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Exploring the Potential Clinical Applications of Salivary Cortisol in the Diagnosis and Management of Cushing's Syndrome, Diabetes, Depression, and Periodontal Disease: A Systematic Review

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Table S1. PRISMA 2020 checklist.

Section and Topic	Item #	Checklist Item	Location where Item is Reported
TITLE			-
Title	1	Identify the report as a systematic review.	Title
ABSTRACT			-
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	-
INTRODUCTION			-
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Initial introduction
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	End of introduction
METHODS			-
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Dedicated section in M&M
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Dedicated section in M&M
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Dedicated table

Section and Topic	Item #	Checklist Item	Location where Item is Reported
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Dedicated section in M&M
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Dedicated section in M&M
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (*e.g.* for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Dedicated section in M&M
	10b	List and define all other variables for which data were sought (*e.g.* participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Dedicated section in M&M
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	4 reviewers assessed the risk of bias – specified in M&M
Effect measures	12	Specify for each outcome the effect measure(s) (*e.g.* risk ratio, mean difference) used in the synthesis or presentation of results.	Mean difference (M&M)
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (*e.g.* tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Type of intervention
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Procedure described M&M
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Procedure described M&M
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Answer to PICO
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (*e.g.* subgroup analysis, meta-regression).	N/A
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	N/A
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	N/A
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Reported conclusions of the included studies
RESULTS			-
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Dedicated table
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Dedicated table
Study characteristics	17	Cite each included study and present its characteristics.	Dedicated table
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Dedicated table
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (*e.g.* confidence/credible interval), ideally using structured tables or plots.	Dedicated table
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Dedicated table
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (*e.g.* confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	N/A
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Dedicated table
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Dedicated table
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Dedicated table
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Dedicated table
DISCUSSION			-

Section and Topic	Item #	Checklist Item	Location where Item is Reported
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Followed
	23b	Discuss any limitations of the evidence included in the review.	Followed
	23c	Discuss any limitations of the review processes used.	Followed
	23d	Discuss implications of the results for practice, policy, and future research.	Followed
OTHER INFORMATION			-
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	DOI No 10.17605/OSF.IO/WFEP4
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	OSF
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	N/A
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	None
Competing interests	26	Declare any competing interests of review authors.	None
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	N/A

Table S2. Search strategies for electronic databases.

Database	Search Strategy
PubMed (MEDLINE)	#1 “Cushing Syndrome” [MESH] OR (Syndrome, Cushing) OR (Cushing's Syndrome) OR (Syndrome, Cushing's) OR (Hypercortisolism) #2 “Diabetes Mellitus, Type 2” [MESH] OR (Diabetes Mellitus, Noninsulin-Dependent) OR (Diabetes Mellitus, Ketosis-Resistant) OR (Diabetes Mellitus, Ketosis Resistant) OR (Ketosis-Resistant) OR (Diabetes Mellitus) OR (Diabetes Mellitus, Non-Insulin Dependent) OR (Diabetes Mellitus, Non-Insulin-Dependent) OR (Non-Insulin-Dependent Diabetes Mellitus) OR (Diabetes Mellitus, Stable) OR (Stable Diabetes Mellitus) OR (Diabetes Mellitus, Type II) OR (NIDDM) OR (Diabetes Mellitus, Noninsulin Dependent) OR (Diabetes Mellitus, Maturity-Onset) OR (Diabetes Mellitus, Maturity Onset) OR (Maturity-Onset Diabetes Mellitus) OR (Maturity Onset Diabetes Mellitus) OR (Noninsulin-Dependent Diabetes Mellitus) OR (Noninsulin Dependent Diabetes Mellitus) OR (Maturity-Onset Diabetes) OR (Diabetes, Type 2) OR (Diabetes Mellitus, Adult-Onset) #3 “Hydrocortisone” [MESH] OR (Cortisol) OR (Epicortisol) #4 “Saliva” [MESH] OR (Salivas) #5 “Biomarkers” [MESH] OR (Biological Markers) OR (Biomarker) OR (Serum Markers) OR (Clinical Markers) OR (Biochemical Markers) OR (Laboratory Markers) #6 “Depression” [MESH] OR (Depressive Symptoms) OR (Depressive Symptom) or (Emotional Depression) OR (Depression, Emotional) #7 “Periodontal diseases” [MESH] OR (Disease, Periodontal) OR (Diseases, Periodontal) OR (Periodontal Disease) OR (Parodontosis) OR (Pyorrhea Alveolaris) #8 #1 AND #4 AND #5 #9 #2 AND #4 AND #5 #10 #3 AND #4 AND #7
SCOPUS	#1 “Cushing Syndrome” [MESH] OR (Syndrome, Cushing) OR (Cushing's Syndrome) OR (Syndrome, Cushing's) OR (Hypercortisolism) #2 “Diabetes Mellitus, Type 2” [MESH] OR (Diabetes Mellitus, Noninsulin-Dependent) OR (Diabetes Mellitus, Ketosis-Resistant) OR (Diabetes Mellitus, Ketosis Resistant) OR (Ketosis-Resistant) OR (Diabetes Mellitus) OR (Diabetes Mellitus, Non-Insulin Dependent) OR (Diabetes Mellitus, Non-Insulin-Dependent) OR (Non-Insulin-Dependent Diabetes Mellitus) OR (Diabetes Mellitus, Stable) OR (Stable Diabetes Mellitus) OR (Diabetes Mellitus, Type II) OR (NIDDM) OR (Diabetes Mellitus, Noninsulin Dependent) OR (Diabetes Mellitus, Maturity-Onset) OR (Diabetes Mellitus, Maturity Onset) OR (Maturity-Onset Diabetes Mellitus) OR (Maturity Onset Diabetes Mellitus) OR (Noninsulin-Dependent Diabetes Mellitus) OR (Noninsulin Dependent Diabetes Mellitus) OR (Maturity-Onset Diabetes) OR (Diabetes, Type 2) OR (Diabetes Mellitus, Adult-Onset) #3 “Hydrocortisone” [MESH] OR (Cortisol) OR (Epicortisol) #4 “Saliva” [MESH] OR (Salivas) #5 “Biomarkers” [MESH] OR (Biological Markers) OR (Biomarker) OR (Serum Markers) OR (Clinical Markers) OR (Biochemical Markers) OR (Laboratory Markers) #6 “Depression” [MESH] OR (Depressive Symptoms) OR (Depressive Symptom) or (Emotional Depression) OR (Depression, Emotional) #7 “Periodontal diseases” [MESH] OR (Disease, Periodontal) OR (Diseases, Periodontal) OR (Periodontal Disease) OR (Parodontosis) OR (Pyorrhea Alveolaris) #8 #1 AND #4 AND #5 #9 #2 AND #4 AND #5 #10 #3 AND #4 AND #7

Table S3. Summary table of studies excluded in this systematic review.

Excluded Studies	Exclusion Reasons
Pires *et al.*, 2022 [1]	Systematic review and meta-analysis
Bargues-Navarro *et al.*, 2022 [2]	Systematic Review
Špiljak *et al.*, 2022 [3]	Narrative Review
Botelho *et al.*, 2018 [4]	Systematic Review
Neupane *et al.*, 2022 [5]	Systematic Review
Song *et al.*, 2023 [6]	Narrative Review
Noh *et al.*, 2022 [7]	Systematic Review
Bastin *et al.*, 2018 [8]	Narrative Review
Boroumand *et al.*, 2021 [9]	Narrative Review
Findling *et al.*, 2017 [10]	Narrative Review
Zhang *et al.*, 2013 [11]	Systematic Review
Choromańska *et al.*, 2015 [12]	Narrative Review

Table S4. Criteria for judging risk of bias in the "Risk of bias" assessment tool.

Random Sequence Generation	
Criteria for a judgement of 'Low risk' of bias.	The investigators describe a random component in the sequence generation process.
Criteria for the judgement of 'High risk' of bias.	The investigators describe a non-random component in the sequence generation process. Usually, the description would involve some systematic, non-random approach. Other non-random approaches happen much less frequently than the systematic approaches mentioned above and tend to be obvious. They usually involve judgement or some method of non-random categorization of participants.
Allocation Concealment	-
Criteria for a judgement of 'Low risk' of bias.	Participants and investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation.
Criteria for the judgement of 'High risk' of bias.	Participants or investigators enrolling participants could possibly foresee assignments and thus introduce selection bias.
Blinding	-
Criteria for a judgement of 'Low risk' of bias.	Any one of the following: - No blinding or incomplete blinding, but the review authors judge that the outcome is not likely to be influenced by lack of blinding; - Blinding of participants and key study personnel ensured, and unlikely that the blinding could have been broken; - No blinding of outcome assessment, but the review authors judge that the outcome measurement is not likely to be influenced by lack of blinding; - Blinding of outcome assessment ensured, and unlikely that the blinding could have been broken.
Criteria for the judgement of 'High risk' of bias.	Any one of the following: - No blinding or incomplete blinding, and the outcome is likely to be influenced by lack of blinding; - Blinding of key study participants and personnel attempted, but likely that the blinding could have been broken, and the outcome is likely to be influenced by lack of blinding; - No blinding of outcome assessment, and the outcome measurement is likely to be influenced by lack of blinding; - Blinding of outcome assessment, but likely that the blinding could have been broken, and the outcome measurement is likely to be influenced by lack of blinding.
Incomplete Outcome Data	-

Random Sequence Generation	
Criteria for a judgement of 'Low risk' of bias.	Any one of the following: - No missing outcome data; - Reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias); - Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; - For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; - For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes not enough to have a clinically relevant impact on observed effect size; - Missing data have been imputed using appropriate methods.
Criteria for the judgement of 'High risk' of bias.	Any one of the following: - Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups; - For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; - For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size; - 'As-treated' analysis done with substantial departure of the intervention received from that assigned at randomization; - Potentially inappropriate application of simple imputation.
Selective Reporting	-
Criteria for a judgement of 'Low risk' of bias.	Any one of the following: - The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way; - The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon).
Criteria for the judgement of 'High risk' of bias.	Any one of the following: - Not all of the study's pre-specified primary outcomes have been reported; - One or more primary outcomes is reported using measurements, analysis methods or subsets of the data (*e.g.*, subscales) that were not pre-specified; - One or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); - One or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis; - The study report fails to include results for a key outcome that would be expected to have been reported for such a study.

Table S5. Criteria for judging risk of bias in ROBINS-I assessment tool.

1. Reaching risk of bias judgements for bias due to confounding	
Low risk of bias (the study is comparable to a well-performed randomized trial with regard to this domain)	The investigators describe a random component in the sequence generation process.
Moderate risk of bias (the study is sound for a non-randomized study with regard to this domain but cannot be considered comparable to a well-performed randomized trial)	The investigators describe a non-random component in the sequence generation process. Usually, the description would involve some systematic, non-random approach. Other non-random approaches happen much less frequently than the systematic approaches mentioned above and tend to be obvious. They usually involve judgement or some method of non-random categorization of participants.
Serious risk of bias (the study has some important problems)	-
Critical risk of bias (the study is too problematic to provide any useful evidence on the effects of intervention)	Participants and investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation.
No information on which to base a judgement about risk of bias for this domain	Participants or investigators enrolling participants could possibly foresee assignments and thus introduce selection bias.
2. Reaching risk of bias judgements for bias in selection of participants into the study	-
Low risk of bias (the study is comparable to a well-performed randomized trial with regard to this domain)	Any one of the following: - No blinding or incomplete blinding, but the review authors judge that the outcome is not likely to be influenced by lack of blinding; - Blinding of participants and key study personnel ensured, and unlikely that the blinding could have been broken; - No blinding of outcome assessment, but the review authors judge that the outcome measurement is not likely to be influenced by lack of blinding; - Blinding of outcome assessment ensured, and unlikely that the blinding could have been broken.

Moderate risk of bias (the study is sound for a non-randomized study with regard to this domain but cannot be considered comparable to a well-performed randomized trial)	Any one of the following: - No blinding or incomplete blinding, and the outcome is likely to be influenced by lack of blinding; - Blinding of key study participants and personnel attempted, but likely that the blinding could have been broken, and the outcome is likely to be influenced by lack of blinding; - No blinding of outcome assessment, and the outcome measurement is likely to be influenced by lack of blinding; - Blinding of outcome assessment, but likely that the blinding could have been broken, and the outcome measurement is likely to be influenced by lack of blinding.
Serious risk of bias (the study has some important problems)	-
Critical risk of bias (the study is too problematic to provide any useful evidence on the effects of intervention)	Any one of the following: - No missing outcome data; - Reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias); - Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; - For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; - For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes not enough to have a clinically relevant impact on observed effect size; - Missing data have been imputed using appropriate methods.
No information on which to base a judgement about risk of bias for this domain	Any one of the following: - Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups; - For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; - For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size; - 'As-treated' analysis done with substantial departure of the intervention received from that assigned at randomization; - Potentially inappropriate application of simple imputation.
3. Reaching risk of bias judgements for bias in classification of interventions	-
Low risk of bias (the study is comparable to a well-performed randomized trial with regard to this domain)	Any one of the following: - The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way; - The study protocol is not available but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon).
Moderate risk of bias (the study is sound for a non-randomized study with regard to this domain but cannot be considered comparable to a well-performed randomized trial)	Any one of the following: - Not all of the study's pre-specified primary outcomes have been reported; - One or more primary outcomes is reported using measurements, analysis methods or subsets of the data (*e.g.*, subscales) that were not pre-specified; - One or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); - One or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis; - The study report fails to include results for a key outcome that would be expected to have been reported for such a study.

Table S6. Bias analysis using the ROBINS-I-tool for observational studies [13].

Reference First Author et al. Year	D1	D2	D3	D4	D5	D6	D7
[14] Bäcklund *et al.*, 2020							
[15] Lages *et al.*, 2019							
[16] Salehi *et al.*, 2019							
[17] Liu *et al.*, 2005							
[18] Pulopulos *et al.*, 2020							
[19] Mesa *et al.*, 2014							
[20] Bawankar *et al.*, 2018							
[21] Khan, 2020							
[22] Khan *et al.*, 2020							
[23] Garrahy *et al.*, 2021							
[24] Johar *et al.*, 2016							
[25] Hackett *et al.*, 2014							
[26] Hackett *et al.*, 2016							
[27] Fenol *et al.*, 2017							
[28] Obulareddy *et al.*, 2018							
[29] Naghsh *et al.*, 2019							
[30] Refulio *et al.*, 2013							
[31] Rahate *et al.*, 2022							
[32] Ueland *et al.*, 2021							
[33] Lin *et al.*, 2019							

Reference First Author et al. Year	D1	D2	D3	D4	D5	D6	D7
[34] Yonekura *et al.*, 2014	~	~	~	✓	✓	~	✓
[35] Develioglu *et al.*, 2020	✓	✓	✓	✓	✓	~	✓
[36] Mohamed *et al.*, 2022	~	✓	✓	✓	✓	~	✓

Note: Questions
D1: Bias due to confounding
D2: Bias due to selection of participants
D3: Bias in classification of interventions
D4: Bias due to deviations from intended intervention
D5: Bias due to missing data
D6: Bias in measurement of outcomes
D7: Bias in selection of the reported results
Possible Answers (1)Low risk of bias (the study is comparable to a well-performed randomized trial with regard to this domain): Green Symbol
(2)Moderate risk of bias (the study is sound for a non-randomized study with regard to this domain but cannot be considered comparable to a well-performed randomized trial): Yellow Symbol
(3)Serious risk of bias (the study has some important problems in this domain): Orange Symbol
(4)Critical risk of bias (the study is too problematic in this domain to provide any useful evidence on the effects of intervention): Red Symbol
(5)No information on which to base a judgement about risk of bias for this domain: No Symbol.

Table S7. Evidence of studies included in this systematic review.

Authors and Year of Publication	Study Design and Aim	Methods	Results	Conclusions
Bäcklund *et al.*, 2020 [14]	A 13-month prospective clinical study, to establish reference intervals for, and compare the diagnostic accuracy of, salivary cortisol and cortisone in late-night samples and after a low-dose (1 mg) dexamethasone suppression test (DST).	Saliva samples were collected at 8 and 23 h, and at 8 am, after a DST, from 22 patients with CS and from 155 adult reference subjects. Then were collected samples at 8 pm and 10 pm from 78 of the reference subjects. Salivary cortisol and cortisone were analyzed with liquid chromatography-tandem mass spectrometry.	The upper reference limits of salivary cortisol and cortisone at 11 pm were 3.6 nmol/L and 13.5 nmol/L, respectively. Using these reference limits, CS was detected with a sensitivity of 90% and specificity of 96% for cortisol, and a 100% sensitivity and 95% specificity for cortisone. After DST, cortisol and cortisone upper reference limits were 0.79 nmol/L and 3.5 nmol/L, respectively. CS was detected with 95% sensitivity and 96% specificity with cortisol, and 100% sensitivity and 94% specificity with cortisone. No differences in salivary cortisol or cortisone levels were found between samples collected at 10 and 11 pm.	Salivary cortisol and cortisone in late-night samples and after DST showed high accuracy for diagnosing CS, salivary cortisone being slightly, but significantly better.
Lages *et al.*, 2019 [15]	A 25-month retrospective study to analyze late-night salivary cortisol as a screening tool for Cushing's syndrome in the Portuguese population.	157 subjects were divided in 3 groups: 57 normal subjects, 39 with suspected and 31 with proven Cushing's syndrome. The functional sensitivity of the automated electrochemiluminescence assay is 0.018 µg/dL for salivary cortisol. The diagnostic cut-off level was defined by Receiver Operating Characteristic curve and Youden's J index.	2.5th - 97.5th percentile of the late-night salivary cortisol concentrations in normal subjects was 0.054 to 0.1827 µg/dL. Receiver Operating Characteristic curve analysis showed an area under the curve of 0.9881 ($p <$ 0.0001). A cut-off point of 0.1 µg/dL provided a sensitivity of 96.77% and specificity of 91.23%.	Late-night salivary cortisol has excellent diagnostic accuracy, making it a highly reliable, noninvasive, screening tool for outpatient assessment. Given its convenience and diagnostic accuracy, late-night salivary cortisol may be added to other traditional screening tests on hypercortisolism.

Authors and Year of Publication	Study Design and Aim	Methods	Results	Conclusions
Lages *et al*., 2019 [15]	A 25-month retrospective study to analyze late-night salivary cortisol as a screening tool for Cushing's syndrome in the Portuguese population.	157 subjects were divided in 3 groups: 57 normal subjects, 39 with suspected and 31 with proven Cushing's syndrome. The functional sensitivity of the automated electrochemiluminescence assay is 0.018 µg/dL for salivary cortisol. The diagnostic cut-off level was defined by Receiver Operating Characteristic curve and Youden's J index.	There was a significant correlation between late night salivary cortisol and late-night serum cortisol (R = 0.6977; $p < 0.0001$) and urinary free cortisol (R = 0.5404; p = 0.0025) in proven Cushing's syndrome group. In our population, the late-night salivary cortisol cut-off was 0.1 µg/dL with high sensitivity and specificity.	Late-night salivary cortisol has excellent diagnostic accuracy, making it a highly reliable, noninvasive, screening tool for outpatient assessment. Given its convenience and diagnostic accuracy, late-night salivary cortisol may be added to other traditional screening tests on hypercortisolism.
Salehi *et al*., 2019 [16]	A 1-year retrospective study to compare salivary cortisol level in type 2 diabetic patients and pre-diabetics with healthy people.	132 patients were divided into 3 groups (44 with type 2 diabetes, 44 pre diabetic people, and 44 healthy subjects) and their salivary samples were collected. The samples were transferred to the laboratory, and salivary cortisol level was measured using ELISA. Data were analyzed using SPSS 22 and Chi 2 tests.	The mean salivary cortisol level in type 2 diabetic patients was 3.14 ± 1.17, in pre-diabetic cases was 1.83 ± 0.68, and in healthy controls was 0.86 ± 0.43 ($p < 0.001$). The mean DMFT in type 2 diabetic patients was 19.6 ± 6.5, in the pre-diabetic group was 13.43 ± 4.5, and in healthy controls was 9.38 ± 3.72 ($p < 0.001$).	Salivary cortisol level in type 2 diabetic patients is more than pre-diabetic people, and in pre-diabetic people is more than healthy people. Also, there was a significant relation between salivary cortisol level and DMFT index.
Liu *et al*., 2005 [17]	A 1-year retrospective study to evaluate late-night salivary cortisol (LNSC) levels in elderly male veterans with and without diabetes.	154 participants with type 2 diabetes were recruited for the "case" group, while 52 participants without diabetes were recruited for the "control" group. Participants underwent outpatient LNSC (23:00 h) testing. Participants with elevated LNSC (≥ 4·3 nmol/l) underwent secondary testing, including 24-h urine free cortisol (24UFC, > 60 µg/day) and dexamethasone suppression testing (DST, serum cortisol > 50 nmol/l). Participants with positive secondary testing had a morning ACTH level analyzed and either pituitary or adrenal imaging performed.	141 diabetics and 46 controls returned samples (91% overall). Average LNSC levels (nmol/l) in diabetics were significantly higher than in non-diabetics. 31 participants required secondary testing. 79% of participants who underwent secondary testing had normal 24UFC and DST. No cases of CS have been diagnosed to date. Increasing age, current diabetes mellitus and elevated blood pressure were associated with abnormal LNSC results.	LNSC has been shown to be sensitive and specific in diagnosing CS in certain high-risk populations, primarily the young and middle-aged. The development of age- and comorbidity-adjusted thresholds may be warranted for LNSC testing in elderly subjects and in those with significant comorbidity.
Pulopulos *et al*., 2020 [18]	A randomized prospective clinical study, to investigate whether manipulation of expectancy (High *vs*. Low expectancy) affects the cortisol response to stress.	52 women in young adulthood anticipated and performed a laboratory-based stress task after receiving positive or negative feedback on their abilities to deal with stressful events. Heart rate variability and salivary cortisol were assessed throughout the experimental protocol.	Participants receiving positive bogus feedback (*i.e*., High Expectancy group) showed a more positive anticipatory cognitive stress appraisal and they showed a lower cortisol response to stress. Moreover, a more positive anticipatory cognitive stress appraisal was associated with better anticipatory stress regulation (indexed as less decrease in heart rate variability), leading to a lower cortisol response.	The results of this study indicate that people with positive expectancy initiate mechanisms of anticipatory stress regulation that enhance the regulation of the physiological stress response. Expectancy and anticipatory stress regulation may be key mechanisms in the development and treatment of stress-related disorders.
Mesa *et al*., 2014 [19]	A 4-month retrospective study to investigate the association between stress and periodontitis by determining stress biomarkers in saliva and urine and to determine whether oral hygiene, gingival inflammation, and tooth loss are correlated with stress biomarkers in patients with periodontitis.	77 patients divided in 41 cases and 36 controls participated in this study. Periodontal examination findings included probing depth, clinical attachment loss, bleeding on probing (BOP), plaque index (PI), and tooth loss. Secretory immunoglobulin (sIg)A and cortisol were determined in saliva. Cortisol, creatinine-adjusted cortisol, metanephrine, normetanephrine, and total metanephrines were measured in urine.	Urinary metanephrine and total metanephrine levels were higher in the case group. In cases, salivary cortisol was correlated with PI, BOP, and tooth loss. Urinary metanephrine levels above the median were associated with a 3.4-fold higher risk of periodontitis, with an 82% increase in risk for each increment of 0.05 µg/24 hours. Urinary total metanephrine levels above the median were associated with a five-fold higher risk of periodontitis.	The results of this study offer new evidence of the association between urinary concentrations of catecholamine metabolites (metanephrine and total metanephrines) and chronic periodontitis. Salivary IgA level showed no statistical difference between the cases and controls. Salivary cortisol levels in the patients with periodontitis were correlated with worse PI, higher gingival inflammation, and greater tooth loss.

Authors and Year of Publication	Study Design and Aim	Methods	Results	Conclusions
Bawankar *et al.*, 2018 [20]	A 10-month prospective clinical study to evaluate the effects of stress, salivary and serum, cortisol and interleukin-1β levels in smokers with CP.	75 patients were divided in 3 groups, healthy controls (Group I), smokers and non-smokers with CP (Group 2 and Group 3) respectively were evaluated for clinical parameters, biochemical parameters of salivary and serum cortisol and IL-1β levels *via* enzyme linked immunosorbent assay (ELISA). Zung's self-rating depression scale questionnaire was used to determine the stress levels amongst the patients.	Smokers with CP exhibited higher values of probing pocket depth, clinical attachment level, plaque index while lower papillary bleeding index, and gingival index scores as compared to non-smokers with CP. The salivary cortisol and IL-1 β were relatively higher as compared to serum values in Group 2 than Group 3. The Group 2 patients revealed higher depression scores as compared to Group 3 patients. The depression scores positively and significantly correlated with the salivary cortisol in Group 2 patients.	The results indicate that smokers with CP exhibit a significantly higher serum and salivary cortisol, IL-1β, and stress levels and thus they may show an increased risk and periodontal disease severity. Further exploration of relationships between periodontitis and stress is required.
Khan, 2020 [21]	An 8-month comparative and cross-sectional study to analyze salivary cortisol level as a depression biomarker.	60 participants were included. Saliva specimens were collected and processed for enzyme-linked immunoassay (ELISA), and absorbance was calculated on a microtiter plate reader.	The mean cortisol level was 1.46±0.91 ng/ml among non-depressive patients, while it was 2.23±1.69 in the depressive group, with no statistical difference in mean ages.	These findings proved the cortisol level directly linked with severe depression and useful for depression diagnostics and management.
Khan *et al.*, 2020 [22]	A 6-month cross sectional study to establish a correlation between major depression, BMI and salivary cortisol.	60 participants were included in the present study. They were divided equally into two groups as normal healthy individuals with no physical or mental illness and severely depressed group. BMI was estimated using the formula: BMI = weight (kg) / (height in m)$^{2.}$ Early morning saliva samples were collected. Estimation of cortisol levels in saliva was done through ELISA.	The mean BMI in normal healthy group was 22.02 ± 4.21, while the mean BMI in severely depressive group was 24.64 ± 3.58. The difference was statistically significant (p = 0.012). The mean salivary cortisol level was significantly raised in patients with major depression (2.23 ± 1.69 nmol/L) in contrast to healthy normal individuals (1.46 ± 0.91 nmol/L), with p-value = 0.031.	BMI and depression has a very noteworthy correlation and there is a remarkable link between raised salivary cortisol, greater BMI and development of major depression.
Garrahy *et al.*, 2021 [23]	A 7-year retrospective observational study to determine the utility of measuring LNSF and LNSE in patients with confirmed Cushing's syndrome compared with other diagnostic tests and to analyze serial late-night salivary cortisol measurements for evidence of variable hormonogenesis.	23 patients with confirmed Cushing's syndrome were included, 21 with Cushing's disease. Saliva samples were collected between 11 pm and midnight and analyzed in the laboratory. Then statistical analysis was performed.	LNSF had a sensitivity of 92%, LNSE 87% and combined LNSF/LNSE 94% per sample. 4 patients had cyclical hormonogenesis, and a fifth patient fell just outside the criteria. 6 patients had evidence of variable hormonogenesis. Sensitivity of 24-h urinary free cortisol (UFC) was 89% per collection. 16 patients had simultaneous measurements of LNSF and UFC; in 3 patients, they provided discordant results.	LNSF appears more sensitive than LNSE and UFC in the diagnosis of CS, combining LNSF and LNSE results leads to superior sensitivity. Half of our cohort had evidence of cyclical or variable hormonogenesis. Fluctuations in LNSF did not always correlate with changes in UFC concentration, emphasizing the importance of performing more than one screening test, particularly if pretest clinical suspicion is high.
Johar *et al.*, 2016 [24]	A 1-year randomized cross sectional study to examine the association of cortisol levels and diurnal secretion patterns with prevalence of type 2 diabetes and HbA1c levels as well as the potential impact of sex and adiposity on this association.	757 patients with type 2 diabetes were selected and underwent saliva sampling collection. Multivariate regression analyses were employed to examine the association between salivary cortisol (measured upon waking (M1), 30 min after awakening (M2), and in the late night (LNSC)) and type 2 diabetes as well as glycated hemoglobin (HbA1c) with adjustments for potential confounders.	In the total sample population, an elevated LNSC level was observed in type 2 diabetes patients compared to non-patients. In sex-stratified analyses, diabetic men showed a greater Cortisol Awakening Response (CAR). Diabetic women had significantly elevated LNSC levels. HbA1c was positively associated with both CAR and LNSC levels but was negatively associated with M1 to LNSC ratio.	In this aged population, type 2 diabetes is associated with dysregulated cortisol secretion characterized by distinct sex specific diurnal patterns.
Hackett *et al.*, 2014 [25]	A 2-year cross sectional study to examine the association of cortisol patterns throughout the day with T2D status in a community-dwelling population.	238 participants with type 2 diabetes (T2D) were recruited and their diurnal cortisol (nmol/L) patterns from six saliva samples obtained over the course of a normal day: at waking, +30 min, +2.5, +8, +12 hours, and bedtime. The cortisol awakening response and slope in diurnal secretion were calculated.	T2D status was associated with a flatter slope in cortisol decline across the day and greater bedtime cortisol independent of a wide range of covariates measured at the time of cortisol assessment. There was no association between morning cortisol, the cortisol awakening response, and T2D.	In this nonclinical population, T2D was associated with a flatter slope in cortisol levels across the day and raised bedtime cortisol values.

Authors and Year of Publication	Study Design and Aim	Methods	Results	Conclusions
Hackett *et al*., 2016 [26]	A prospective cohort study to examine the association of diurnal cortisol secretion with future T2D and impaired glucose metabolism in a community-dwelling population.	A total of 3270 participants underwent salivary cortisol measurement (nmol/l) from six saliva samples obtained over the course of a day: at waking, +30 minutes, +2.5 hours, +8 hours, +12 hours, and bedtime. Participants who were normoglycemic in 2002-2004 (phase 7) were reexamined in 2012-2013 (phase 11).	Raised evening cortisol at phase 7 was predictive of new-onset T2D at phase 11 (odds ratio [OR], 1.18; 95% confidence interval [CI], 1.01-1.37) with a trend for a flatter slope in participants with incident T2D (odds ratio, 1.15; 95% CI, 0.99-1.33). When expanding this analysis to a broader category of glucose disturbance we found that a flattened diurnal cortisol slope at phase 7 was predictive of future impaired fasting glucose or T2D at phase 11, as was high bedtime cortisol.	In this nonclinical population, alterations in diurnal cortisol patterns were predictive of future glucose disturbance.
Fenol *et al*., 2017 [27]	A cross-sectional study to evaluate the association between stress, salivary cortisol, and periodontitis among the inmates of the central prison.	70 participants were grouped depending on their pocket depth into Group A (pocket depth >4 mm and <6 mm), Group B (at least four sites with pocket depth ≥6 mm), and Group C (pocket depth ≤3 mm). The clinical parameters such as the oral hygiene index-simplified, gingival index, pocket depth, and the clinical attachment levels (CALs) were recorded. Stress was measured using the Depression, Anxiety, and Stress Scale along with prison time served. Saliva samples were collected, and cortisol levels were determined using electrochemiluminescence assay.	The CALs, the stress score and the salivary cortisol levels were significantly higher in Group B ($P <$ 0.001). Pearson's correlation showed a positive correlation between stress, cortisol level, and pocket depth. A positive correlation which was statistically significant was obtained between salivary cortisol level and prison time served by the inmates.	It can be concluded that there is a positive relation between stress and periodontal disease. The study suggests that salivary cortisol level can be used as a marker to assess stress.
Obulareddy *et al*., 2018 [28]	A 6-month randomized cross-sectional study to evaluate saliva cortisol levels (SCLs) in chronic periodontitis (CP) patients with and without stress.	92 participants underwent saliva samples collection and cortisol levels were analyzed, using ELISA method. The participants were divided into four groups based on periodontal condition (number of teeth present, plaque index (PI), bleeding on probing (BOP), probing pocket depth, and clinical attachment level) and stress levels into Group 1 (no periodontitis and no stress), Group 2 (with periodontitis and no stress), Group 3 (without periodontitis and with stress), and Group 4 (with periodontitis and stress).	Participants with stress and periodontitis have high mean SCL when compared to other groups (Group 1: 15.01 ± 2.62, Group 2: 31.92 ± 6.80, Group 3: 34.47 ± 13.47, and Group 4: 60.13 ± 6.68). Group I shows a significant negative correlation of cortisol to BOP, stress to PI, and stress to cortisol level, whereas there is a positive correlation of SCL to PD in Group 4 which is not statistically significant.	SCL showed differences among the groups. SCL were associated with both CP and psychological stress. Increase in inflammation and stress levels enhances the SCL.
Naghsh *et al*., 2019 [29]	A 1-year randomized cross-sectional study to evaluate the association between the salivary cortisol level (SCL) of unstimulated saliva and CP in patients referred to Isfahan Dental Faculty.	90 patients were divided in 2 groups: 45 in the parodontitis group and 45 in non-periodontitis group. First, by evaluating the level of anxiety with the Spielberger State-Trait Anxiety Inventory questionnaire, each group was divided into three subgroups, each containing 15 persons. To measure the SCL in all subgroups by the enzyme-linked immunosorbent assay method, saliva samples were collected with unstimulated spitting method between 9 and 11 AM. Periodontal evaluation was done using the mean probing depth (PD), plaque index, and bleeding on probing.	The mean level of salivary cortisol (P = 0.048) and PD (P = 0.009) in patients with periodontitis was significantly higher than those without periodontitis. There was a direct and meaningful correlation between PD and SCL. In both groups of participants with and without periodontitis, the mean SCL in patients with high anxiety was significantly more than patients with medium and low anxiety.	The results showed that there is an increased level of salivary cortisol (as anxiety index) in patients with CP. Therefore, it seems that the probability of the occurrence of periodontitis is higher in those with increased cortisol level.

Authors and Year of Publication	Study Design and Aim	Methods	Results	Conclusions
Refulio *et al*., 2013 [30]	A 1-year cross-sectional study to evaluate the correlation between emotional stress, SCL (salivary cortisol level), and CP (chronic periodontitis).	70 patients, systemically healthy, were selected for the study. 36 subjects presented CP while the other 34 didn't. Parameters such as: 1) probing depth; 2) clinical attachment level; 3) bleeding on probing; and 4) tooth mobility, were recorded. Saliva samples were collected for the evaluation of SCL (*via* a highly sensitive electrochemiluminescence immunoassay), and all subjects also answered the Zung Self-rating Depression Scale questionnaire.	Subjects with moderate CP had statistically significantly higher levels of SCL than subjects with a diagnosis of slight CP. Also, subjects with severe CP showed the same outcome when compared to those with slight CP (p=0.012). In addition, 46 subjects presented high SCL whereas 24 had a normal level. CP was found to be correlated with the SCL.	Subjects with a high SCL and depression may show an increased risk for CP.
Rahate *et al*., 2022 [31]	A 15-month randomized cross-sectional study to investigate the serum and salivary ghrelin and cortisol levels in smokers and non-smokers with Stage III Periodontitis.	90 systemically healthy patients were recruited for this study. They were divided in 3 groups: Group I-Periodontally healthy patients; Group II-Non-smokers with Stage III Periodontitis and Group III-Smokers with Stage III periodontitis. Clinical parameters of Probing pocket depth (PPD), Clinical attachment levels (CAL), Plaque Index (PI), Gingival Index (GI) and Papillary Bleeding Index (PBI) were recorded and biochemical parameters of serum and salivary ghrelin and cortisol levels were analyzed *via* Enzyme Linked Immunosorbent Assay (ELISA). Stress levels were assessed using Zung's self-rating depression scale.	Serum and salivary ghrelin values were found to be higher in Group II (620.25 ± 260.86 pg/mL, 892.40 ± 271.65 pg/mL respectively) as compared to Group III. Similarly, salivary as well as serum cortisol levels were higher in Group III (20.78 ± 9.23 pg/mL, 399.37 ±189.21 pg/mL respectively) as compared to Group II (16.36 ± 8.88 pg/mL, 320.68 ± 107.01 pg/mL respectively). In Group III, a direct correlation was observed between stress, serum and salivary cortisol levels while an inverse correlation was found between stress, serum and salivary ghrelin levels. Group III showed a greater number of depressed patients followed by Group II and I. Serum and salivary ghrelin values were found to be higher in Group II (620.25 ± 260.86 pg/mL, 892.40 ± 271.65 pg/mL respectively) as compared to Group III. Similarly, salivary as well as serum cortisol levels were higher in Group III (20.78 ± 9.23 pg/mL, 399.37 ±189.21 pg/mL respectively) as compared to Group II (16.36 ± 8.88 pg/mL, 320.68 ± 107.01 pg/mL respectively). In Group III, a direct correlation was observed between stress, serum and salivary cortisol levels while an inverse correlation was found between stress, serum and salivary ghrelin levels. Group III showed a greater number of depressed patients followed by Group II and I.	The results show that smokers with Stage III Periodontitis exhibit an elevated stress and cortisol levels, lower serum and salivary ghrelin levels as compared to the non-smokers.
Ueland *et al*., 2021 [32]	A prospective cohort clinical study to define liquid chromatography tandem mass spectrometry (LC-MS/MS)-based cutoff values for bedtime and morning salivary cortisol and cortisone in children, and validate the results in children with and without CS.	320 healthy, 54 obese and 3 pituitary Cushing syndrome children were recruited respectively. Steroid hormones were assayed by LC-MS/MS. Cutoff levels for bedtime salivary cortisol and cortisone were defined by the 97.5% percentile in healthy subjects.	Bedtime cutoff levels for cortisol and cortisone were 2.4 and 12.0 nmol/L, respectively. 1 child from the obesity clinic had bedtime salivary cortisol exceeding the defined cutoff level, but normal salivary cortisone. All 3 children with pituitary CS had salivary cortisol and cortisone far above the defined bedtime cutoff levels. Healthy subjects showed a significant decrease in salivary cortisol from early morning to bedtime.	Results suggest that bedtime salivary cortisol measured by LC-MS/MS with a diagnostic threshold above 2.4 nmol/L can be applied as a screening test for **CS** in children. Age- and gender-specific cutoff levels are not needed.

Authors and Year of Publication	Study Design and Aim	Methods	Results	Conclusions
Lin *et al*., 2019 [33]	A 12-year retrospective clinical study, to investigate midnight salivary cortisol for Cushing's syndrome in the Chinese population.	61 Chinese patients suspected to have CS were evaluated. 48 patients were confirmed to have Cushing's syndrome. Then it was analyzed the midnight salivary cortisol, midnight serum cortisol and 24-hour urine free cortisol excretion for diagnosis. Midnight salivary cortisol was collected from 21 healthy volunteers for control purposes.	In the patient group, mean urine free cortisol excretion and midnight salivary cortisol levels were 296.50 ± 47.99 µg/day and 10.18 ± 1.29 ng/mL, respectively. Among the control group and normal participants, mean midnight salivary cortisol level was 0.53 ± 0.13 ng/mL and 0.50 ± 0.12 ng/mL, respectively. The cut-off value for midnight salivary cortisol was 1.7 ng/mL for diagnosing Cushing's syndrome, with a sensitivity of 98% and specificity of 100%. The diagnostic performance of midnight salivary cortisol (area under the curve [AUC] = 0.99) was superior to that of urine free cortisol (AUC = 0.89).	The study confirmed the good diagnostic performance of midnight salivary cortisol for diagnosing Cushing's syndrome in a Chinese population. Correlation between midnight salivary cortisol and either urine free cortisol or midnight serum cortisol was good. Midnight salivary cortisol is a convenient and precise tool for diagnosing Cushing's syndrome and can be the screening test of choice for Chinese populations.
Yonekura *et al*., 2014 [34]	A 3-day retrospective clinical study to determine the utility of salivary cortisol levels for screening mental states such as depression in adolescents following a natural disaster.	63 adolescents' survivors who were administered the GHQ and provided saliva samples thrice daily (morning, afternoon, and evening) over the course of 3 days. Based on the GHQ-depression subscores, subjects were divided into low and high depression groups. About 22% of the subjects were classified into the high symptom group.	A significant difference in the collected data was observed between the two groups in the salivary cortisol levels at the evening time point the ratio of the morning/evening levels as well. Analyzed by means of receiver-operating characteristic curves, the morning/evening ratios showed a good power in discriminating between subjects with and without depressive symptoms.	The study suggests that repeated measurement of salivary cortisol levels over 3 days has utility in screening for depressive states in adolescents following a natural disaster.
Develioglu *et al*., 2020 [35]	A prospective clinical study, to determine the salivary levels of cortisol, α-amylase, β-endorphin, and chromogranin (CgA) in saliva and to investigate their relationship with periodontitis.	80 patients with periodontitis were recruited for this study. The individuals were divided into three groups: mild, moderate, or severe chronic periodontitis. Plaque index (PI), gingival index (GI), clinical attachment loss (CAL), and probing depth (PD) measurements were recorded for all the participants. All participants underwent the State–Trait Anxiety Inventory test (STAI 1 and 2). Between 09:00 and 11:00 a.m., saliva samples from the participants were collected into tubes within an average of five minutes.	Higher cortisol measurements were detected in the saliva samples of participants with severe chronic periodontitis than in those who had mild chronic periodontitis. There were statistically significant age differences among patients with mild–moderate, moderate–severe, and mild chronic periodontitis, the severity of the disease increasing with age. There was also a positive correlation between STAI 1 stress scores and cortisol levels. Similarly, there was a positive correlation between CAL and cortisol levels. However, a significant difference was found among groups only in terms of salivary cortisol levels.	Within the limitations of the study, there was found to be a relationship between saliva cortisol levels and periodontitis and between salivary cortisol levels and stress.
Mohamed *et al*., 2022 [36]	A 4-year retrospective study to compare LNSC, LNS cortisone, overnight dexamethasone suppression test, low-dose dexamethasone suppression test and 24-h urinary free cortisol results of patients investigated for CS.	55 patients were divided in 2 groups: those individuals who had Cushing syndrome (21) and those who did not (33). Patients collected their saliva between 23h and midnight. Salivary cortisol and cortisone analysis was carried out by liquid chromatography and tandem mass spectroscopy (LC-MS/MS).	CS was diagnosed in 21 patients and CS was excluded in 33 patients. 10 patients were diagnosed with CD first presentation, 6 with recurrent CD, 2 with CS due to adrenal adenomas, 2 with CS due to adrenocortical cancers and 1 patient had CS due to an ectopic ACTH source. The latter patient underwent inferior petrosal sinus sampling to exclude a central source of ACTH with a confirmed ACTH source from diffuse idiopathic pulmonary neuroendocrine cell hyperplasia.	LNSC had a sensitivity of 95% and a specificity of 91%. LNS cortisone had a specificity of 100% and a sensitivity of 86%. With an optimal cut-off for LNS cortisone of >14.5 nmol/L the sensitivity was 95.2%, and the specificity was 100% with an area under the curve of 0.997, for diagnosing CS. Saliva collection is non-invasive and can be carried out at home. We therefore advocate simultaneous measurement of LNSC and LNS cortisone as the first-line screening test to evaluate patients with suspected CS.

Authors and Year of Publication	Study Design and Aim	Methods	Results	Conclusions
Zhang *et al*., 2022 [37]	A 30-month double-blind randomized controlled clinical trial to evaluate the salivary cortisol level and interleukin-1 B level in patients of Chronic periodontitis in smokers and stress and nonsmokers without stress.	600 subjects were recruited for the study. The sample size was divided into 300 males and 300 females. Out of 600 subjects, 200 subjects comprised of subjects with chronic periodontitis with positive depression level with a history of smoking (Group I), 200 subjects comprised of subjects with chronic periodontitis without depression and without smoking (Group II), and 200 subjects who were taken as the control group comprised of healthy subjects without chronic periodontitis, without depression level, and no smoking history (Group III). Salivary cortisol levels were determined by enzyme-linked immunosorbent assay (ELISA).	The result showed that there was a positive correlation between morning and evening salivary cortisol level in all the groups with correlation coefficient. There was a significantly higher value of salivary cortisol in Group I patients when compared with Group II and Group III. However, when the comparison of salivary cortisol levels was done between the Group II and Control group, the result showed nonsignificant P value.	It is suggested that stress is positively correlated with the salivary cortisol levels in smokers and nonsmokers.
Vrshek-Schallhorn *et al*., 2013 [38]	A 53-month randomized clinical study to analyze if CAR biomarker for prediction of major depressive disorders: (*a*) is stable over longer periods of time; (*b*) is independent of prospective stressful life events; and (*c*) differentially predicts first onsets or recurrences of MDEs.	270 older adolescents completed baseline diagnostic and life stress interviews, questionnaires, and a 3-day salivary cortisol sampling protocol measuring the CAR and diurnal rhythm, as well as up to four annual follow-up interviews of diagnoses and life stress.	Non-proportional person-month survival analyses revealed that higher levels of the baseline CAR significantly predict MDEs for 2.5 years following cortisol measurement. However, the strength of prediction of depressive episodes significantly decays over time, with the CAR no longer significantly predicting MDEs after 2.5 years. Elevations in the CAR did not significantly increase vulnerability to prospective major stressful life events. They did, however, predict MDE recurrences more strongly than first onsets.	The results suggest that a high CAR represents a time-limited risk factor for onsets of MDEs, which increases risk for depression independently of future major stressful life events. Possible explanations for the stronger effect of the CAR for predicting MDE recurrences than first onsets are discussed.

Abbreviations: ACS: autonomous cortisol secretion, BMI: body mass index, BOP: probing, CAL: clinical attachment loss, CAR: cortisol awakening response, CD: Cushing disease, CP: chronic periodontitis, CS: Cushing syndrome, CD: Cushing disease, DMFT: Mean number of Decayed, Missing, and Filled Permanent Teeth, DST: dexamethasone suppression test, ELISA: enzyme-linked immunoassay, GI: gingival index, LNS: late-night salivary, LNSC: late-night salivary cortisol, MDE: major depressive disorder, PBI: Papillary Bleeding Index, PD: probing depth, PI: plaque index, PPD: probing pocket depth, SCLs: saliva cortisol levels, sIg: secretory immunoglobulin, TOP: termination of pregnancy, T2D: type 2 diabetes.

Table S8. NHLBI quality assessment of controlled intervention studies.

NHLBI Quality Assessment of Controlled Intervention Studies																
First Author *et al*., Year	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Total Score	Quality Rating
Zhang *et al*., 2021 **[367**	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	13/14 (92.85%)	Good
Vrshek-Schallhorn *et al*., 2013 [38]	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	N	**Y**	N	11/14 (78.57%)	Good

Note: Q1: Was the study described as randomized, a randomized trial, a randomized clinical trial, or an RCT?, Q2: Was the method of randomization adequate (*i.e*., use of randomly generated assignment)?, Q3: Was the treatment allocation concealed (so that assignments could not be predicted)?, Q4: Were study participants and providers blinded to treatment group assignment?, Q5: Were the people assessing the outcomes blinded to the participants' group assignments?, Q6: Were the groups similar at baseline on important characteristics that could affect outcomes (*e.g*., demographics, risk factors, co-morbid conditions)?, Q7: Was the overall drop-out rate from the study at endpoint 20% or lower of the number allocated to treatment?, Q8: Was the differential drop-out rate (between treatment groups) at endpoint 15 percentage points or lower?, Q9: Was there high adherence to the intervention protocols for each treatment group?, Q10: Were other interventions avoided or similar in the groups (*e.g*., similar background treatments)?, Q11: Were outcomes assessed using valid and reliable measures, implemented consistently across all study participants?, Q12: Did the authors report that the sample size was sufficiently large to be able to detect a difference in the main outcome between groups with at least 80% power?, Q13: Were outcomes reported or subgroups analyzed prespecified (*i.e*., identified before analyses were conducted)?, Q14: Were all randomized participants analyzed in the group to which they were originally assigned, *i.e*., did they use an intention-to-treat analysis?; Total Score: Number of yes; CD: cannot be determined; NA: not applicable; NR: not reported; N: no; Y: yes. Quality Rating: Poor <50%, Fair 50–75%, Good ≥75%.

Table S9. NHLBI quality assessment tool for observational cohort and cross-sectional studies.

-	NHLBI Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies															
First Author *et al.*, Year	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Total Score	Quality Rating
Bäcklund *et al.*, 2020 [14]	Y	Y	N	N	N	Y	Y	Y	Y	Y	Y	N	Y	N	9/14 (64.29)	Bäcklund *et al.*, 2020 [13]
Lages *et al.*, 2019 [15]	Y	Y	N	N	Y	Y	N	Y	Y	N	Y	N	Y	N	7/14 (50.00%)	Fair
Salehi *et al.*, 2019 [16]	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	N	Y	N	11/14 (78.57%)	Good
Liu *et al.*, 2005 [17]	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	11/14 (78.57%)	Good
Pulopulos *et al.*, 2020 [18]	Y	Y	Y	N	N	Y	Y	Y	Y	N	Y	N	Y	Y	10/14 (71.42%)	Fair
Mesa *et al.*, 2014 [19]	Y	Y	N	N	Y	Y	Y	Y	Y	Y	Y	N	Y	N	11/14 (78.57%)	Good
Bawankar *et al.*, 2018 [20]	Y	Y	N	Y	N	Y	N	Y	Y	Y	Y	Y	Y	N	10/14 (71.42%)	Fair
Khan, 2020 [21]	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y	N	Y	N	10/14 (71.42%)	Fair
Khan *et al.*, 2020 [22]	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y	N	Y	N	10/14 (71.42%)	Fair
Garrahy *et al.*, 2021 [23]	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	Y	11/14 (78.57%)	Good
Johar *et al.*, 2016 [24]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	13/14 (92.85%)	Good
Hackett *et al.*, 2014 [25]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	13/14 (92.85%)	Good
Hackett *et al.*, 2016 [26]	Y	Y	Y	Y	Y	N	N	Y	Y	Y	Y	N	Y	Y	11/14 (78.57%)	Good
Fenol *et al.*, 2017 [27]	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	Y	12/14 (85.71%)	Good
Obulareddy *et al.*, 2018 [28]	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	11/14 (78.57%)	Good
Naghsh *et al.*, 2019 [29]	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	11/14 (78.57%)	Good
Refulio *et al.*, 2013 [30]	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	11/14 (78.57%)	Good
Rahate *et al.*, 2022 [31]	Y	Y	Y	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	11/14 (78.57%)	Good
Ueland *et al.*, 2021 [32]	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	10/14 (71.42%)	Fair
Lin *et al.*, 2019 [33]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	12/14 (85.71%)	Good
Yonekura *et al.*, 2014 [34]	Y	Y	Y	Y	Y	N	Y	N	Y	Y	Y	N	Y	N	10/14 (71.42%)	Fair
Develioglu *et al.*, 2020 [35]	Y	Y	Y	Y	Y	N	Y	N	Y	N	Y	N	Y	N	9/14 (64.29)	Fair
Mohamed *et al.*, 2022 [36]	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N	Y	N	11/14 (78.57%)	Good

Note: Q1: Was the research question or objective in this paper clearly stated?, Q2: Was the study population clearly specified and defined?, Q3: Was the participation rate of eligible persons at least 50%?, Q4: Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?, Q5: Was a sample size justification, power description, or variance and effect estimates provided?, Q6: For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?, Q7: Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?, Q8: For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (*e.g.*, categories of exposure, or exposure measured as continuous variable)?, Q9: Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?, Q10: Was the exposure(s) assessed more than once over time?, Q11: Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?, Q12: Were the outcome assessors blinded to the exposure status of participants?, Q13: Was loss to follow-up after baseline 20% or less?, Q14: Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?; Total Score: Number of yes; CD: cannot be determined; NA: not applicable; NR: not reported; N: no; Y: yes. Quality Rating: Poor <50%, Fair 50–75%, Good ≥75%.

REFERENCES

[1] Pires ALPV, Alves LDB, da Silva AM, *et al*. Salivary biomarkers to evaluate psychological disorders in oral lichen planus: A systematic review with meta-analysis. Oral Dis 2022.

[2] Bargues-Navarro G, Ibáñez-Del Valle V, El Mlili N, Cauli O. Salivary Biomarkers Associated with Psychological Alterations in Patients with Diabetes: A Systematic Review. Medicina (Kaunas) 2022; 58: 1091.

[3] Špiljak B, Vilibić M, Glavina A, Crnković M, Šešerko A, Lugović-Mihić L. A Review of Psychological Stress among Students and Its Assessment Using Salivary Biomarkers. Behav Sci (Basel) 2022; 12: 400.

[4] Botelho J, Machado V, Mascarenhas P, *et al*. Stress, salivary cortisol and periodontitis: A systematic review and meta-analysis of observational studies. Arch Oral Biol 2018; 96: 58-65.

[5] Neupane SP, Virtej A, Myhren LE, Bull VH. Biomarkers common for inflammatory periodontal disease and depression: A systematic review. Brain Behav Immun Health 2022; 21100450

[6] Song M, Bai H, Zhang P, Zhou X, Ying B. Promising applications of human-derived saliva biomarker testing in clinical diagnostics. Int J Oral Sci 2023; 15: 2.

[7] Noh JW, Jang JH, Yoon HS, *et al*. Evaluation of Salivary Biomarkers of Periodontal Disease Based on Smoking Status: A Systematic Review. Int J Environ Res Public Health 2022; 19: 14619.

[8] Bastin P, Maiter D, Gruson D. Le dosage du cortisol salivaire: aspects pré-analytiques et analytiques. Ann Biol Clin (Paris) 2018; 76: 393-405. [Salivary cortisol testing: preanalytic and analytic aspects].

[9] Boroumand M, Olianas A, Cabras T, *et al*. Saliva, a bodily fluid with recognized and potential diagnostic applications. J Sep Sci 2021; 44: 3677-90.

[10] Findling JW, Raff H. DIAGNOSIS OF ENDOCRINE DISEASE: Differentiation of pathologic/neoplastic hypercortisolism (Cushing's syndrome) from physiologic/non-neoplastic hypercortisolism (formerly known as pseudo-Cushing's syndrome). Eur J Endocrinol 2017; 176: R205-16.

[11] Zhang Q, Dou J, Gu W, Yang G, Lu J. Reassessing the reliability of the salivary cortisol assay for the diagnosis of Cushing syndrome. J Int Med Res 2013; 41: 1387-94.

[12] Choromańska K, Choromańska B, Dąbrowska E, *et al*. Saliva of obese patients - is it different? Postepy Hig Med Dosw 2015; 69: 1190-5.

[13] 2016.http://www.riskofbias.info [accessed {12 May 2024}]

[14] Bäcklund N, Brattsand G, Israelsson M, *et al*. Reference intervals of salivary cortisol and cortisone and their diagnostic accuracy in Cushing's syndrome. Eur J Endocrinol 2020; 182: 569-82.

[15] Lages AS, Frade JG, Oliveira D, *et al*. Late-Night Salivary Cortisol: Cut-Off Definition and Diagnostic Accuracy for Cushing's Syndrome in a Portuguese Population. Acta Med Port 2019; 32: 381-7.

[16] Salehi M, Mesgarani A, Karimipour S, *et al*. Comparison of Salivary Cortisol Level in Type 2 Diabetic Patients and Pre-Diabetics with Healthy People. Open Access Maced J Med Sci 2019; 7: 2321-7.

[17] Liu H, Bravata DM, Cabaccan J, Raff H, Ryzen E. Elevated late-night salivary cortisol levels in elderly male type 2 diabetic veterans. Clin Endocrinol (Oxf) 2005; 63: 642-9.

[18] Pulopulos MM, Baeken C, De Raedt R. Cortisol response to stress: The role of expectancy and anticipatory stress regulation. Horm Behav 2020; 117104587

[19] Mesa F, Magán-Fernández A, Muñoz R, *et al*. Catecholamine metabolites in urine, as chronic stress biomarkers, are associated with higher risk of chronic periodontitis in adults. J Periodontol 2014; 85: 1755-62.

[20] Bawankar PV, Kolte AP, Kolte RA. Evaluation of stress, serum and salivary cortisol, and interleukin-1β levels in smokers and non-smokers with chronic periodontitis. J Periodontol 2018; 89: 1061-8.

[21] Khan QU. Relationship of Salivary Cortisol Level With Severe Depression and Family History. Cureus 2020; 12e11548

[22] Khan QU, Zaffar S, Rehan AM, Rashid RR, Ashraf H, Hafeez F. Relationship of Major Depression with Body Mass Index and Salivary Cortisol. Cureus 2020; 12e6577

[23] Garrahy A, Forde H, O'Kelly P, *et al*. The diagnostic utility of late night salivary cortisol (LNSF) and cortisone (LNSE) in Cushing's syndrome. Ir J Med Sci 2021; 190: 615-23.

[24] Johar H, Emeny RT, Bidlingmaier M, Kruse J, Ladwig KH. Sex-related differences in the association of salivary cortisol levels and type 2 diabetes. Findings from the cross-sectional population based KORA-age study. Psychoneuroendocrinology 2016; 69: 133-41.

[25] Hackett RA, Steptoe A, Kumari M. Association of diurnal patterns in salivary cortisol with type 2 diabetes in the Whitehall II study. J Clin Endocrinol Metab 2014; 99: 4625-31.

[26] Hackett RA, Kivimäki M, Kumari M, Steptoe A. Diurnal Cortisol Patterns, Future Diabetes, and Impaired Glucose Metabolism in the Whitehall II Cohort Study. J Clin Endocrinol Metab 2016; 101: 619-25.

[27] Fenol A, Jebi S, Krishnan S, *et al*. Association of stress, salivary cortisol level, and periodontitis among the inmates of a central prison in Kerala. Dent Res J (Isfahan) 2017; 14: 288-92.

[28] Obulareddy VT, Chava VK, Nagarakanti S. Association of Stress, Salivary Cortisol, and Chronic Periodontitis: A Clinico-biochemical Study. Contemp Clin Dent 2018; 9: S299-304.

[29] Naghsh N, Mogharehabed A, Karami E, Yaghini J. Comparative evaluation of the cortisol level of unstimulated saliva in patients with and without chronic periodontitis. Dent Res J (Isfahan) 2019; 16: 421-7.

[30] Refulio Z, Rocafuerte M, de la Rosa M, Mendoza G, Chambrone L. Association among stress, salivary cortisol levels, and chronic periodontitis. J Periodontal Implant Sci 2013; 43: 96-100.

[31] Rahate PS, Kolte RA, Kolte AP, Lathiya VN, Gupta M, Chari S. Evaluation of stress, serum, and salivary ghrelin and cortisol levels in smokers and non-smokers with Stage III periodontitis: A cross-sectional study. J Periodontol 2022; 93: 1131-40.

[32] Ueland GÅ, Kellmann R, Jørstad Davidsen M, *et al*. Bedtime Salivary Cortisol as a Screening Test for Cushing Syndrome in Children. J Endocr Soc 2021; 5bvab033

[33] Lin DC, Tsai PS, Lin YC. Midnight salivary cortisol for the diagnosis of Cushing's syndrome in a Chinese population. Singapore Med J 2019; 60: 359-63.

[34] Yonekura T, Takeda K, Shetty V, Yamaguchi M. Relationship between salivary cortisol and depression in adolescent survivors of a major natural disaster. J Physiol Sci 2014; 64: 261-7.

[35] Develioglu H, Korkmaz S, Dundar S, Schlagenhauf U. Investigation of the levels of different salivary stress markers in chronic periodontitis patients. J Oral Biol Craniofac Res 2020; 10: 514-8.

[36] Mohamed RS, Abuelgasim B, Barker S, *et al*. Late-night salivary cortisol and cortisone should be the initial screening test for Cushing's syndrome. Endocr Connect 2022; 11e220050

[37] Zhang H, Chen B, Pan C, Zhang A. To evaluate the serum cortisol, salivary cortisol, and serum interleukin-1 B level in patients of chronic periodontitis with smoking and stress and without smoking and stress. Medicine (Baltimore) 2021; 100e26757

[38] Vrshek-Schallhorn S, Doane LD, Mineka S, Zinbarg RE, Craske MG, Adam EK. The cortisol awakening response predicts major depression: predictive stability over a 4-year follow-up and effect of depression history. Psychol Med 2013; 43: 483-93.