (SOD), an antioxidant enzyme, is progressively reduced with the advancement of TMDs. This reduction may be related to an insufficient cleansing capacity of free radicals. However, another study showed levels of SOD in synovial fluid and increased lipid peroxidation, suggesting that the increased production of free radicals should cause an increase in the generation of antioxidant enzymes. The current study showed increased salivary SOD levels among 60% of the subjects indicating an elevated antioxidant activity after the soft splint therapy.

A study done by Harkins et al. concluded that soft splints had a reduction in facial myalgia. A study done by Nevarro et al. (1985) had concluded that soft splints are ineffective, and in another study done by Okeson (1987) on nocturnal electromyogram comparison of hard and soft reported significantly less effect with soft splints.

Our study found that soft splints were effective in reducing the symptoms of temporomandibular joint myalgia, however biochemical analysis of salivary oxidative stress were statistically insignificant within the sample of this study though individual variation of stress markers seen among temporomandibular joint myalgia patients.

#### CONCLUSIONS

This study involves the use of occlusal soft splint therapy for the management of myofascial pain. It is simple, with fewer side effects, cost effective, noninvasive, and better patient compliance. Certain TMD symptoms improve more quickly after soft splint therapy. The minimal time frame for splint therapy to reduce TMD symptoms is thought to be three months. Therefore, this study supports the use of splint therapy for managing temporomandibular myalgia patients.

Furthermore, clinical success, in terms of reduction of depressive symptoms, which correlates with the reduction of oxidative stress markers in the myalgia patients, indicates that oxidative stress might be related to psychological factors in TMD patients

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