

## Comparative Evaluation of Salivary Cortisol Levels in Bruxism Patients Before and After Using Soft Occlusal Splint: An *in vivo* Study

### Abstract

**Introduction:** Bruxism is defined as a nonfunctional activity or a parafunctional habit characterized by the unconscious repetitive motion of clenching and/or grinding of the teeth. Soft occlusal splints have been considered as the first-line strategy for treating nocturnal bruxism. Cortisol is a major steroid hormone secreted by fascicular zone of the adrenal cortex, belonging to the glucocorticosteroid group of hormones. Clinical studies have shown that when humans are placed under stress, the hypothalamic–pituitary–adrenocortical axis is activated, leading to an increase in cortisol secretion. However, the effect of splint is questionable by some researchers, and best way to perceive the effect of soft splints in patients with bruxism is by evaluating the stress levels.

**Materials and Methods:** A total of 20 individuals suffering from bruxism were selected from the Outpatient Department of Prosthodontics and Crown and Bridge, JSS Dental College and Hospital, Mysore. Saliva samples were collected before and after using the occlusal soft splints and subjected to enzyme-linked immunosorbent assay for determining the salivary cortisol levels. **Results:** The collected data were subjected to appropriate descriptive statistics, paired sample *t*-test, and one sample *t*-test. The paired sample *t*-test shows that intragroup comparison of the mean cortisol level in the study group shows near significant values, which means there is a decrease in the salivary cortisol levels in patients after using soft occlusal splint, but statistically nonsignificant. **Conclusion:** In the present study, it has been found that 70% of individuals after using the soft occlusal splint showed decreased cortisol levels.

**Keywords:** *Bruxism, enzyme-linked immunosorbent assay, salivary cortisol levels, soft occlusal splints*

### Introduction

Bruxism is defined as a nonfunctional activity or a parafunctional habit characterized by the unconscious repetitive motion of clenching and/or grinding of the teeth.<sup>[1]</sup> The most predominant form could occur when the patient is awake (daytime bruxism) or more commonly, during sleep (nocturnal bruxism), in which it can be considered as a stereotyped and periodic motion disorder.<sup>[2-4]</sup> Bruxism is a potentially destructive habit that results in tooth wear, damage to the structure of the surrounding teeth, inflammation of the gums, increased risk of periodontal disease, muscle pain, and temporomandibular joint (TMJ) dysfunction.<sup>[5]</sup> Primary etiological factors of bruxism are tooth interference in dental occlusion, psychosocial influences such as stress or anxiety, central or pathophysiological causes involving brain neurotransmitters or basal ganglia, systemic

diseases, occupational, and genetic factors.<sup>[6]</sup> Secondary factors are also considered, such as medication, tobacco, alcohol, other drugs, and the use of caffeine.<sup>[7]</sup> To treat bruxism, different modalities such as behavioral techniques, intraoral devices, medications, and contingent electrical stimulation have been applied. Occlusal splints have been considered as the first-line strategy for treating nocturnal bruxism.<sup>[8]</sup> An occlusal splint is a removable appliance, most often designed to cover the occlusal and incisal surfaces of all the teeth in the upper or lower jaw. Soft splints easily distribute the heavy loads encountered during parafunctional activities, and they have been associated with a high degree of patient tolerance.<sup>[9]</sup> Soft splints have some advantages, such as their relative simplicity, reversibility, noninvasiveness, and cost. These splints could be made to fit either the maxillary or mandibular arch. Cortisol is a major steroid hormone

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secreted by fascicular zone of the adrenal cortex, belonging to the glucocorticosteroidal group of hormones. The name cortisol is derived from cortex. Cortisol has its dispersion throughout all body fluids being detected in the urine, plasma, and saliva. Cortisol is responsible for important processes in the human body such as glucose production and activation of anti-inflammatory processes.<sup>[10,11]</sup> Clinical studies have shown that when humans are placed under stress, the hypothalamic–pituitary–adrenocortical axis is activated, leading to an increase in cortisol secretion. Based on this fact, several studies have used changes in salivary cortisol levels as an indicator of stress, anxiety, and depression.<sup>[12]</sup> During stressful situations, there is an increase in the level of cortisol, the so-called stress hormone. However, the effect of splint is questionable by some researchers,<sup>[9,13]</sup> and the best way to perceive the effect of soft occlusal splints in patients with bruxism is by evaluating the stress levels. Therefore, the aim of the study is to evaluate the salivary cortisol levels in patients with bruxism before giving soft occlusal splint and to evaluate and compare the salivary cortisol levels in patients with bruxism 15 days after giving soft occlusal splint.

## Materials and Methods

The present study was carried out in the Department of Prosthodontics, Crown and Bridge, JSS Dental College and Hospital, a Constituent College of JSS Academy of Higher Education and Research, Mysore. Ethical clearance was obtained from the Institutional Ethical committee. The study involved measurement of saliva cortisol levels in bruxism patients before and after using the soft occlusal splint using a salivary cortisol enzyme-linked immunosorbent assay (ELISA) kit.

A total of 20 individuals suffering from bruxism were selected from the Outpatient Department of Prosthodontics and Crown and Bridge, JSS Dental College and Hospital, Mysore, for the study based on the inclusion criteria. Individuals in the age group of 20–50 years with clinical finding of tooth wear were included in the study after signing the consent form. Individuals with any preexisting conditions affecting cortisol levels, such as adrenal gland insufficiency, pregnant women on oral contraceptives, asthmatic patients who are on steroid inhalers, individuals with xerostomia and those taking B-blockers, antihistamines, anticholinergics, antiparkinson agents, antidepressants or antipsychotic medication, diuretics, and sedatives were excluded.

## Methodology

After taking the written consent, individuals were instructed not to eat and drink 30 min before the sample collection. Individuals were given distilled water and asked to rinse for 30 s. Saliva was collected by spitting method in a sterile microcentrifuge tube [Figure 1] and stored in a refrigerator at  $-80^{\circ}\text{C}$  [Figure 2]. After collecting saliva,

an alginate impression was made on the mandibular arch, and it was poured immediately with dental stone. With a Sta-Vac vacuum machine [Figure 3], a 2 mm thick, thermoplastic polyethylene sheet [Figure 3] was adapted to the mandibular cast, and then, the outline of the sheet was trimmed. After fabrication of soft splint [Figure 3], the patient was instructed to wear splint at night for a period of 15 days. After 15 days, individuals were recalled and saliva was collected by using sterile microcentrifuge tubes and was stored at  $-80^{\circ}\text{C}$ . Stored saliva samples were subjected to ELISA test to detect the salivary cortisol levels. On the day of assay, the saliva samples were prepared by centrifugation [Figure 4] at 7000 rpm for 10 min and then used for the analysis. The measurement of saliva cortisol was performed using a 96 well salivary cortisol enzyme immunoassay kit [Figure 5] according to the manufacturer's instructions. The immune enzymatic reaction indicating the cortisol level was measured by optical density that was read at 450 nm in a multimode plate reader. After collecting the data, results were tabulated, statistically analyzed, and compared.

## Results

The collected data were entered from the paper-based records into Excel data sheet. The data were subjected to appropriate descriptive statistics, paired *t*-test, and one sample *t*-test using SPSS- statistical package for the social sciences version 22 IBM Corporation, Washington, D.C, united states of America. Intragroup comparison of mean cortisol levels in the study groups before and after using the occlusal splints shows  $21.548472 \pm 8.1402835$ ,  $20.799619 \pm 10.5274207$ , respectively [Table 1]. The one sample test shows that there is a statistically significant difference in the mean of the study groups before and after giving the soft occlusal splint [Table 2]. The percentage of change in the cortisol levels  $-70\%$  of individuals after using the soft occlusal splint showed decreased cortisol levels and  $30\%$  of individuals after using the soft occlusal splint showed increased cortisol levels [Graph 1]. With  $95\%$  confidence interval and  $19^{\circ}$  of freedom, difference in the cortisol levels shows *P* value was statistically



Figure 1: Sterile microcentrifuge tubes

nonsignificant [Table 3]. The mean difference in the cortisol levels among the study group after using soft occlusal splint shows decrease in the cortisol levels in 14 patients, whereas in 6 patients, increased cortisol levels were observed which can be seen in the graph as a negatively plotted axis [Graph 2].

### Discussion

“Bruxism is defined as the parafunctional grinding of teeth (or) an oral habit consisting of involuntary rhythmic or spasmodic nonfunctional gnashing, grinding, or clenching of teeth, in other than chewing movements of the mandible, which may lead to occlusal trauma.” It is also called grinding or occlusal neurosis (GPT 9).<sup>[14]</sup> Bruxism can occur during wakefulness or during sleep. Bruxism during daytime is commonly a semivoluntary “clenching” activity and is also known as “Awake Bruxism” or Diurnal Bruxism. Bruxism during sleep either during daytime or

during night is termed as “Sleep Bruxism.”<sup>[15]</sup> It is a potentially destructive habit that results in tooth wear damage to the structure of the surrounding teeth, damage to the structure of the surrounding teeth, inflammation of the gums, increased risk of periodontal disease, muscle pain, and TMJ dysfunction.<sup>[5]</sup> Previous studies reported that individuals under stress are more likely to exhibit bruxism;<sup>[16,17]</sup> others failed to confirm this correlation.<sup>[18,19]</sup> An earlier study by Rugh and Solberg reported that sleep bruxism (SB) seemed to appear after exhausting and stressful days. Similarly, Hicks and Chancellor showed an association between bruxism and an overtly ambitious character or behavior (Type A), which in turn is related to a stressful lifestyle. Furthermore, in another study on 1339 individuals, frequent bruxism was significantly associated with severely stressful situations at work, and it was

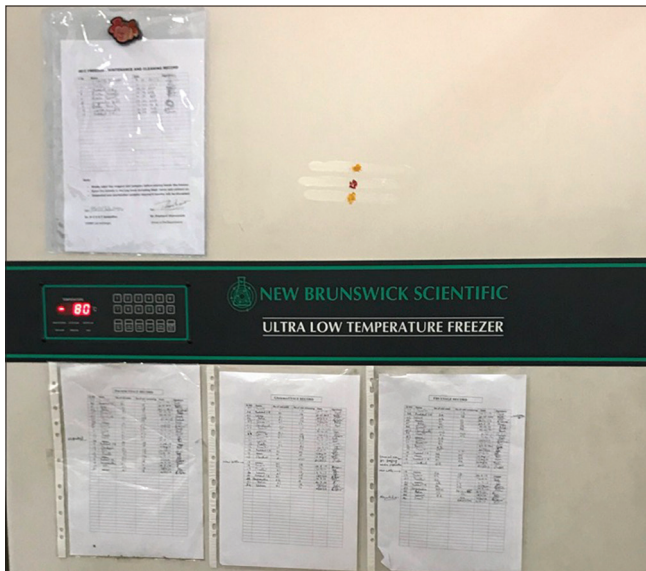


Figure 2: Ultra-low temperature freezer (-80°C)



Figure 4: Centrifuge

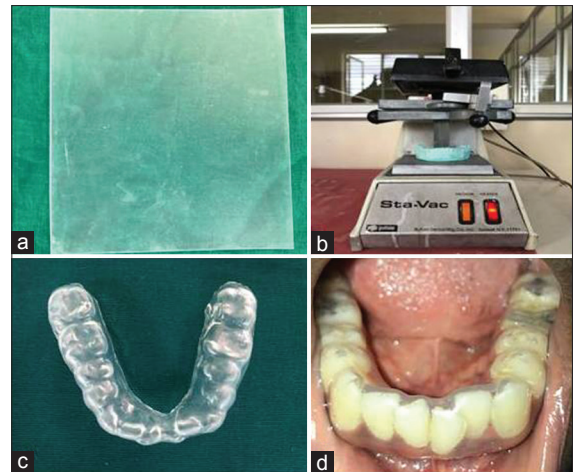


Figure 3: 2-mm polyethylene sheet and Sta-Vac vacuum machine

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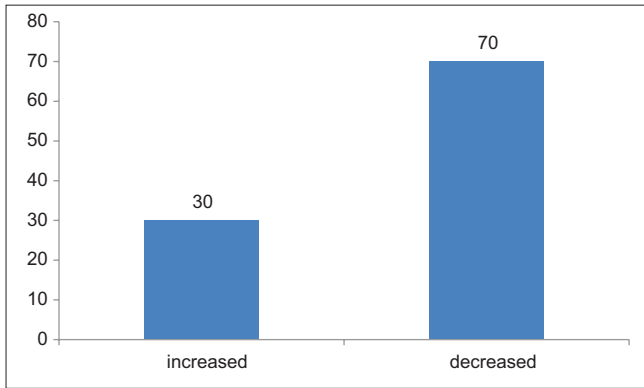
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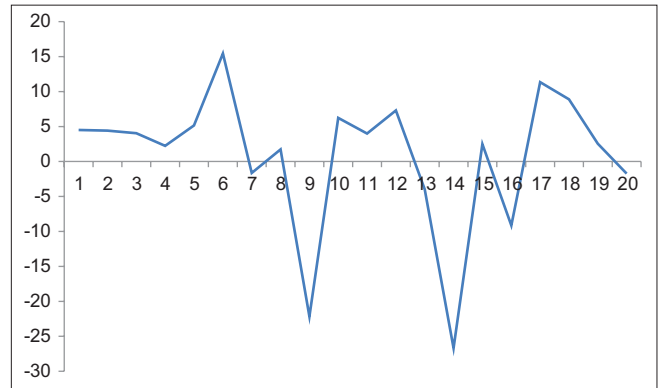
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Figure 5: Enzyme-linked immunosorbent assay kit-CAN-C-290



Graph 1: Percentage of change in the cortisol levels



Graph 2: Mean difference in the cortisol levels among the study group after using soft occlusal splint

**Table 1: Comparison of mean cortisol levels**

Pair 1	Descriptive statistics			
	Mean	n	SD	SEM
Before	21.548472	20	8.1402835	1.8202227
After	20.799619	20	10.5274207	2.3540028

SD: Standard deviation; SEM: Standard error of mean

**Table 2: One-sample test**

Before and after giving soft occlusal splint	One-sample test			
	t	df	Significant (two-tailed)	Mean difference
Pre	4.680	19	0.000	8.5184724
Post	3.301	19	0.004	7.7696194

**Table 3: Mean difference among the study group**

Pair 1	Paired samples test				
	Paired Differences		t	df	Significant (two-tailed)
	Mean	SD			
Before - after	0.7488530	10.1437310	0.330	19	0.745

SD: Standard deviation

concluded that bruxism may show ongoing stress in normal work life. In contrast, Nakata *et al.* examined the relationship between psychological job stress and SB in a Japanese population of 1944 male and 736 female factory workers found that SB was weakly associated with some aspects of job stress in men.<sup>[19]</sup> Soft splints, which are more convenient for patients than hard splints, can be used immediately after provisional diagnosis.<sup>[20]</sup> The rationale for using soft splints is that the soft resilient material may help in distributing the heavy load associated with parafunctional habits.<sup>[21]</sup> Soft occlusal splints have been considered as the first-line strategy for treating dental grinding noise and tooth wear in SB.<sup>[8]</sup> In general, the design of the device is simple; it covers the whole maxillary or mandibular dental arch and is well tolerated by the patient.<sup>[8]</sup> They should be replaced after 4–6 months as they lose their resilience with the passage of time.<sup>[22]</sup> By using occlusal splint, the patient is obliged to place his mandible in a new posture, thus resulting in a new muscular and

articular balance. This prevents the patient from clenching his teeth, which protects his TMJ and teeth. The forces generated during bruxism can be as much as six times the maximal force generated by normal chewing. The splints distribute these forces across the masticatory system. These appliances can decrease the frequency of bruxing episodes but not the intensity.<sup>[22]</sup> However, the effect of soft occlusal splint is questionable by some researchers.<sup>[9,13]</sup> Cortisol is a hormone produced by fascicular zone of the adrenal cortex, belonging to the glucocorticosteroidal group of hormones. Its secretion is controlled by the adrenocorticotropic hormone, produced in the pituitary gland, and indirectly by corticotrophin-releasing hormone of the hypothalamus. The secretion of cortisol is subject to a diurnal rhythm, and the highest concentration can be observed in the morning hours, around 9 o'clock. It is also considered as a “stress hormone” because in stressful situations, its levels in the blood and the saliva considerably raise.<sup>[23]</sup> In general, cortisol levels are marked in the blood, but it is also possible to mark the level of this hormone in other biological specimens, such as urine and saliva. It was discovered that there is a close correlation between blood cortisol levels and nonstimulated saliva. Saliva collection is considered to be an easy, noninvasive, and effective method, as salivary cortisol levels show a high correlation with serum cortisol levels. Moreover, cortisol in serum is known to be rapidly transferred to saliva (within 5 min) and is not affected by salivary flow rate.<sup>[24]</sup> Therefore, for the purpose of this study, measurement of salivary cortisol levels was selected as the most appropriate method for properly assessing stress. In the present study, the effect of soft occlusal splint in patients with bruxism was evaluated using salivary cortisol as a biomarker. A total of 20 individuals with bruxism were selected after history and clinical examination. Saliva sample of 20 patients were collected at two different intervals. The method of collecting saliva was spitting method which is in accordance with the studies done before in which they had evaluated four traditional assessment methods (suction, swab, spit, and draining) among the recruited individuals. The spit method required the individual to pool saliva in

the mouth and then expectorate into airtight containers. Previous studies have shown that swab method was unreliable and suction method had the highest test reliability, but consistently yielded more saliva volume than the spit or draining methods. Because of simplicity, the spit method was preferable to the draining method.<sup>[25]</sup> After collection of saliva samples by spitting method in a sterile microcentrifuge tube, it was stored in a refrigerator at  $-80^{\circ}\text{C}$ . Based on the previous study done by Garde and Hansen in 2009, long-term storage of saliva at room temperature was not recommended. However, no effects on cortisol concentrations were found after storage of saliva at  $5^{\circ}\text{C}$  for up to 3 months or at  $-20^{\circ}\text{C}$  and  $-80^{\circ}\text{C}$  for up to 1 year.<sup>[26]</sup> Repeated cycles of freezing and thawing did not appear to affect the concentrations of cortisol. Therefore, saliva was stored at  $-80^{\circ}\text{C}$  till it was analyzed. In the present study, after collecting saliva, an alginate impression was made on the mandibular arch and it was poured immediately with dental stone. With a Sta-Vac vacuum machine, a 2 mm thick, thermoplastic polyethylene sheet was adapted to the mandibular cast and then the outline of the sheet was trimmed. Manns *et al.*<sup>[27]</sup> showed that splints that increased vertical dimension 4.4 mm and 8.2 mm were more effective in producing muscular relaxation in patients with bruxism and myofascial pain dysfunction patients than >2-mm splints. Piper<sup>[28]</sup> suggested a 12- to 15-mm distance (incisal edge to incisal edge) to decrease clenching efficiency. These studies suggest that a minimum of 2-mm increase in vertical dimension is necessary to protect bruxing patients. If the patient is wearing a splint of 2 mm in thickness and still experiences muscular soreness, headache, and/or facial muscle tightness immediately after waking, splint thickness should be increased incrementally until symptoms disappear, indicating the appropriate splint thickness has been determined. Therefore, in this study, 2-mm soft occlusal splint was used. After fabrication of soft occlusal splint patient was instructed to wear soft splint at night for a period of 15 days. Its efficacy in reducing the number of masticatory episodes per hour of sleep seems to be transient, with a maximal effect observed during first 2 weeks, and returning to baseline after long period of use.<sup>[8]</sup> Hence, in the present study after 15 days, individuals were recalled and saliva samples were collected and stored at  $-80^{\circ}\text{C}$  till it was analyzed. On the day of assay, the saliva samples were prepared by centrifugation at 7000 rpm for 10 min and then analyzed for cortisol levels. Stored saliva samples were subjected to CAN-C-290 ELISA KIT biomarker cortisol (Supplied by Diagnostics Biochem Canada Inc, CORTISOL SALIVA ELISA KIT, Version: 4.1) test to detect the salivary cortisol levels. In this study, the link connecting SB activity and soft occlusal splint was investigated using levels of cortisol in saliva, which is a biomarker for assessment of stress level. The results indicate that intragroup comparison of the mean cortisol level in the study group shows near significant values, which means there is a decrease in the salivary cortisol

levels in patients after using soft occlusal splint, but statistically nonsignificant. Earlier investigations studying the relationship between bruxism and perceived stress through the estimation of stress-related biomarkers (cortisol and  $\alpha$ -amylase) in saliva reported that patients with bruxism showed higher levels of cortisol than normal individuals. On the contrary, salivary  $\alpha$ -amylase levels were not significantly different in patients with bruxism and normal individuals. These findings suggest that SB activity was related to higher levels of perceived psychological stress and salivary cortisol.<sup>[29]</sup> Furthermore, another study done by Miletic *et al.*<sup>[30]</sup> showed that patients with SB have higher levels of salivary cortisol. The findings of the present study are in agreement with previous investigations that showed that SB patients experienced higher levels of perceived psychological stress. Results in percentage of change in the cortisol levels in 70% of individuals after using the soft occlusal splint showed decreased cortisol levels and 30% of individuals after using the soft occlusal splint showed increased cortisol levels. Increase in cortisol levels in 30% of individuals inspite of using soft occlusal splints could have been due to anxiety or negative mood changes<sup>[31,32]</sup> of patients during saliva sample collection. Therefore, based on the results, soft occlusal splint could be one of the treatment modalities advised for a patient with bruxism to treat bruxism and reduce stress levels.

## Conclusion

It may be concluded that:

- There was a generalized increase in salivary cortisol level in patients with bruxism before using soft occlusal splint
- A decrease in salivary cortisol level was seen in 70% of the patients with bruxism after using soft occlusal splint.

Present findings might support the hypothesis that use of soft occlusal splint could be one of the treatment modalities advised for a patient with bruxism to treat bruxism and reduce stress levels.

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## Conflicts of interest

There are no conflicts of interest.

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