

Original Research Article

Salivary cortisol levels and temporomandibular disorders – A systematic review and meta-analysis of 13 case-control studies

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Sent for review: 12 February 2022

Revised accepted: 25 May 2022

Abstract

Purpose: To compare salivary cortisol levels between patients with temporomandibular disorders (TMD) and healthy controls.

Methods: Pubmed, EMBASE, Web of Science and PsycINFO databases were employed to screen for the included studies. RevMan 5.3 software and RStudio software were used for meta-analysis, while a random-effect model was selected to synthesize the effect with the mean difference (MD).

Results: Thirteen studies were eligible and a total of 504 TMD patients and 410 controls were included. The pooled MD of salivary cortisol levels in TMD patients compared to controls was 0.05 (95 %CI = 0.01 - 0.09, $p = 0.02$), indicating a significantly higher level of salivary cortisol in TMD patients than in the controls. Subgroup analysis suggested studies published later than 2014 showed significant increase of salivary cortisol level in TMD patients when compared to controls (MD = 0.07, 95 % CI = 0.01-0.13, $p = 0.03$). Besides, high-quality studies presented significant differences with regard to the cortisol level in saliva among individuals with or without TMD (MD = 0.04, 95 %CI = 0.03-0.05, $p < 0.01$). However, the instability of the results showed by the sensitivity analysis was a hindrance to reaching a definitive conclusion.

Conclusion: The findings of this study indicate that salivary cortisol level in TMD patients is significantly higher than in controls. Consequently, supportive psychological treatment is recommended to prevent TMD patients from mood disorders. More high-quality studies are, however, needed to confirm the relationship, considering the high degree of heterogeneity among the studies.

Keywords: Salivary cortisol, Temporomandibular disorders, Mood disorders, Psychotherapy

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INTRODUCTION

Temporomandibular disorder (TMD) is an umbrella term for a heterogeneous series of musculoskeletal and neuromuscular disorders involving the temporomandibular joint (TMJ),

masticatory muscles and the related structures. With a prevalence of approximately 31 % in adults or the elderly and 11 % in children or adolescents, TMD is the most common reason for chronic orofacial pain of nondental origin [1]. The symptoms of TMD are joint noise, pain in the

joint area, restricted movement of the mandible, headache and tinnitus [2]. The research diagnostic criteria for temporomandibular disorders (RDC/TMD) and the diagnostic criteria for temporomandibular disorders (DC/TMD) are two accepted tools worldwide for diagnosing TMD [3]. The etiology of TMD is thought to be multifarious. Except for biological and social factors, psychological factors like anxiety, depression and stress may play important roles [4]. Individuals with anxiety are up to five times more prone to develop TMD, and stress seems to worsen the TMD symptoms [5].

Cortisol is a vital glucocorticoid hormone secreted by zona fasciculata of adrenal cortex. As the end product of the Hypothalamic-Pituitary-Adrenal axis (HPA axis), cortisol participates in the activation of anti-inflammatory and anti-stress processes, and is considered as the biological marker for stress and anxiety [6]. Plasma, serum, urine, hair and saliva are the candidate specimens for the measurement of cortisol level. Considering the number of published articles, this study focused on salivary cortisol because of its potential to be noninvasive and a readily available method for collection, and especially suitable for at-home test or large-scale epidemiological studies. Cortisol concentration in saliva has been studied in people with or without TMD, but with contradictory results [7,8], and there has been no meta-analysis targeted at salivary cortisol in TMD yet. This study was the first attempt to investigate the relationship between TMD and the perceived stress or anxiety determined by salivary cortisol levels. This present study aims to analyze the salivary cortisol level between individuals with or without TMD.

METHODS

Study design and registration

This systematic review was developed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) 2015 statement [9]. The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) database with number CRD42021288457.

Eligibility criteria

Based on the PRISMA guideline, the main research question was constructed according to the PECO strategy. Population refers to general population, and exposure refers to TMD. Healthy individuals without TMD were set as Control and

the outcome was salivary cortisol level in TMD patients as well as controls.

Inclusion criteria were as follows: (a) original articles published in scientific journals, (b) case-control, comparative cross-sectional or cohort studies written in English, (c) studies conducted in humans; (d) studies which used the research diagnostic criteria for temporomandibular disorders (RDC/TMD) or the diagnostic criteria for temporomandibular disorders (DC/TMD) for the diagnostic tool for TMD, and (e) studies which evaluated salivary cortisol levels in TMD patients and healthy controls with no less than 15 participants each.

Exclusion criteria were as follows: (a) studies that gave participants extra stimulations like exam stress; (b) studies that involved less than 15 participants in each group; (c) studies in which the participants underwent surgery, orthodontic treatment or other treatments like splints, medications and physiotherapy; (d) studies in which TMD diagnosis was not clarified; (e) studies that failed to provide prevalence data; (f) reviews, meeting abstracts, letters, books, case reports, opinion pieces and patents.

Information sources and search strategy

The databases including PubMed/MEDLINE, EMBASE, Web of Science and PsycINFO was searched up for potential eligible studies published up to October 2021 with key terms ("TMD" OR "Temporomandibular disorder" OR "Temporomandibular joint dysfunction" OR "orofacial pain" OR "Temporomandibular joint disorder") AND ("cortisol" OR "HPA axis") AND ("salivary" OR "saliva"). And manual searching was conducted according to the reference list of included articles. EndNote X9 software was used for reference management and duplicates exclusion.

Selection of studies

Two researchers (LL and BY) independently evaluated the search results for completeness. Full texts of the articles which met the selection criteria were screened independently. Disagreements regarding the eligible studies were resolved through discussion with a third reviewer (MHL).

Data collection

Two reviewers (LL and BY) independently extracted the data using a predefined data extraction form, and all discrepancies were resolved by discussions with a third reviewer

(LMH). The following information were extracted for record: first author, year, country, title, type of study, sample characteristics (population, gender and age), TMD diagnostic criteria, saliva collection, storage and detection details, salivary cortisol levels in TMD patients and controls.

Risk of bias in individual studies

The methodological quality of each eligible study was evaluated by independent reviewers (LL and BY) following the Joanna Briggs Institute Critical Appraisal Checklist for case control studies, with score ≥ 8 being high quality, and all discrepancies were resolved by discussions with a third reviewer (LMH).

Data synthesis and statistical analysis

Review Manager (RevMan 5.3) (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, 2014) was used for statistical analysis. The random-effects model was chosen and mean differences (MD) of salivary cortisol level and its 95 % confidence intervals (CIs) were calculated as the main outcome, and the mean (range) or median (quartile) was estimated to mean (SD). The unit of measurement of salivary cortisol was $\mu\text{g/dL}$. The Z-test showed the significance of pooled MD, and Inconsistency index (I^2) was used to perform the quantitative analysis of heterogeneity. Heterogeneity was determined to be $I^2 > 50\%$, and P -value of χ^2 test were also considered. Subgroup analysis was performed as a result of high heterogeneity, dividing studies into groups according to the publication year and the quality of the study. Rstudio Desktop software (Rstudio Inc.) was used for evaluating publication bias, and using funnel plot and Egger's test. Sensitivity analysis was applied to identify the stability of the results. Statistical significance was assumed at p -value < 0.05 .

RESULTS

Selection of studies

The search identified a total of 248 studies with 90 duplicates. One hundred and fifty-eight articles were directed to the reading of the title and abstract by two independent researchers (LL and BY) and 125 were excluded for not meeting the eligibility criteria, leaving 33 studies for full text reading. Thereafter, several articles were discarded: 6 articles failed to present healthy control groups; 3 studies included TMD patients less than 15; 2 studies did not diagnose TMD with RDC/TMD or DC/TMD; 5 studies gave extra stimulation or treatment to the participants; 4

studies did not record the salivary cortisol level in TMD patients. Finally, 13 studies were included in this systematic review (Figure 1).

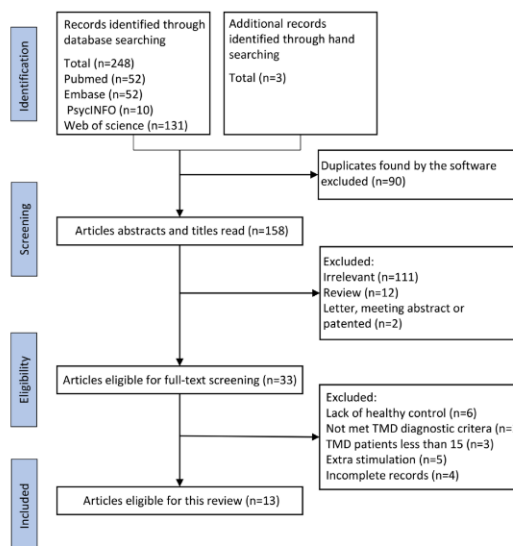


Figure 1: Flow chart of the study selection

Characteristics of studies

All the included studies were case-control studies which are published between 2008 and 2020. A total of 914 subjects (504 TMD patients and 410 controls) (244 males and 670 females; female-to-male ratio 2.75) with a mean age ranging from 10 [10] to 46 [11] years. The sample size ranged from 35 [12] to 145 [13]. All TMD patients were diagnosed with RDC/TMD or DC/TMD. The studies were conducted in eight countries: 5 [13-17] studies were conducted in Brazil, 2 [7,18] in Sweden, 1 in India [8], Syria [19], Norway [11], Croatia [12], Korea [20] and Thailand [21]. Table 1 summarizes the descriptive characteristics of the studies.

Risk of study bias

Using the Joanna Briggs Institute Prevalence Critical Appraisal Checklist for case control studies, the overall risk of bias was evaluated. Four studies were classified as moderate risk of bias while 9 were classified as low risk of bias. The detailed descriptions were shown in Table 2.

Data from individual studies

All studies were case-control studies. In 13 studies, 6 [8,11-13,19,21] indicated that TMD group had a significantly higher level of salivary cortisol than the controls, with MD varied from 0.05 [13] to 0.23 [19], while other 7 studies

Table 1: Characteristics of the studies involved in this meta-analysis (n = 13)

First Author, Year	Country	n (TMD/Ctl)	Mean age (TMD/Ctl)	Gender (Male/ Female)	Collection method	Measurement method	Sampling time (a.m.)	TMD diagnosis	S
Almeida 2014	Brazil	48(25/23)	21.80/21.26	12/36	Stimulated	Spectrophotometry	9:00 – 9:25	RDC/TMD	N
Andrade 2008	Brazil	40(20/10)	22.54/22.86	20/20	Stimulated	ELISA	7:00 – 8:00	RDC/TMD	N
Barbosa 2012	Brazil	145(86/59)	Matched (8-14)	49/96	Stimulated	ELISA	30min after awakening	RDC/TMD	Y
Goyal 2020	India	60(40/20)	Matched (18-30)	30/30	Unstimulated	ELISA	7:00 – 8:00	RDC/TMD	Y
Jasim 2014	Sweden	78(51/27)	44.37/45.7	0/78	Unstimulated	ELISA	7:45-12:15	RDC/TMD	N
Kobayashi 2017	Brazil	76(38/38)	10.63/10.63	48/28	Stimulated	ELISA	7:00; 7:30; 8:00	RDC/TMD	N
Nilsson 2010	Sweden	60(30/30)	19.75/21.2	0/60	Stimulated	Radioimmunoassay	Immediately upon waking	RDC/TMD	N
Salameh 2014	Syria	120(60/60)	Matched (19-44)	36/84	NA	ELISA	Awakening; 30min after; 60min after	RDC/TMD	Y
Staniszewski 2018	Norway	88(44/44)	44/46	12/76	Stimulated	Spectrophotometry	Morning	RDC/TMD	Y
Vrbanović 2018	Croatia	35(20/15)	39.30/34.33	0/35	Unstimulated	ELISA	Awakening; 30min and 60min after	DC/TMD	Y
Chinthakanan 2018	Thailand	45(21/23)	26.05/22.00	12/32	Unstimulated	ELISA	Morning	RDC/TMD	Y
Kyung B 2016	Korean	48 (30/18)	Matched (20-40)	0/48	Unstimulated	Radioimmunoassay	Awakening; 30min and 60min after	RDC/TMD	N
Moreira 2016	Brazil	72(39/33)	Matched (18-39)	25/46	Unstimulated	ELISA	9:00 – 10:00	RDC/TMD	N

Abbreviations: S = Significance; ELISA, enzyme-linked immunosorbent assay; NA, not available; N, no; Y, Yes; RDC/TMD, the research diagnostic criteria for temporomandibular disorders; DC/TMD, diagnostic criteria for temporomandibular disorders

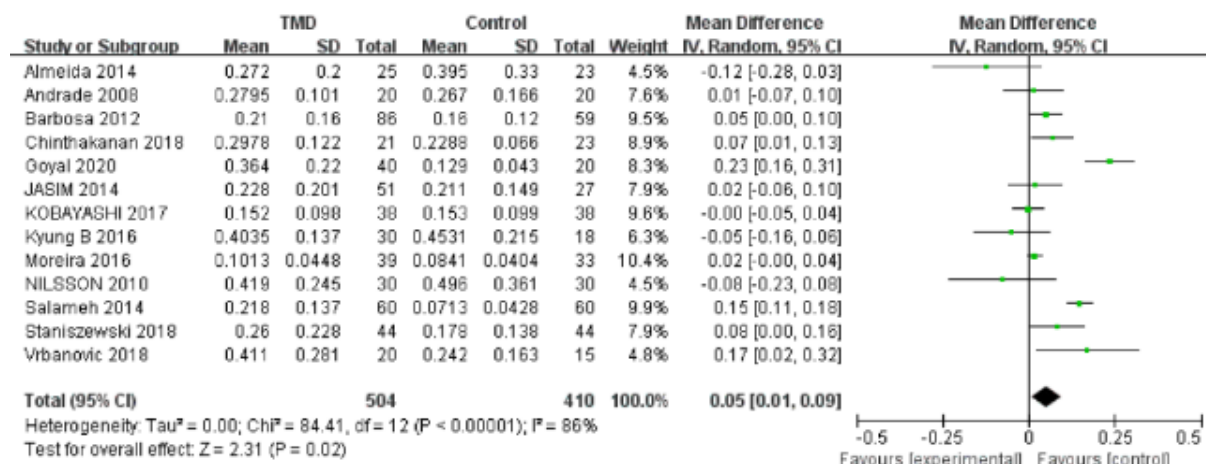
Table 2: JBI Critical Appraisal Checklist for case control studies

Variable	Almeida	Andrade	Barbosa	Goyal	Jasim	Kobayashi	Nilsson	Salameh	Staniszewski	Vrbano vi' c	Chinth akanan	Kyung B	Moreira
(1) Were the groups comparable other than the presence of disease in cases or the absence of disease in control?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
(2) Were cases and controls matched appropriately?	N	Y	N	Y	Y	Y	Y	Y	Y	Y	N	Y	N
(3) Were the same criteria used for identification of cases and controls?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
(4) Was exposure measured in a standard, valid and reliable way?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
(5) Was exposure measured in the same way for cases and controls ?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
(6) Were confounding factors identified?	N	Y	Y	Y	Y	N	Y	Y	N	N	N	N	Y
(7) Were strategies to deal with confounding factor stated?	N	Y	Y	Y	Y	N	Y	N	N	N	N	N	Y
(8) Were outcomes assessed in a standard, valid and reliable way for cases?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
(9) Was the exposure period of interest long enough to be meaningful?	N	N	U	U	Y	U	U	U	U	Y	U	Y	Y
(10) Was appropriate statistical analysis used?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Total number of "Y"	6	9	8	9	10	7	9	8	7	8	6	8	9
Quality assessment	Medium	High	High	High	High	Medium	High	High	Medium	High	Mediu m	High	High

Abbreviations: Y, yes; N, no; U, unclear

Table 3: Random-effects analysis of salivary cortisol level in TMD patients and the controls based on the publication year and the quality of the study

Parameter		N	MD (95%CI)	P-value	I ² (%)	P
Publication year	>2014	7	0.07 (0.01, 0.13)	0.03	87	<0.001
	≤2014	6	0.03 (-0.04, 0.09)	0.42	90	<0.001
Quality	High	9	0.04 (0.03, 0.5)	<0.001	90	<0.001
	Medium	4	0.03 (-0.00, 0.06)	0.09	66	0.03

**Figure 2:** Forest plot of random-effects analysis: salivary cortisol levels in TMD patients and the controls

showed no significant difference in cortisol level in saliva between TMD group and controls, with MD varied from -0.12 [15] to 0.02 [17]. Barbosa [13] and Kobayashi [10] focused on children and adolescents aged 7-14 years, while the other studies had adults as participants with the mean age ranging from 19 [7] to 46 [11]. 9 studies had both male and female subjects, among which female subjects were reported to have higher TMD prevalence, and a higher level of anxiety/depression and salivary cortisol than male subjects. Jasim [18], Nilsson [7], Kyung B [20] and Vrbanovic [12] only included female subjects. Except for salivary cortisol concentration, 11 studies, except for Andrade [16] and Vrbanovic [12], also studied the relationship between mood disorders and TMD or cortisol level, and the results showed that TMD patients had significantly higher scores for anxiety, depression, perceived stress or somatic symptoms than those of controls. Goyal [8] further verified that significantly higher value of salivary cortisol was detected in TMD patients with depression than TMD patients without depression and the control group. Six studies [8,12,17,18,20,21] collected saliva without extra stimulation, while 6 collected saliva stimulated by chewing the swab or cotton roll, and 1 [19] was unclear. Most studies evaluated the cortisol level using an ELISA kit, except for Almeida [15] and Staniszewski [11] which used spectrometry, as well as Nilsson [7] and Kyung B [20] which used radioimmunochemical assay. Seven studies [7]

[11,15,17-19,21] only collected saliva samples in the morning and the others collected samples both in the morning and at night. In this systematic review, only records in the morning were calculated considering the secretion rhythm of cortisol.

Synthesis of results

A total of 13 studies were included for meta-analysis. The pooled MD was of salivary cortisol levels in TMD patients compared to controls was 0.05 (95 % CI = 0.01 - 0.09, $p = 0.02$) though high statistical heterogeneity existed between the studies ($I^2 = 86%$) (Figure 2). In order to explore the statistical heterogeneity, subgroup analysis was done using three terms: the publication year and the quality of the studies (Table 3). There was a significant difference in the studies published later than 2014 (MD = 0.07, 95%CI = 0.01-0.13, $p = 0.03$), but not in studies reported earlier than or in 2014. In high-quality studies, cortisol level of TMD patients was significantly higher than the controls (MD = 0.04, 95%CI = 0.03-0.05, $p < 0.01$), while no significant difference was found in medium-quality studies. These results showed that publication year and quality of the study may contribute to the statistical heterogeneity.

As to publication bias, Figure 3 presents the funnel plot. The p -value for Egger's tests is 0.774, which confirm the absence of publication

bias across the studies. The sensitivity analysis was done by removing one study per time and the cumulative analysis was done (Figure 4). The graph showed the instability of the results, indicating the need of more high-quality studies for definitive conclusions.

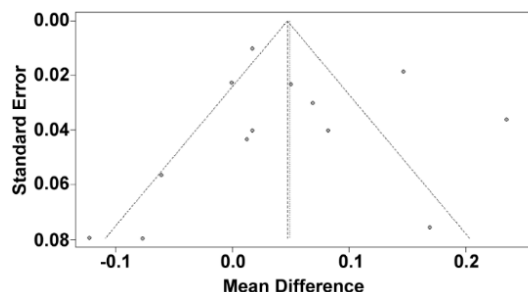


Figure 3: Funnel plot of random-effects analysis: salivary cortisol levels in TMD patients and controls

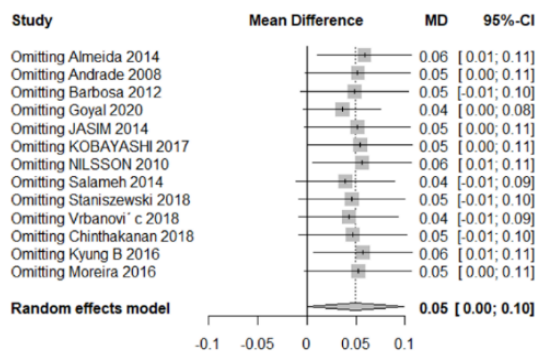


Figure 4: Sensitive analysis shown in forest plot by omitting one study each time

DISCUSSION

This systematic review compared the salivary cortisol level in individuals with or without TMD, and the results showed that TMD patients had a significantly higher level of salivary cortisol than the controls. The possible relationship between increased cortisol level in saliva and TMD patients might be due to its etiology. Mental disorders like anxiety and depression are believed to be potential causes of TMD symptoms and they might contribute to initiating TMD as well as its perpetration [4]. The psychosocial comorbid conditions also predicted a negative response to classical therapy [22]. The underlying mechanisms between TMD and psyche is still unclear. One explanation was the dysregulation of the HPA axis, resulting in the production of excessive level of stress hormones like cortisol and catecholamines [23].

Another possible reason was the alteration of the threshold of pain perception by stress, thus

increasing the fatigue and tightness of the masticatory muscle and subsequently initiated the TMD [22]. Many studies have been conducted to explore the cortisol level in TMD patients and healthy controls, producing contradictory results. Among the 13 studies involved, 6 published showed significantly higher level of salivary cortisol in subjects with TMD than the ones without, while 7 showed no significant difference. Though the pooled MD was 0.05 with p -value as 0.02, the instability of the results detected by the sensitivity analysis should be considered. Cortisol is a glucocorticoid hormone and the end product of HPA axis activation. The HPA axis is of great importance in physiological coping, and helps in mediating stress-related disease and its effects on health, mood and behavior [24]. The neurochemicals involved in the HPA axis were secreted in response to increased stress in the patient. Therefore, cortisol is used for evaluating an individual's response to stressful stimuli. Salivary cortisol has been widely used in researches due to its advantage of being easy-to-collect and relatively inexpensive. Compared to plasma cortisol, samples of salivary cortisol can be collected using noninvasive techniques, and subsequently can be timed without the support of a laboratory or health care institute, and this is suitable for daily detection or large-scale epidemiological investigation. But cortisol is highly variable and sensitive to a wide range of factors during the collection. For example, the secretion of cortisol has a known circadian rhythm, fluctuating in a predictable cycle during the day. Generally, the levels of cortisol peak approximately 20 - 30 min after waking in the morning, and decreases to half at mid-afternoon, reaching its lowest levels by midnight [25].

Therefore, standardized collection time for all subjects should be considered by researchers. The articles were included in this meta-analysis, and saliva collection time ranged from 7:00 a.m. to 12:15 a.m., which some just described as awakening or morning. The variation of the time for sample collection might have contributed to the heterogeneity of the study. Besides, subgroup analysis indicated that the publication year and the quality of the studies might be two explanations as to its heterogeneity. Pooled MD of studies published later than 2014 got a positive result, and the standardized and commercial collection as well as detection methods could be the possible reason. High-quality studies also tended to have significant difference compared to the medium-quality ones, because in most high-quality studies, the confounding factors were identified, and enough

exposure time to TMD was required (6 months at least).

There were also some limitations in this study. A total of 914 individuals were included, which was not that sufficient due to the limited amounts of participants included in every study, ranging from 35 [12] to 145 [13]. Besides, Barbosa [13] and Kobayashi [10] included children and adolescents aged 7-14, while the others all had adults as subjects. Whether age contributes to the statistical heterogeneity is worth being explored, but there were insufficient number of articles which studied children and adolescents available for subgroup analysis. Females were believed to have higher salivary cortisol level than men, so the matched number of each gender involved in the TMD group and the control group was of great importance. Four studies [13,15,17,21] failed to provide same female-to-male ratio in two groups, which might also affect the homogeneity of the results. In spite of the shortcomings of the study, this study was the first attempt to explore salivary cortisol levels in TMD patients and controls with meta-analysis, and our results advocate for more focus on the emotional disorders of TMD patients. This study also indicated that with the improvement of collection and assessment methods, salivary cortisol had great prospects for development.

CONCLUSION

This meta-analysis demonstrates that TMD patients tend to have higher salivary cortisol level than healthy controls, which is probably linked to exposure to perceived stress, anxiety, depression and somatic symptoms. Therefore, supportive psychological treatment is suggested in therapy for TMD patients. Considering the high heterogeneity across the studies and instability of the results, more high-quality studies with bigger sample size are required to confirm the relationship.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors met the criteria for authorship established by the International Committee of Medical Journal

Editors. Lin Lu was responsible for designing, drafting and critically revising the manuscript. Bo Yang and Menghuan Li assisted to data analysis, systematic search strategies development and revised the manuscript. Baicheng Bao contributed to the conception and design and critically revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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