Saliva as a tool for monitoring hemodialysis: a systematic review and meta-analysis

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Abstract: This study aimed to assess whether the reductions in serum urea and creatinine levels are different from the reductions in salivary urea and creatinine levels that occur after hemodialysis in chronic renal patients. The systematic review protocol was registered in the PROSPERO database. Eight databases were searched to identify pretest-posttest studies of chronic kidney disease patients undergoing hemodialysis, with no language or year restrictions. The JBI Critical Appraisal Tool was used to assess the risk of bias. Meta-analyses using random-effect models were conducted to compare salivary and serum correlations and to pooled mean and proportion differences from pre- to posthemodialysis urea and creatinine levels by subgroup analysis. The I^2 test was used to assess heterogeneity, and a meta-regression was performed to statistically assess correlations and differences in the pooled effects pre- and postdialysis. The Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) was used to assess the certainty of evidence. The search resulted in 1404 records, and only six studies (n = 252 participants) fulfilled the eligibility criteria and were included. The studies were published between 2013 and 2018. All studies showed a significant reduction in both salivary and serum urea/creatinine levels. All eligible studies presented a low risk of bias. The meta-analysis shows a moderate to high correlation between salivary and blood levels of urea (r: 0.79; 95% CI: 0.56-1.00) and creatinine (r: 0.64; 95%CI: 0.16–1.00), with a very low level of certainty. The reductions in salivary urea and creatinine levels are similar to and correlated with the reductions in blood urea and creatine levels after hemodialysis among chronic kidney disease patients.

Keywords: Renal Insufficiency, Chronic; Saliva; Urea; Creatinine.

Introduction

Hemodialysis is the treatment option commonly indicated for purifying blood in patients diagnosed with advanced chronic kidney disease (CKD).[1] Hemodialysis is responsible for filtering toxic substrates from the bloodstream, such as creatinine, urea, and phosphorus, thus decreasing the signs and symptoms of these patients.[2]

The glomerular filtration rate (GFR) is an assessment of the filtering capacity of the functioning nephrons in the kidneys. GRF is a sensitive

method to detect and monitor changes in renal function.[3] Changes in GFR are a good predictor for determining the need for renal replacement therapy such as dialysis. The clearance of exogenous substances such as inulin or various radiolabeled markers is considered the gold standard for determining GFR. In addition, despite the use of creatinine and urea levels, cystatin C has been considered a candidate marker to evaluate patients with CDK, and interestingly, this protein can also be found and precisely quantified in saliva using state-of-the-art mass spectrometry. Monitoring the efficacy of hemodialysis is of utmost importance for determining the clinical prognosis of the patient and for follow-up on regression or the development of renal failure.[4] Laboratory analysis of blood is used to identify substrates in the blood, such as creatinine and urea.[5] However, blood collection is an invasive procedure that may lead some patients into a state of anxiety.[6] Moreover, repeated venous punctures increase the chances of infection, which explains why CKD patients present higher risks of infection from hepatitis B and C.[7]

The collection of saliva has been presented as an excellent alternative to collecting blood, considering that saliva is an alternative for the presence of bioproducts containing several blood serum components.[8] Recent studies have presented promising results regarding the correlation of saliva and blood analytes in different clinical conditions.[9,10,11] Specifically, for chronic kidney disease, saliva has been shown to be a promising tool for diagnosing this condition in early stages.[12] Thus, this easy collection procedure is a noninvasive and low-cost method, making saliva an optimum fluid for monitoring hemodialysis effects from home.[6]

In this context, the present systematic review aims to assess whether changes in the levels of urea and creatinine that occur in the serum will also occur in saliva after hemodialysis in patients diagnosed with chronic kidney disease.

Methodology

Protocol and registration

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations[13] and the Joanna Briggs Institute Manual.[14] The systematic review protocol was registered in the PROSPERO database (CRD42018116775).

Research question and eligibility criteria

The performance of the study was supported by the following research question: "Is there a correlation between salivary and serum urea and creatinine levels after hemodialysis in chronic renal patients?"

Inclusion criteria

a. Population: Adult patients (> 18 years old) with end-stage CKD (glomerulation filtration rate <15 mL/min/1.73 m^2) undergoing hemodialysis treatment.
b. Intervention: Salivary analysis.
c. Comparator: Serum analysis.
d. Outcome: Assessment of urea and creatinine levels after hemodialysis.
e. Study design: Clinical studies of pretest-posttest design with or without healthy patients as a control group to observe both salivary and blood levels of urea and creatinine pre- and posthemodialysis, with no restrictions on language or year of publication.

Exclusion criteria

a. Review articles, letters to the editor/editorials, personal opinions, books/book chapters, textbooks, reports, conference abstracts, and patents.
b. Studies including patients with other kidney diseases in addition to CKD;
c. Studies with pediatric patients.

Sources of information and search

The Embase, Latin-American and Caribbean Health Sciences Literature (LILACS), PubMed/MEDLINE, SciELO, Scopus, and Web of Science databases were used as primary study sources. OpenThesis and OpenGrey were used to partially search the "grey literature". Additionally, the reference lists of the eligible studies were manually searched to obtain additional studies. All steps were performed to minimize study selection bias.

The Medical Subject Heading (MeSH) terms, Health Sciences Descriptors (DeCS), and Embase Subject Headings (Emtree) resources were used to select the search descriptors. In addition, synonyms and free terms were used to enhance the search. The Boolean operators "AND" and "OR" were used to enhance the search strategy through several combinations according to each database. The search terms and strategy were adapted for each database (Table 1). The bibliographic research was performed in December 2018 and updated on March 21st, 2020. The results obtained were exported to EndNote Web™ software (Thomson Reuters, Toronto, Canada), in which duplicates were removed automatically. The remaining results were exported to Microsoft Word™ 2010 (Microsoft™ Ltd, Washington, USA), in which any remaining duplicates were removed manually.

Study selection

The studies were selected in three phases. In the first phase, as a calibration exercise, the reviewers discussed the eligibility criteria and applied them to

Table 1. Strategies for database search.

Database	Search Strategy (March, 2020)
PubMed http://www.ncbi.nlm.nih.gov/pubmed	("Kidney Diseases" OR "Kidney Disease" OR "Chronic Kidney Disease" OR "Chronic Renal Disease" OR "Renal Insufficiencies" OR "Kidney Insufficiency" OR "Renal Function" OR "Kidney Function" OR "Renal Failure" OR "Renal Injury") AND ("Saliva" OR "Salivary Creatine" OR "Salivary Urea" OR "Salivary Biomarkers")
Scopus http://www.scopus.com/	(("Kidney Diseases" OR "Kidney Disease" OR "Chronic Kidney Disease" OR "Chronic Renal Disease") AND ("Saliva" OR "Salivary Creatine" OR "Salivary Urea"))
LILACS	tw:("Kidney Diseases" AND "Saliva") AND (instance:"regional") AND (db:("LILACS"))
http://lilacs.bvsalud.org/	tw:("Renal Insufficiencies" AND "Saliva") AND (instance:"regional") AND (db:("LILACS"))
	tw:("Kidney Insufficiency" AND "Saliva") AND (instance:"regional") AND (db:("LILACS"))
	tw:("Kidney Diseases" AND "Salivary Creatine") AND (instance:"regional") AND (db:("LILACS"))
	tw:("Renal Insufficiencies" AND "Salivary Creatine") AND (instance:"regional") AND (db:("LILACS"))
	tw:("Kidney Diseases" AND "Salivary Urea") AND (instance:"regional") AND (db:("LILACS"))
SciELO	Kidney Diseases AND Saliva
http://www.scielo.org/	Renal Insufficiencies AND Saliva
	Kidney Insufficiency AND Saliva
	Kidney Diseases AND Salivary Creatine
	Hemodialíse AND Saliva
	Kidney Insufficiency AND Salivary Creatine
	Kidney Diseases AND Salivary Urea
	Renal Insufficiencies AND Salivary Urea
	Kidney Insufficiency AND Salivary Urea
Embase http://www.embase.com	('kidney diseases' OR 'kidney disease' OR 'chronic kidney disease' OR 'chronic renal disease' OR 'renal insufficiencies' OR 'kidney insufficiency' OR 'renal function' OR 'kidney function' OR 'renal failure' OR 'renal injury') AND ('saliva' OR 'salivary creatine' OR 'salivary urea' OR 'salivary biomarkers')
Web Of Science http://apps.webofknowledge.com/	(("Kidney Diseases" OR "Kidney Disease" OR "Chronic Kidney Disease" OR "Chronic Renal Disease" OR "Renal Insufficiencies" OR "Kidney Insufficiency" OR "Renal Function") AND ("Saliva" OR "Salivary Creatine" OR "Salivary Urea"))
OpenGrey http://www.opengrey.eu/	("Kidney Diseases" OR "Kidney Disease" OR "Chronic Kidney Disease" OR "Chronic Renal Disease" OR "Renal Insufficiencies" OR "Kidney Insufficiency" OR "Renal Function") AND ("Saliva" OR "Salivary Creatine" OR "Salivary Urea")
OpenThesis http://www.openthesis.org/	("Kidney Disease") AND ("Saliva")

a sample of 20% of the studies retrieved to determine the interexaminer agreement. After achieving a proper level of agreement (Kappa ≥ 0.81), two eligibility reviewers (RPCBR and WAV) methodically analyzed the titles of the studies independently. The reviewers were not blind to the names of authors and journals. Titles not related to the topic were eliminated in this phase. In the second phase, the reviewers (RPCBR and WAV) read the abstracts independently for the initial application of the aforementioned exclusion criteria. Those results with titles that met the objectives of the study but did not have abstracts available were fully analyzed in phase three.

In the third phase, the preliminary eligible studies had their full texts obtained and evaluated to verify whether they fulfilled the eligibility criteria. When reviewers disagreed, a third reviewer (LRP) was consulted to make a final decision. The studies rejected in this phase were registered separately, and the reasons for exclusion were specified.

Data collection

The following information was extracted from the studies selected: study identification (author, year, location), sample characteristics (number of patients in each study, distribution by sex, average age), sample collection and processing characteristics (saliva collection method, collection time of the biological material, type of salivary and blood analysis, type of statistical analysis), and specific results (concentrations of salivary and blood urea, concentrations of salivary and blood creatinine, percentage of posthemodialysis reduction, main conclusions). In case of incomplete or insufficient information, the corresponding author was contacted via e-mail. There were no language restrictions, but articles in languages other than English or Portuguese were translated to ensure that data were properly extracted.

To ensure consistency among reviewers, training was performed with both reviewers, in which information was extracted jointly from an eligible study. Disagreements between the reviewers were resolved through discussion and consensus. When this was not possible, a third reviewer (LRP) was consulted to make a final decision.

Risk of individual bias of the studies

The Joanna Briggs Institute Critical Appraisal Tools for use in JBI Systematic Reviews for quasi-experimental studies[15] assessed the risk of bias and the individual quality of the studies selected. The tool for quasi-experimental studies was chosen because it addresses the evaluation of pretest-posttest studies where the participants are not randomized.[16]

Two authors (WAV and LRP) independently assessed each domain regarding their potential risk of bias, as recommended by the PRISMA statement.[12] Any disagreement between the reviewers was solved through discussions on the topics assessed, and a third reviewer was consulted to make a final decision.

The risk of bias was ranked as high when the study reached 49% of the "yes" score, moderate when the study reached 50% to 69% of the "yes" score, and low when the study reached over 70% of the "yes" score.

Summary measures and syntheses of results

To summarize the data, a descriptive analysis of the findings was performed. The effectiveness of the salivary measure in comparison to serum values was evaluated using meta-analysis. To compare salivary and serum agreement, a meta-analysis was conducted of correlation according to correlation (r) values available for each study. Standard errors and confidence intervals (95%CI) of correlation coefficients were estimated to perform the meta-analysis. To compare salivary and serum concentrations of urea and creatinine, a subgroup meta-analysis was conducted evaluating the pooled mean and proportion differences from pre- to posthemodialysis results. Only articles with data from salivary and serum concentrations were included in the meta-analysis of each renal function indicator to ensure proper comparison of pooled estimates using the same samples. The measures described in mg/dL were converted to mmol/L using the MediCalc tool (http://www.scymed.com/) to standardize the data.[17] To compare salivary and serum concentrations of urea and creatinine on the same scale, the results of potential changes in the mean concentration were presented as a standard mean difference with Hedges correction (g), and conversions were performed using the formulas presented by Borenstein et al.[18]

Additionally, a meta-regression was performed to statistically assess differences in the pooled effects estimated (*g;* and proportion). The meta-analysis of all outcomes was performed using fixed- and random-effect models. The selection of each effect, either random or fixed, was based on the presence of heterogeneity ($p < 0.05$, chi-square or $I^2 > 50\%$). When this occurred, the random effects model was preferred.[19] Moreover, salivary and serum percentage reductions were measured for each renal function indicator, and the pooled effects were estimated.

Certainty of evidence

Certainty of evidence was assessed with the Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) tool.[20] GRADE pro GDT software (http://gdt.guidelinedevelopment.org) was used to summarize the results. This assessment was based on study design, risk of bias, inconsistency, indirect evidence, imprecision, and other considerations. The certainty of evidence was characterized as high, moderate, low, or very low.[20]

Results

Study selection

During the first phase of study selection, 1,404 results were found distributed in eight electronic databases, including the grey literature. After removing the duplicates, 745 results remained for analysis of titles and abstracts. After applying eligibility criteria to the titles and abstracts, only eight results were eligible for full-text analysis. The references of the eight potentially eligible studies were carefully assessed, and one additional study was selected, resulting in nine studies for full-text reading. After reading the full text, the studies of Khanum et al.,[4] Suzuki et al.[21] and Chen et al.[22] were excluded for not performing blood analysis after hemodialysis. Thus, six studies[23,24,25,26,27,28] were selected for the qualitative analysis. Figure 1 reproduces the process of search, identification, inclusion, and exclusion of articles.

Characteristics of eligible studies

The studies were published between 2013 and 2018 and were performed in China,[23] India,[24] Finland,[25] Bulgaria,[26] Italy,[27] and Turkey.[28] The total sample included 252 chronic kidney disease (CKD) patients undergoing hemodialysis. Only one study presented a control group with healthy participants (n = 40).[28] The average age of the sample ranged from 43.9 to 60.66. Men prevailed in all the eligible studies containing sex data.

Saliva and blood samples were collected simultaneously in five studies[23,24,26,27,28] before and after hemodialysis. The study by Bilancio et al.[26] also collected blood and saliva during hemodialysis. The collection of saliva samples was unstimulated in four studies[23,25,26,27] and stimulated in two studies.[23,25] Saliva and blood were analyzed with the colorimetric method in four eligible studies.[23,24,25,26] The study by Chen et al.[25] also observed the relationship between salivary urea concentration and ammonia in the breath of CKD patients. Table 2 shows detailed information about each eligible study.

Specific results of the eligible studies

All eligible studies[23,24,25,26,27,28] assessed the levels of urea. In the saliva analysis predialysis, the concentration of urea ranged from 15.85 mmol/L to 46.89 mmol/L, while in the saliva analysis postdialysis, the concentration ranged from 5.94 mmol/L to 46.78 mmol/L. The differences pre- and postdialysis ranged from 0.3% to 68%. When blood was analyzed, urea ranged from 21.24 mmol/L to 43.9 mmol/L before dialysis and from 6.4 mmol/L to 14.8 mmol/L after dialysis. Three[23,25,27] studies found a strong correlation between blood urea and salivary urea, and one study[27] showed a moderate correlation. The other two studies[23,25] did not perform correlation statistics (Table 3).

Four eligible studies[23,24,26,28] assessed the levels of creatinine. In the saliva analysis predialysis, the concentration of creatinine ranged from 0.6363 mg/dL to 1.13 mg/dL, while in the saliva analysis postdialysis, the concentration ranged from 0.34 mg/dL to 0.6343 mg/dL. The differences pre- and postdialysis ranged from 0.4% to 70%. When blood was analyzed, creatinine ranged from 7.24 mg/dL to 11.28 mg/dL before dialysis and from 2.99 mg/dL to 4.12 mg/dL after dialysis. The differences in blood creatinine pre- and postdialysis ranged from 31%

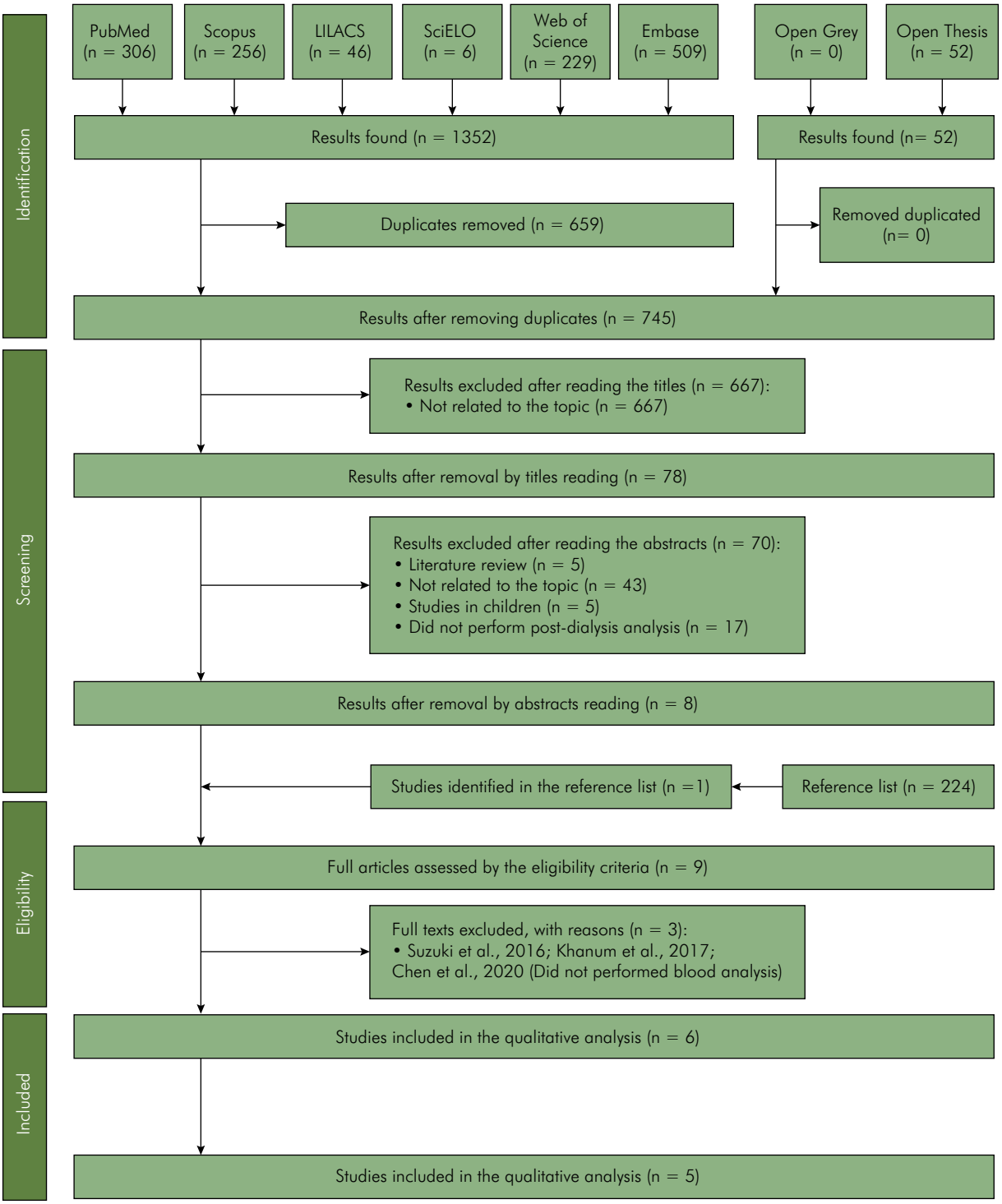

Figure 1. Flowchart of the literature search and selection process adapted from the PRISMA statement.

to 72%. The differences in blood creatinine pre- and postdialysis ranged from 52% to 64%. One study[23] observed a strong correlation between serum and salivary creatinine, while one study[28] found a low correlation. The other studies[24,26] did not examine correlation coefficients (Table 4).

Table 2. Summary of the main characteristics of the eligible studies.

Author, year, and location	Sample (n)	Mean age in years (SD)	Saliva collection method	Time of blood collection relative to saliva collection	Type of salivary analysis	Type of blood analysis	Statistical analysis
Cheng et al., 2013. China[23]	47	48.0 (13.5)	Stimulated saliva was collected (2 ml) using the spitting method before and after dialysis.	Simultaneously	Automatic analyzer (colorimetric method).	Automatic analyzer (colorimetric method).	Student's t test, paired t test, and Pearson's correlation analysis.
Seethalakshmi et al., 2014. India[24]	30 (16♂ 14♀)	50.33	Unstimulated whole saliva was collected (approximately 5 ml) using the spitting method before and after dialysis.	Simultaneously	Automatic analyzer (colorimetric method).	Automatic analyzer (colorimetric method).	Paired t test analysis.
Chen et al., 2016. Finland[25]	12 (8♂ 4♀)	52.41	Stimulated saliva was collected after 1 minute by chewing a paraffin pellet 1 minute before, during, and after dialysis.	Simultaneously	Colorimetric method.	Photometric enzymatic method.	Spearman's rank correlation test.
Alpdemir et al., 2018. Turkey[28]	Dialysis patients: 88 (32♀ 56♂) Healthy: 40 (16♀ 24♂)	Dialysis patients: 45.8 (13.3) Healthy: 43.9 (8.5)	Unstimulated saliva was collected using the spitting method after 5 min of relaxation.	Simultaneously	Spectrophotometric method.	Spectrophotometric method.	Student's t test, paired t test, and Pearson's correlation analysis.
Bilancio et al., 2018. Italy[27]	5	*	Saliva samples were collected using a synthetic swap (Salivette, Sarstedt, Germany).	1-2 minutes before	Urease/NADH method for urea.	Automated biochemistry and commercially available kits.	Student's t test, simple correlation coefficient, and linear regression.
Nogalcheva et al., 2018. Bulgaria[26]	70 (32♀ 38♂	60.66 (14.46)	The subjects were instructed to spit saliva every minute for approximately 5 minutes without causing prior stimulation of the salivary secretion.	Simultaneously	UV kinetic method for urea and colorimetric method for creatinine.	UV kinetic method for urea and colorimetric method for creatinine.	Descriptive statistics, Phi and Cramer's V nominal associations, and Pearson's chi-square test.

*Not cited by the authors.

Risk of individual bias of the studies

All eligible studies[23,24,25,26,27,28] presented a low risk of bias. Item 4 was considered 'No' for four eligible studies[23,24,25,26] due to lack of a control group. Item 5 was considered 'Uncertain' for all studies because they did not make it clear how many pre-posttest measurements were performed.[23,24,25,26,27,28] Item 6 was considered 'Not Applicable' for all studies[23,24,25,26,27,28] because the posttests were performed immediately after hemodialysis, with no follow-up. Table 5 shows detailed information on the risk of bias of the studies included.

Q1. Is it clear in the study what is the 'cause' and what is the 'effect' (i.e., there is no confusion about which variable comes first)? Q2. Were the participants included in any comparisons similar? Q3. Were the participants included in any comparisons receiving

Table 3. Main results of the eligible studies investigating urea levels.

Authors	Mean Concentration of SaU predialysis in mmol/L (SD)	Mean Concentration of SaU postdialysis (SD)	Difference (%)	Mean concentration of SU predialysis in mmol/L (SD)	Mean concentration of SU postdialysis in mmol/L (SD)	Difference (%)	r	Conclusion
Cheng et al., 2013[23]	28.33 (±7.53)	9.91 (±4.21)	65	28.54 (± 5.85)	10.57 (± 4.22)	63	0.909	There was a strong correlation (r=0.90, p<0.001) between blood urea and salivary urea.
Seethalakshmi et al., 2014[24]	15.85 (± 4.99)	6.73 (± 2.62)	58	22.03 (± 5.46)	8.52 (± 2.69)	62	*	Urea levels in serum and saliva were significantly lower after hemodialysis than before hemodialysis.
Chen et al., 2016[25]	16.92 (± 9.73)	5.94 (± 3.48)	65	21.67 (± 5.64)	6.73 (± 2.21)	69	0.77	There was a strong correlation (r=0.77, p<0.001, n=69) between blood urea and salivary urea.
Alpdemir et al., 2018[28]	44.9 (± 21.10)	24.7 (± 12.42)	45	43.9 (± 14.96)	14.8 (± 7.78)	66	0.58	Urea levels in serum and saliva were significantly lower after hemodialysis than before hemodialysis (p<0.001). This study showed a moderate correlation (r=0.58, p<0.001) between blood and salivary urea.
Bilancio et al., 2018[27]	24.6 (± 5.52)	7.8 (± 2.28)	68	23.2 (± 1.30)	6.4 (± 1.51)	72	0.96	The changes in saliva concentrations paralleled the changes in plasma concentrations for urea.
Nogalcheva et al., 2018[26]	46.89	46.78 (± 23.47)	0.3	21.24	14.71	31	[a]	There was a statistically significant reduction in blood urea levels before and after dialysis (p=0.000). However, there was no statistically significant reduction in salivary urea levels (p= 0.240).

*Variable investigated by the author; SaU: salivary urea; SU: serum urea.

similar treatment/care, other than the exposure or intervention of interest? Q4. Was there a control group? Q5. Were there multiple measurements of the outcome both pre and post the intervention/exposure? Q6. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed? Q7. Were the outcomes of participants included in any comparisons measured in the same way? Q8. Were outcomes measured in a reliable way? Q9. Was appropriate statistical analysis used? √ yes; × no; U: Unclear.

Synthesis of results and meta-analysis

Five studies were selected for quantitative assessment in at least one meta-analysis. Table 3 and Table 4 summarize all values of concentration, proportion differences, and the correlation (r) used to perform meta-analysis for each outcome. The study by Nogalcheva et al.[26] was not included in any meta-

Table 4. Main results of the eligible studies investigating creatinine levels.

Authors	Mean concentration of SaCr predialysis in mg/dL (SD)	Mean concentration of SaCr postdialysis in mg/dL (DS)	Difference (%)	Mean concentration of SCr predialysis in mg/dL (SD)	Mean concentration of SCr postdialysis in mg/dL (SD)	Difference (%)	Correlation (r)	Conclusion
Cheng et al., 2013.[23]	1.13 (± 0.4)	0.34 (± 0.1)	70	11.28 (± 1.98)	4.01 (± 1.48)	64	0.87	Creatinine levels in serum and saliva were significantly lower after hemodialysis than before hemodialysis, and a high correlation (r = 0.87, p < 0.01) were observed between blood and salivary creatinine.
Seethalakshmi et al., 2014.[24]	0.89 (± 0.47)	0.639 (± 0.34)	29	8.68 (± 2.77)	4.12 (± 1.48)	53	*	Creatinine levels in serum and saliva were significantly lower after hemodialysis than before hemodialysis.
Alpdemir et al., 2018.[28]	0.66 (± 0.5)	0.43 (± 0.23)	35	7.24 (± 2.5)	2.99 (± 1.06)	59	0.38	Creatinine levels in serum and saliva were significantly lower after hemodialysis than before hemodialysis (p < 0.001). This study showed a low correlation (r = 0.38, p < 0.001) between blood and salivary creatinine.
Nogalcheva et al., 2018.[26]	0.6363	0.6343	0.4	8.31	4.02	52	*	There was a statistically significant reduction in blood creatinine levels before and after dialysis (p =0.000). However, there was no statistically significant reduction in salivary creatinine levels (p = 0.065).

*The authors did not perform this analysis; SaCr: salivary creatinine; SCr: serum creatinine.

Table 5. Risk of bias assessed by the Joanna Briggs Institute Critical Appraisal Tools for use in JBI Systematic Reviews for Quasi-experimental studies.

Authors	Q.1	Q.2	Q.3	Q.4	Q.5	Q.6	Q.7	Q.8	Q.9	% yes/risk
Cheng et al., 2013[23]	√	√	√	x	U	N/A	√	√	√	75%/low risk
Seethalakshmi et al., 2014.[24]	√	√	√	x	U	N/A	√	√	√	75%/low risk
Chen et al., 2016[25]	√	√	√	x	U	N/A	√	√	√	75%/low risk
Alpdemir et al., 2018[28]	√	√	√	√	U	N/A	√	√	√	88%/low risk
Bilancio et al., 2018[27]	√	√	√	√	U	N/A	√	√	√	88%/low risk
Nogalcheva et al., 2018[26]	√	√	√	x	U	N/A	√	√	√	75%/low risk

analysis since it did not present standard deviation values to calculate standard errors for differences properly or the correlation coefficient (r).

Bioproducts from salivary samples showed different magnitudes of correlation. Urea collected from saliva was similar to the serum values. According to the pooled correlation coefficient from the meta-analysis of urea correlation, there is a high correlation value (r: 0.79; 95%CI: 0.56–1.00). Only two studies showed data for creatinine correlation, presenting moderate correlation with high confidence interval (r: 0.64; 95%CI: 0.16–1.00). Data from all estimated correlation coefficients are presented in Figure 2.

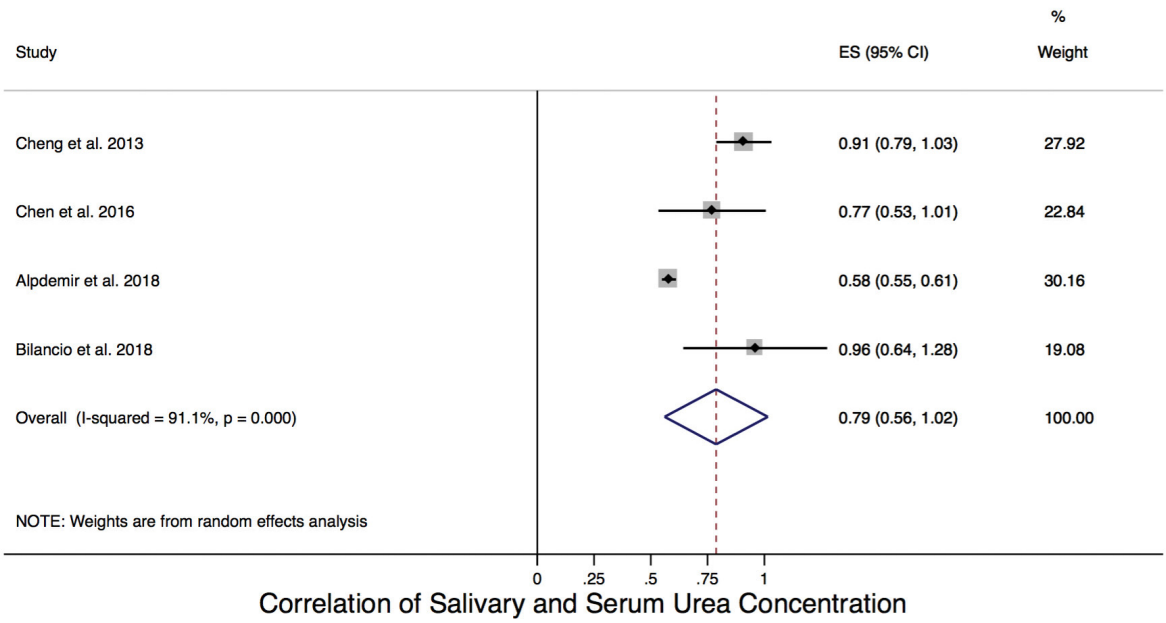

Figure 2A. Forest plot of the correlation between salivary and serum urea concentration. Weights are from random effects analysis.

The mean difference and proportion differences subgroup meta-analysis and the meta-regression did not show significant differences between salivary and serum differences in renal function indicators from pre- to posthemodialysis. When urea concentration reduction was analyzed,[23,24,25,27,28] the serum urea values showed a standardized mean difference (g) of 3.37 mmol/L (g; 3.00; 95%CI: 2.25–4.50), which was greater than that of the salivary values (g: 1.95; 95%CI: 2.14–-0.81). However, there were no significant differences between serum and salivary values. The meta-regression analysis did not show statistical significance (p = 0.157); thus, the source of bioproducts did not explain possible heterogeneity in mean concentration reduction. Moreover, the same pattern was observed for the percentage reduction, considering the pooled reduction of 65.6% (95%CI: 57.7–73.6) for serum and 58.6% (95%CI: 49.3–67.8) for salivary urea concentration pre- to posthemodialysis. The meta-regression analysis also did not show statistical significance (p = 0.251). Figure 3 shows all estimates of urea indicators of renal function considering the salivary and serum samples of each study.

Only three studies[23,24,28] allowed for proper calculation of the salivary and serum mean concentrations and the subsequent standard errors for creatinine estimates. The results of salivary and serum creatinine mean and proportion reductions were even more similar than urea, and there was no difference in either *g* or the percentage of reduction between pre- and posthemodialysis results. The salivary *g* reduction of creatinine concentration was 0.56 (g: 0.56; 95%CI: 0.03–1.08), and serum values presented almost the same reduction at 0.58 (g: 0.58; 95%CI: -2.28–6.02). There was no statistical significance in the meta-regression as well (p = 0.571). Considering the proportion of reduction, a higher value was evident in serum reduction (60.7%; 95%CI: 51.3–70.1) than in saliva (45.4%; 95%CI: 18.2–72.6) but was not a significant difference. In agreement, meta-regression analysis showed no statistical significance (p = 0.405). Figure 4 shows the summary of the meta-analysis for creatinine concentration and proportion differences.

Certainty of identified evidence

The GRADE approach[20] was used to assess the certainty of the summary evidence of the correlation of salivary and serum levels of urea and creatinine (Table 6). Both summary estimates presented a very low level of certainty. The analysis of the certainty of evidence started as "low" because it only included

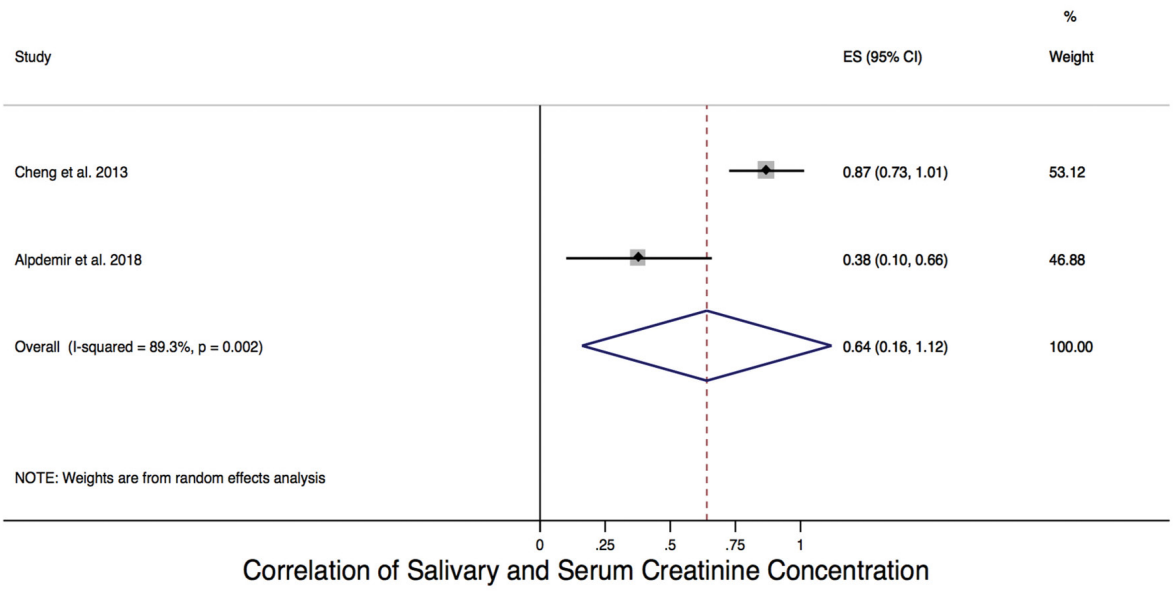

Figure 2B. Forest plot of the correlation between salivary and serum creatinine concentrations. Weights are from random effects analysis.

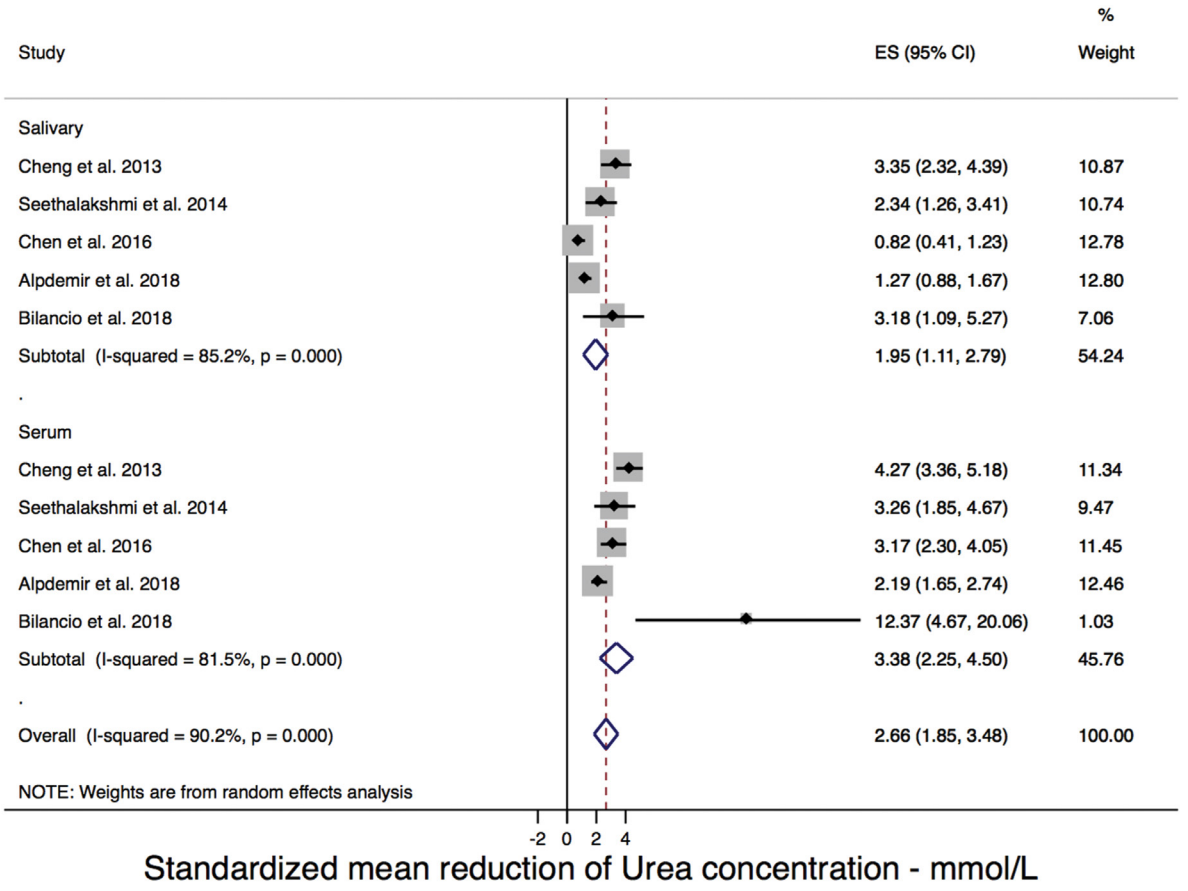

Figure 3A. Forest plot of the difference in urea concentrations from pre- to posthemodialysis meta-analysis of Hedges standard mean difference (g) of urea concentration. Weights are from random effects analysis.

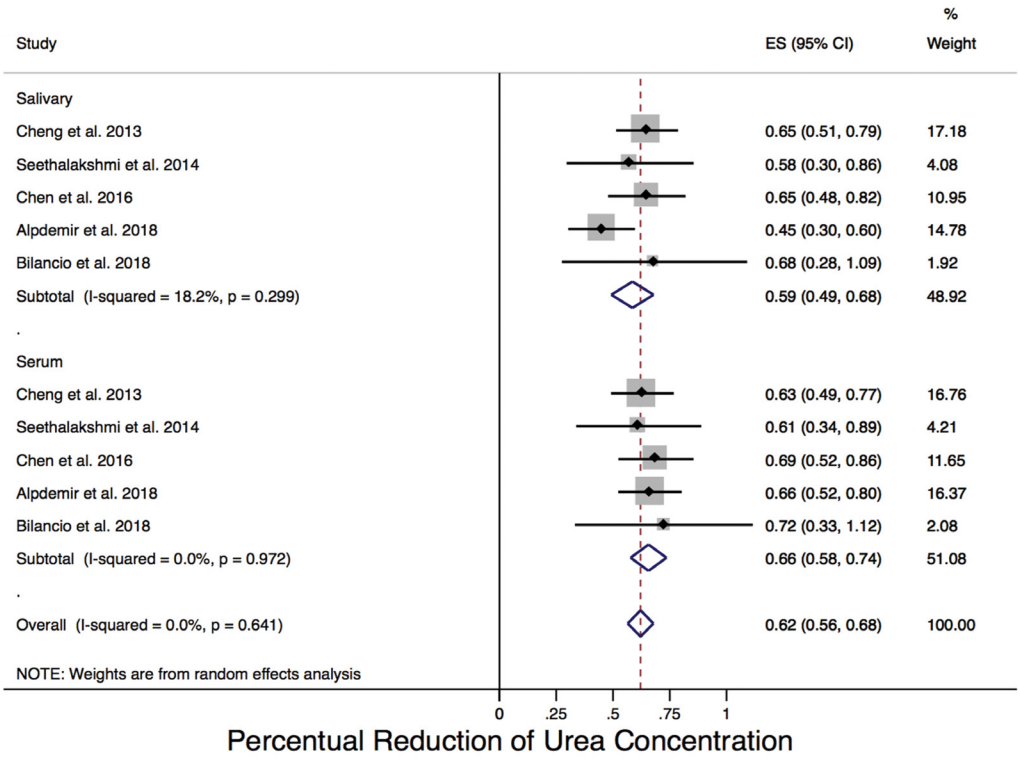

Figure 3B. Forest plot of the subgroup meta-analysis of the percentage reduction of urea concentration from pre- to posthemodialysis. Weights are from fixed effects analysis.

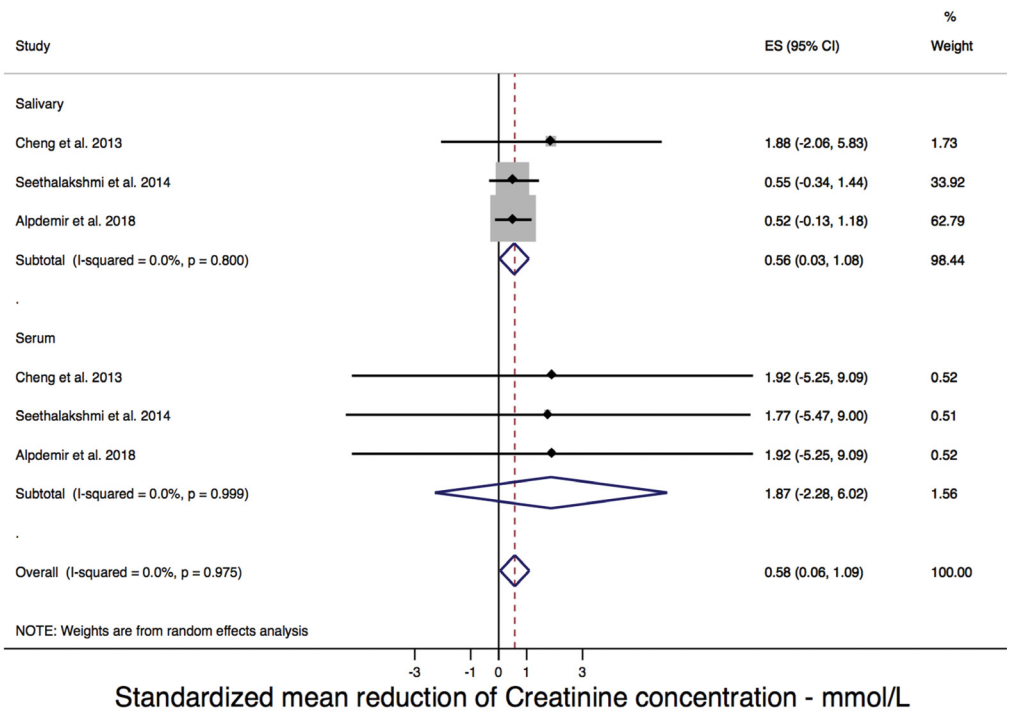

Figure 4A. Forest plot of the subgroup meta-analysis of Hedges standard mean difference (*g*) of creatinine concentration from pre- to posthemodialysis. Weights are from random effects analysis.

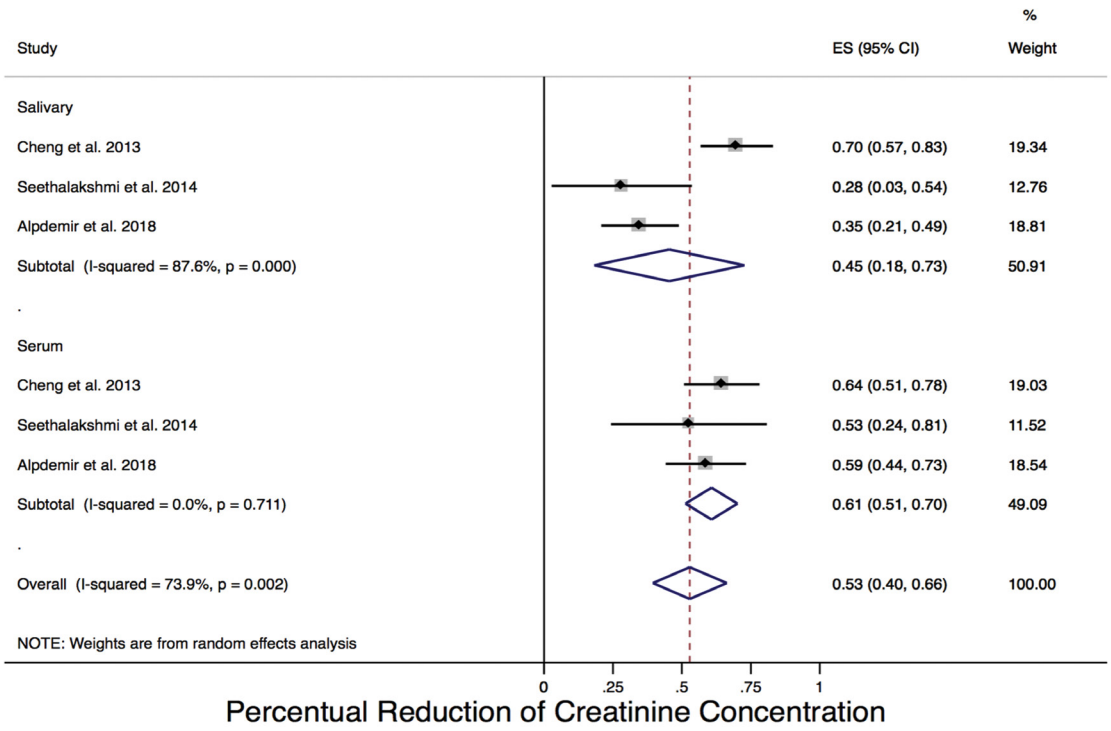

Figure 4B. Forest plot of the subgroup meta-analysis of the percentage reduction of creatinine concentration from pre- to posthemodialysis. Weights are from fixed effects analysis.

Table 6. Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Summary of Findings Table for the Outcomes of the Systematic Review and Meta-Analysis.

Quality Assessment							Summary of Results			Importance
Number of studies	Study design	Methodological limitations	Inconsistency	Indirectness	Imprecision	Publication biases	Number of participants	Effect	General quality	
Outcome 1: Correlation between saliva and serum – Urea levels										
4	pretest-posttest studies	Not serious[a]	Serious[b]	Not serious[c]	Very serious[d]	none	152	*r*: 0.79 (95% CI: 0.56-1.00)	⊕ VERY LOW	Critical
Outcome 2: Correlation between saliva and serum – Creatinine levels										
2	pretest-posttest studies	Not serious[a]	Serious[b]	Not serious[c]	Very serious[d]	none	135	*r*: 0.64 (95% CI: 0.16-1.00)	⊕ VERY LOW	Critical

a: All studies presented a low risk of bias; b: The I^2 was high (> 75%) - Downgraded by one level; c: Evidence came from studies with populations suitable for PICO; d: The total number of participants is less than 400, and there is a wide confidence interval in the effect estimates - Downgraded by two levels.
GRADE Working Group grades of evidence; High certainty: We are very confident that the true effect lies close to that of the estimate of the effect; Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different; Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect; Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

observational studies. Subsequently, inconsistency and imprecision downgraded the certainty by one and two levels, respectively. Inconsistency was judged by the I^2 test of each analysis, and the imprecision was judged by the number of participants and by the confidence interval of the estimates.

Discussion

This systematic literature review aimed to investigate the use of salivary components for monitoring the efficacy of hemodialysis in chronic kidney disease (CKD) patients. The meta-analysis results confirmed a reduction in urea and creatinine salivary indicators from pre- to posthemodialysis. Despite saliva not presenting the same magnitude of the reduction observed in serum measures, there was no significant difference between the two approaches.

Hemodialysis is a procedure indicated for patients with glomerular filtration rate (GFR) lower than 15 ml/min/1.73 m^2,[29] in which the excess of metabolic products in the blood is filtered by diffusion through a superpermeable membrane, simulating an artificial kidney.[1] Controlling the efficacy of the procedure is crucial, considering that it is essential to ensure the quality of life of individuals with renal impairment. In the present review, the efficacy of hemodialysis was assessed with creatinine and urea indicators pre- and posthemodialysis, as suggested in the literature.[1] All eligible studies[23,24,25,26,27,28] showed a significant reduction in urea and creatinine levels in both blood and saliva after hemodialysis, reinforcing the importance of this procedure for controlling the signs and symptoms of CKD patients.

Urea is a nitrogenated organic compound produced from the oxidation of amino acids, especially in the liver.[30] The normal rates of urea in the blood and saliva are 30-40 mg/dL and 12–70 mg/dL, respectively, and these values are used as an indirect form of assessing renal function.[4] Several studies observed a positive correlation between blood and salivary urea indicators, in which increased levels of urea in the blood were also high in saliva.[8,31,32] The present meta-analysis showed a significant reduction in urea indicators in both saliva (g: -1.45; 95%CI: -2.14–-0.81) and blood (g: -3.00; 95%CI: -4.12–-1.88), without a significant difference between them. This means that the reduction of urea molecules in the blood is similar to the reduction of urea in saliva. Additionally, the meta-analysis showed a high correlation value between salivary and serum levels of urea. These results may be explained by the molecular correlation that exists between these fluids. Urea is a molecule of small size and low weight, and it easily passes through the intercellular junctions of salivary glands by passive diffusion.[33] Thus, once the concentration of urea in the blood decreases, fewer urea molecules are filtered to saliva, consequently reducing the levels of salivary urea.

However, urea indicators may be modified by bacteria related to dental caries and periodontitis, as well as enzymes present in saliva (arginase and urease), which degrade urea in ammonia (NH_3).[34] This is common in CKD patients[27] and consequently masks the actual levels of urea in saliva. Thus, assessing the amount of NH_3 in the breath of chronic renal patients under hemodialysis may be a complementary tool for assessing the levels of salivary urea.

Creatinine levels are used to determine the GFR; these levels are high in patients with CKD, indicating a lowered GFR.[1] Similar to the results obtained for urea, the present meta-analysis showed a reduction in creatinine levels posthemodialysis in saliva and blood, with moderate correlation between them. Although the reduction in creatinine in blood levels was more extensive than that in salivary levels, the meta-regression did not find significant differences between the fluids, indicating that the reduction of creatinine molecules in the blood is similar to the reduction in saliva. A potential explanation for the reduction in salivary indicators is that after hemodialysis, the blood creatinine indicators are reduced, and there is not a concentration gradient that positively affects the diffusion of creatinine molecules from blood to saliva, decreasing the passage of creatinine to the salivary flow.[35]

Although all of the eligible studies[23,24,25,26,27,28] presented a low risk of bias, some limitations may explain the heterogeneity obtained in the meta-analysis. First, there was a lack of detailed data for the included patients, such as age, race, sex, and other systemic diseases associated with CKD. Moreover, the eligible studies[23,24,25,26,27,28] did not precisely confirm the GFR of the patient when comparing blood and saliva; the GFR may interfere directly with creatinine and urea indicators and with the effects of hemodialysis.

Nonetheless, this is the first systematic review of the literature that observed the potential use of salivary biomarkers as a tool for monitoring the

efficacy of hemodialysis in chronic renal patients. The meta-analysis of the data obtained from the studies also represents a strength of this review, as it provides greater consistency with the results obtained. Moreover, the extensive search in different databases, without restricting the year and using the "grey literature", significantly minimizes the risk of bias in the study selection. Using the GRADE approach[20] and The Joanna Briggs Institute Critical Appraisal tools to assess the certainty of the summary evidence and the methodological quality of the studies, respectively, shows the rigor of data collection of the eligible studies.

According to Dawes and Siqueira,[36] many plasma proteins enter saliva via gingival crevicular fluid, and for this reason, saliva can be considered an important tool for monitoring patients with CKD. We find strong support for our hypothesis, since the amount of urea and creatinine in saliva and plasma were measured in healthy patients and CKD patients before and after dialysis. The amounts of urea (mmol/L) and creatinine (μmol/l) in serum were 13.8 ± 3.25, 43.9 ± 14.96, 14.8 ± 7.78 and 85.7 ± 14.14, 640.1 ± 221.88, and 252.2 ± 94.58 for the control group before and after hemodialysis, respectively. In addition, the amounts of urea (mmol/L) and creatinine (mmol/l) in saliva were 21.1 ± 11.21; 44.9 ± 21.10; 27.4 ± 12.42 and 37.2 ± 26.52; 58.4 ± 45.08 and 38.1 ± 21.21 for the control group before and after hemodialysis, respectively. Even with the presence of proteases in the oral cavity, the relative difference between the groups shows that salivary urea and creatinine are abundant and that saliva testing may be valuable in CKD patient follow-up. In addition, when comparing salivary creatinine and urea concentrations with their concentration in blood, our previous study described a sensitivity of 93.3% (95%CI: 88.6–97.9) for salivary creatinine levels and 87.5% (95%CI: 83.2–91.8) for salivary urea levels, while the overall specificity was 87.1% (95%CI: 82.8–91.3) and 83.2% (95%CI: 65.0–101.4) for salivary creatinine and urea levels, respectively.[12] Another factor that should be analyzed is the absence of data related to the oral health of the study participants, considering that the presence of oral diseases may interfere with the biomarker values analyzed.

The results from this meta-analysis reaffirm the role of saliva as a promising tool for monitoring patients in advanced stages of CKD. Patients with kidney diseases require constant and regular follow-ups and may require lifelong treatment. According to the National Kidney Foundation, CKD can be divided into 5 stages (1- kidney damage with normal or increased GFR to 5- kidney failure). Stage 5 patients are treated with hemodialysis; however, for patients between stages 1 and 4, very close follow-up is required to estimate the disease progression and for evaluation and treatment plans for possible complications. One of the treatments for patients in level 4 is peritoneal dialysis, which can be performed at home. Thus, replacing blood collection with saliva collection to verify urea and creatinine levels may be beneficial in countless aspects, such as cost reduction, easy at-home collection, and decreased patient anxiety. Additionally, the literature reports that patients with CKD have a higher flow rate after hemodialysis (0.8 mL/min) compared to (0.4 mL/min) prior to hemodialysis, but despite this hyposalivation scenario, saliva is still considered an alternative method for CKD patient follow-up.[4] Additionally, given that saliva collection is a less invasive method for collecting biological material than blood collection, using saliva would decrease the risk of infection to which patients are exposed during blood collection; this is positive, keeping in mind that the immunity of these patients is often low due to renal problems potentially presenting secondary diseases.

Thus, the present study provides relevant and essential information that opens avenues for further studies to be performed with greater methodological rigor to adjust the GRF equation to validate the use of saliva as a diagnostic tool and as a monitoring method for kidney disease patients.

Limitations

This study is not free of limitations. The first limitation is the low number of studies included in the meta-analysis. Additionally, the included studies showed a high level of heterogeneity caused mainly by the lack of standardization in the assessment of results. Another limitation in this review is the general quality of the evidence found. According to

the GRADE approach,[20] the summary estimates had a very low level of certainty. This is primarily due to the design of the studies included, considering observational studies already start at lower levels. Second, the strong inconsistencies observed among the studies for each outcome and the wide confidence interval of the summary estimates downgraded the certainty of evidence by two levels. Thus, these results should be interpreted with caution, and more studies should be performed in the future.

Thus, despite the promising results, they should be analyzed with caution, and further studies with an improved design (diagnostic accuracy studies) are required to confirm the use of saliva as a tool for monitoring the efficacy of hemodialysis in CKD patients and to validate a mathematical formula that establishes the GFR through saliva.

Conclusion

There is a reduction in salivary urea and creatinine levels in comparison to the reduction found in the blood after hemodialysis in chronic kidney disease patients. As a clinical outcome, the present study provides relevant and essential information that could serve as a basis for an innovative study to be performed with greater methodological rigor to adjust the glomerular filtration rate (GRF) equation to validate the use of saliva as a diagnostic tool and follow-up method for kidney disease patients.

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