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박사학위 논문

Salivary cortisol of young adult patients with temporomandibular disorders

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천 찬 용

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측두하악장애를 가진 젊은 성인환자의 타액코티졸

2022년 2월 25일

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목 차

표 목 차

도 목 차

초록

측두하악장애를 가진 젊은 성인 환자의 타액 코티졸

천 찬 용
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연구목적: 젊은 성인 측두하악장애 환자와 대조군에서 타액코티졸 농도를 비교분석하여 측두하악장애의 스트레스와 연관된 원인요소를 알아보고자 하였다.

연구대상 및 연구방법: 2021년 6월 1일부터 8월 31일까지 조선대학교 치과병원에 내원한 측두하악장애로 진단된 젊은 성인 환자 32명과 대조군 34명을 대상으로 타액을 채취하였다. 타액코티졸 농도를 측정하기 위하여 ELISA(Enzyme-linked immunosorbent assay)를 시행하였다.

결과: 측두하악장애 환자군과 대조군에서 타액코티졸 농도는 두 군간의 유의한 차이를 보였다 ($p<0.05$). 측두하악장애의 임상증상 기간에 따른 타액코티졸 농도는 남성에서 유의한 차이를 보였다 ($p<0.05$). 느껴지는 스트레스(perceived stress)레벨에 따른 타액코티졸 농도는 미약과 중간정도라고 표현한 군에서 두 군간 유의한 차이를 보였다 ($p<0.05$). 이갈이나 이악물기와 관련된 두 군간의 타액 코티졸 농도는 유의한 차이가 없었다 ($p>0.05$).

결론: 측두하악장애 환자군과 대조군에서 타액코티졸 농도는 통계적으로 유의성을 나타내어 스트레스가 원인요소임을 알 수 있었다.

주제어: 타액 코티졸, 측두하악장애

I. Introduction

Temporomandibular disorders(TMDs) is a musculoskeletal disorder, and it mainly presents symptoms such as pain in the temporomandibular joint and masticatory muscles, joint noise, and mouth opening limitation. Occlusion, trauma, emotional stress, deep pain input, and parafunctional activity are known as the main causes [1]. In recent years, the prevalence of TMDs has been continuously increasing, and the prevalence is particularly high among young adults [1,2]. It is estimated that stress due to the complexity of modern social life is the main cause of such a situation, but it has not been clearly identified.

Stress affects disease through physiological responses to psychological stimuli. In general, an integrated biological response appears after exposure to stress, among which changes in the neuroendocrine system help to overcome the stress response and also affect the onset or course of disease. That is, various hormones are secreted to increase the body's resistance to stress factors, and when these reactions are changed, it is reported that many diseases are caused [3].

What is being used as a measure of hormonal activity against stress is catecholamine, cortisol, sexual hormone, thyroid hormone, growth hormone, and the like. Among them, cortisol is widely used in research as an important indicator that can best represent the changed physiological state in response to stress stimuli [4].

One way to measure stress-related hormones is to measure levels of the hormone in blood, urine, and saliva. Although measurements of hormones in the blood have been widely used, there are factors that stress such as venipuncture to obtain a blood sample. Therefore, recently, a lot of research using saliva has been conducted [3].

In a recent study on salivary cortisol in patients with TMDs, Kobayashi

et al. [5] reported that there was no difference in salivary cortisol concentrations between patients with TMDs and controls, but Saad Alresayes et al. [6] reported that adolescents with TMDs was found to have high salivary cortisol concentrations. The relationship between TMDs and stress has not been clearly elucidated yet.

Therefore, in this study, the causative factors related to the stress of TMDs were investigated through the analysis of salivary cortisol in young adults with TMDs.

II. Materials and Methods

1. Study subjects

From June 1, 2021 to August 31, 2021, 32 patients diagnosed with TMDs(16 males, 16 females) and 34 patients with no history of TMDs(12 men, 22 women) visited Chosun University Dental Hospital were the study subjects. Both the temporomandibular disorder patient group and the control group were studied with young adults in their late teens, 20s and 30s. This study was conducted with the approval of the IRB(Institutional Review Board) of Chosun University Dental Hospital (CUDHIRB-02103-010).

2. Study methods

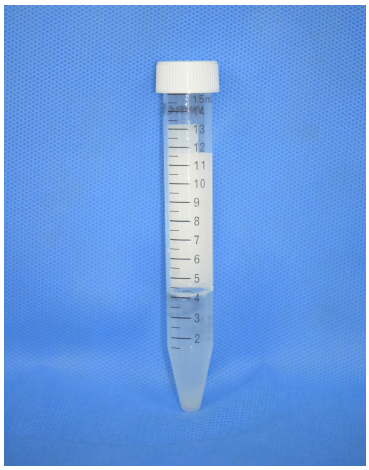
More than 2ml of non-stimulated saliva from the temporomandibular disorder patient group and control group was collected in an experimental tube(Fig.1). Saliva was collected between 3 and 5 pm in order to minimize the difference in salivary cortisol concentration during the day, and the collected saliva in the tube was immediately frozen at -20℃. The age and sex, perceived stress level, and history of bruxism and clenching were investigated in the experimental group and control group. The persistent symptoms of TMDs in the experimental group were classified into less than 3 months and more than 3 months to distinguish acute and chronic.

Recently, the degree of perceived stress was expressed in three stages: +(mild), ++(moderate), and +++(severe) and self-reported history of bruxism and clenching was investigated. Saliva was stored frozen for about 3 months until all samples were collected for salivary cortisol analysis. Salivary cortisol concentration was measured by enzyme-linked immunosorbent assay (ELISA), and for the experiment, it was requested to menopause health center (Leaders union Co. Ltd., Gyeonggi-do, Korea).

3. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 26.0 for Windows (IBM Co., Armonk, NY, USA). Mann-whitney U test and independent t-test were used to compare and analyze the salivary cortisol concentration of the temporomandibular disorder patient group and the control group and the salivary cortisol concentration between the two groups according to the presence or absence of bruxism and clenching. The Mann-whitney U test was used to compare and analyze the salivary cortisol concentration according to the duration of symptoms in the temporomandibular disorder patient group and the salivary cortisol concentration according to the perceived stress level in both groups.

Fig. 1. Salivary sample in plastic tube.



III. Results

1. Distribution of patients.

The average age of the temporomandibular disorder patient group was 28.25 years, and the control group was 25.97 years old. The gender ratio was 50% male and 50% female in the temporomandibular disorder patient group, and 35.3% male and 64.7% female in the control group (Table 1).

2. Comparison of concentration of salivary cortisol in the temporomandibular disorder patients and the control group.

There was statistically significant difference between the two groups in the temporomandibular disorder patients and the control group ($p<0.05$) (Table 2).

3. Comparison of concentration of salivary cortisol according to the symptom duration in the temporomandibular disorder patients.

There was a statistically significant difference ($p<0.05$) in salivary cortisol concentrations only in male, but there was no significant difference according to the symptom duration ($P>0.05$) (Table 3).

4. Comparison of concentration of salivary cortisol accoring to the perceived stress level in the temporomandibular disorder patients and the control group.

There was a statistically significant difference between the two groups in the group with mild and moderate perceived stress level ($p<0.05$) (Table 4).

5. Comparison of concentration of salivary cortisol according to the existence of bruxism/clenching in the temporomandibular disorder patients

and the control group.

There was no statistically significant difference between the two groups in the temporomandibular disorder patients and the control group ($P>0.05$) (Table 5).

Table 1. Demographic characteristics of the Subject.

Characteristic		Group	
		TMDs (n=32)	Control (n=34)
Sex	Male	16 (50%)	12 (35.3%)
	Female	16 (50%)	22 (64.7%)
	Total	32	34
Average age(y)	Male	28.81 ± 5.96	26.42 ± 2.14
	Female	27.69 ± 5.28	25.73 ± 2.61
	Total	28.25 ± 5.66	25.97 ± 2.48

TMDs, temporomandibular disorders; n, number; y, year.

Values are presented as number(%) or mean ± standard deviation.

Table 2. Comparison of concentrations of salivary cortisol between each group.

		Group		Total	p-value
		TMDs (n=32)	Control (n=34)	(n=66)	
Concentration	Male (n=28)	5.34 ± 3.55 (n=16)	3.32 ± 1.22 (n=12)	4.48 ± 2.98 (n=28)	0.009*
	Female (n=38)	6.13 ± 3.10 (n=16)	3.30 ± 0.71 (n=22)	4.49 ± 2.51 (n=38)	0.000*
	Total	5.74 ± 3.36	3.31 ± 0.92	4.49 ± 2.51	0.000*
	p-value	0.523	0.959	0.981	

TMDs, temporomandibular disorders; n, number.

Values are presented as mean ± standard deviation of cortisol concentrations(nmol/L). p-values were obtained by independent t-test and Mann-whitney U test.

*$p<0.05$

Table 3. Comparison of concentrations of salivary cortisol according to the symptom duration in the temporomandibular disorder patients

		Duration		p-value
		< 3 (n=14)	≥ 3 (n=18)	
Sex	Male (n=16)	6.29 ± 4.18 (n=10)	3.76 ± 0.66 (n=6)	0.030*
	Female (n=16)	3.82 ± 0.67 (n=4)	6.90 ± 3.21 (n=12)	0.096
	Total	5.59 ± 3.73	5.86 ± 3.03	0.830

TMDs, temporomandibular disorders; <3, less than 3months; ≥3, more than 3months; n, number.

Values are presented as mean ± standard deviation of cortisol concentrations(nmol/L). p-values were obtained by independent t-test and Mann-whitney U test.

*p<0.05

Table 4. Comparison of concentrations of salivary cortisol according to the perceived stress level in each group

		Group		Total	p-value
		TMDs (n=32)	Control (n=34)	(n=66)	
Stress	+ (n=36)	4.59 ± 2.06 (n=12)	3.36 ± 1.05 (n=24)	4.48 ± 2.98	0.027*
	++ (n=22)	6.28 ± 4.21 (n=13)	3.13 ± 0.47 (n=9)	4.49 ± 2.51	0.006*
	+++ (n=8)	6.71 ± 2.76 (n=7)	3.67 ± 0.00 (n=1)	4.49 ± 2.51	0.188

TMDs, temporomandibular disorders; n, number; +, mild; ++, moderate; +++, severe

Values are presented as mean ± standard deviation of cortisol concentrations(nmol/L). p-values were obtained by independent t-test and Mann-whitney U test.

*$p<0.05$

Table 5. Comparison of concentrations of salivary cortisol according to the existence of bruxism/clenching in each group

Group		Bruxium/clenching		Total (n=66)	p-value
		Yes (n=31)	No (n=35)		
TMDs	Male (n=16)	4.65 ± 1.37 (n=8)	6.04 ± 4.73 (n=8)	5.34 ± 3.55	0.834
	Female (n=16)	5.55 ± 2.96 (n=9)	6.71 ± 3.13 (n=7)	6.13 ± 3.10	0.876
	Total (n=32)	3.28 ± 0.65 (n=17)	3.32 ± 1.08 (n=15)	5.74 ± 3.36	0.678
Control	Male (n=12)	3.43 ± 0.74 (n=6)	3.21 ± 1.55 (n=6)	3.32 ± 1.22	0.423
	Female (n=22)	3.17 ± 0.54 (n=8)	3.37 ± 0.78 (n=14)	3.30 ± 0.71	0.551
	Total (n=34)	3.28 ± 0.65 (n=14)	3.32 ± 1.08 (n=20)	3.31 ± 0.92	0.909

TMDs, temporomandibular disorders; n, number; Yes, existence; No, none-existence.

Values are presented as mean ± standard deviation of cortisol concentrations(nmol/L). p-values were obtained by independent t-test and Mann-whitney U test.

*$p<0.05$

IV. Discussion

TMDs presents pain and dysfunction in the temporomandibular joint and masticatory muscles. It occurs mainly in the teens and 20s, and the prevalence increases with age, and it is reported that it is more common in women than men [1,2]. A recent domestic study also showed a sharp increase in the number of patients in their 20s, but the cause is not clearly known [2]. Therefore, in this study, although there are various causes of TMDs, salivary cortisol was analyzed for the group in their 20s in order to examine the relationship with stress (Table 1).

Many diseases are caused or exacerbated by stress. Diseases known to be caused by stress include tension type headaches, migraine, coronary artery disease, gastric and duodenal ulcers, irritable bowel syndrome, and various psychiatric disorders. In the orofacial area, recurrent aphthous ulcers, oral lichen planus, and TMDs are associated with stress [3].

In the stress response, activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis (HPA axis) plays a central role. The body secretes various hormones to increase resistance due to stress factors, and hormones such as catecholamine and glucocorticoid are best used in stress studies [7]. Acute stress stimulates the adrenal medulla through the activation of the pituitary gland, and secretes catecholamines such as epinephrine and norepinephrine to cause physiological responses. When stress continues, the pituitary gland secretes adrenocorticotropic hormone, which in turn secretes the glucocorticoid hormones(cortisol and aldosterone) from the adrenal cortex [3,8].

Cortisol is an important biomarker that can best indicate the physiological state changed in response to stress stimuli [9]. Cortisol secretion peaks around 8 am and lowest at midnight, and is secreted

spontaneously or by various biochemicals and psychological stimuli [7].

The study of neuroendocrine function is based on the measurement of hormones in the blood and urine. When blood is used for sampling, stress hormone levels can be increased only by the stress of blood collection itself. Therefore, the analysis of stress hormones using saliva is a very good tool for the study of stress-related diseases [10-12]. Since salivary hormone levels reflect blood hormone levels well, saliva has been widely used in recent studies. Storage of saliva samples is not complicated. Kahn et al. [13] found that storing the samples at room temperature for 2 weeks did not change the salivary cortisol levels. Garde and Hansen [14] reported that samples can be stored for up to 3 months at 5°C or at least 1 year at -20°C or -80°C when used for analysis of cortisol after storage. In this study, in order to minimize changes in salivary cortisol concentration during the day, more than 2ml of saliva was collected from a plastic tube between 3 and 5 pm and stored frozen at -20℃ until analysis (Fig. 1).

As the cause of TMDs, one factor that can affect masticatory function is the increase in emotional stress experienced by the patient. The activation of HPA-axis due to stress increases the activity of gamma efferent fibers through a complex neural pathway, causing the fibers in the muscle spindles to contract. The overall effect is to increase muscle tension [15]. In a study by A Da Silva Andrade et al. [14], salivary cortisol was higher in women with TMDs with elevated depression and somatization scores, and in a study by Ebtisam Salameh et al. [17] also reported that adult patients with TMDs had higher salivary cortisol concentrations than controls, and that females showed higher concentrations than males. This study also showed a statistical difference in the salivary cortisol concentration of the male and female temporomanidbular disorder patient group and the control group,

confirming that TMDs are related to psychological stress($p<0.05$)(Table 2).

The level of cortisol in saliva reflects the physiological response to mental stress [18]. The HPA axis is activated when the recipient feels threatened by an upcoming event with negative consequences. Bassett et al. [19] reported that the increased salivary cortisol level was maintained for 2 hours before speaking in front of an audience in a study, and Lehnert et al. [20] also reported that the salivary cortisol level was highest 10 minutes before public speaking.

Salivary cortisol is also increased by various kinds of physical stress or exercise. Stahl and Dörner [21] reported an increase in salivary cortisol after painful medical procedures such as prostatic biopsy and sternal puncture, and in a study by O'Connor and Corrigan,[22], 30 minutes of ergometer exercise at 75% VO_{2max} showed a marked increase in salivary cortisol. Interestingly, the cortisol response to repeated physical stress is not habituated.

In a study on salivary cortisol concentrations in patients with TMDs, Saad Alresayes et al. [6], Andrade et al. [16] and Ebtisam Solameh et al. [17] reported high salivary cortisol concentrations in patients with TMDs. However, in a study by Lambert et al. [23], the Chornic HPA axis response to stress was weak in patients with TMDs, and Jasim et al. [24] reported that salivary cortisol levels in women with chronic and acute orofacial pain were not different from those in the control group.

In this study, the statistical significance of salivary cortisol concentration was shown in male and mild and moderate groups in comparison according to the duration of TMDs symptoms and perceived stress level($P<0.05$)(Table 3,4). A comparison according to the duration of the TMDs symptoms showed a relationship of stress in the male group, but it is thought that a more detailed period setting and a study targeting a large number of subjects are needed in the future. In addition, it was

difficult to clearly understand the relationship between perceived stress level and changes in salivary cortisol concentration because there was no statistical significance in the group expressed as severe and it is thought that studies with various psychological tests are needed in the future.

Causes of temporomandibular disorders include parafunctional activities such as bruxism or teeth clenching. The increase in emotional stress not only increases the tension of the head and neck muscles, but also increases non-functional muscle activity such as bruxism and clenching [25]. Bruxism and clenching may be related to the development of TMDs or torus mandibularis, as they exhibit muscle hyperactivity beyond physiological tolerance in the orofacial area[26]. In a study of salivary cortisol related to bruxism, Mina Khayamzadeh et al. [27] reported that salivary levels were high in patients with parafunction habit. However, in this study, there was no statistical significance of salivary cortisol concentration in the group with and without bruxism or clenching($P>0.05$)(Table 5). This result is thought to be due to the various causes of bruxism or clenching.

In this study, salivary cortisol was analyzed in the young adult temporomandibular disorder patient group in order to investigate the causative factors related to stress in temporomandibular disorder. In the temporomandibular disorder patient group and control group, the salivary cortisol concentration showed statistical significance, suggesting that stress was the causative factor.

Although salivary cortisol concentration related to the clinical symptom duration of temporomandibular disorder, perceived stress level, and bruxism or clenching were not statistically significant between the two groups, it was found that salivary cortisol is a useful marker for stress-related studies.

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