Salivary creatinine and urea in patients with end-stage chronic kidney disease could not be used as diagnostic biomarkers for the effectiveness of dialysis treatment

Articoli originali

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ABSTRACT

Introduction. End-stage chronic kidney disease (CKD) is characterized by kidney failure with the organ's functions reduced or lost completely, where the kidneys are incapable of filtering excess fluids. Renal replacement therapy may be provided by peritoneal dialysis, hemodialysis or renal transplantation. Among the key indicators for tracking patients' current status are urea and creatinine levels.

Aim. The study analyzed saliva as a medium to detect and measure urea and creatinine levels in end-stage CKD patients as well as to use it as criteria for the effectiveness of the dialysis treatment by comparing salivary urea and creatinine levels with their blood levels.

Material and methods. The study targeted 70 end-stage CKD patients from northeastern Bulgaria undergoing hemodialysis treatment. The urea in blood serum was carried out using the UV kinetic method. Creatinine levels were measured using Jaffe reaction colorimetric method without deproteinezation, adapted on an Olympus AU 400 automated biochemical analyzer (Beckman Coulter Inc., USA). Samples from whole unstimulated saliva were collected in a 15 ml sterile test tube as per Navazesh method. The qualitative determination of salivary urea was performed using the UV kinetic method. Creatinine levels in whole unstimulated saliva were measured using Jaffe reaction colorimetric method.

Results. There was a statistically significant reduction in blood urea levels (P=0.000) and in blood creatinine levels (P = 0.000) following hemodialysis. The results revealed that there was no statistically significant dependence between both, the urea levels (P=0.240) and the creatinine levels (P=0.065) in whole unstimulated saliva obtained prior to and after a hemodialysis.

Conclusion. Despite the parallel increase of the urea and creatinine levels in blood serum and in whole unstimulated saliva in end-stage CKD, salivary urea and creatinine levels could notbe used as diagnostic biomarkers for the effectiveness of dialysis treatment.

KEYWORDS: end-stage chronic kidney disease, dialysis, salivary urea, salivary creatinine

Introduction

Chronic kidney disease (*CKD)* is recognised as a health concern globally, leading to ever-increasing rates of *morbidity* [1]. End-stage renal disease is characterized by kidney failure with the organ's functions reduced or lost completely, where the kidneys are incapable of filtering excess fluids. Renal replacement therapy becomes then indispensible and may be provided by peritoneal dialysis, hemodialysis or renal transplantation [2–5]. However, in consequence of the disease and the dialysis treatment itself certain typical oral manifestations are soon detected in CKD patients, such as dry mouth, altered salivary composition, etc. [6–8] Among the key indicators for tracking patients' current status are urea and creatinine levels. [9] The use of blood serum in order to determine those levels is still held up as "the gold standard". Alternative routes of excretion by the body have been sought. Due to its availability and non-invasive method of collection, saliva is gaining wide momentum in recent years as a diagnostic tool [10, 11].

Aim

The study analyzed saliva as a medium to detect and measure urea and creatinine levels in end-stage CKD patients as well as to use it as criteria for the effectiveness of the dialysis treatment by comparing salivary urea and creatinine levels with their blood levels.

Material and methods.

The study targeted 70 end-stage CKD patients from northeastern Bulgaria, aged 32-89 years, undergoing hemodialysis treatment of different duration. 32 patients were men (46.38%), while 38 patients were women (53.62%), with a mean age of 60.66 years (SD = 14.46). There was no statistically significant difference in the mean age across gender (P> 0.05).

Chronic hemodialysis was normally carried out over 3 sessions a week, each with a duration of 4 hours. The duration of the procedure is contingent on factors such as preserved residual renal function (RRF), accumulation of unexcreted waste products, sodium levels and body weight. Blood samples were collected twice for all patients: prior to the procedure and immediately after its completion.

I. Laboratory methods for measuring urea and creatinine levels in blood serum.

The test took place at a certified clinical laboratory at St. Marina Hospital in Varna, Bulgaria.

Urea in blood serum. The quantitative determination of urea in blood serum was carried out using the UV kinetic method. *Urea is hydrolyzed in the presence of water and urease* to produce *ammonia and carbon dioxide. The ammonia then reacts* with *2–oxoglutarate and*NADH in the *presence* of *glutamate dehydrogenase* (GIDH) to produce glutamate and NAD^+. The *decrease* in *absorbance* due to the *decrease* of *NADH concentration* in *unit time is proportional* to the *urea concentration.* For reference values the study adopted 3.2 – 8.2 mmol/L.

Creatinine in blood serum. Creatinine levels were measured using Jaffe reaction colorimetric method without deproteinezation, adapted on an Olympus AU 400 automated biochemical analyzer (Beckman Coulter Inc., USA). By this method blood creatinine levels are traceable to Standard Reference Material 909 b Level 2 of the National Institute of Standards and Technology (NIST-USA). The method showed a linear dependence within a concentration range of 18-2200 mmol/L (0.2-25.0 mg/dL) for serum and plasma. The study adopted the following reference values: 62 -115mcmol/l for men, 44 -97 mcmol/l for women, 18-62 mcmol/l for children.

II. Laboratory methods for determination of urea and creatinine in unstimulated saliva.

Collection of samples from whole unstimulated saliva. The assay preparation commenced on the previous day and involved the following steps: The saliva was collected in the morning after a 10-hour overnight fast, with the last meal not later than 7pm on the previous day, excluding alcohol, carbohydrates and proteins. The patient was asked not to wash their teeth prior to bedtime, and to conduct their evening oral hygiene by simply rinsing the mouth 10 times with lukewarm water, gargling the water in the mouth for a while. The procedure ended with the intake of 100 ml of water. Samples from whole unstimulated saliva were collected in a 15 ml sterile test tube as per Navazesh method [12]. The test tubes were opened right before the assay. Saliva was collected from 7.30am to 8.30am. The subjects were then instructed to spit the saliva every minute for about 5 minutes without causing prior stimulation of the salivary secretion. Thereby the necessary 5 ml of saliva were obtained. The individual samples, labeled with each patient's initials, were stored at -20°C until the laboratory analysis could be conducted. On the day of the lab tests all 4 ml samples were centrifuged for 10 min at 8000 G, then 2 ml of the *supernatant was pipetted* and tested for the parameters under study.

Urea in whole unstimulated saliva. The qualitative determination of urea with urease and glutamate dehydrogenase was performed using the UV kinetic method. *Urea is hydrolyzed by urease* to produce *ammonia and carbon dioxide.* As a second step, the *2–oxoglutarate*binds with an ammonium radical in the presence of glutamate dehydrogenase (GLDH) and coenzyme NAD to produce L-glutamate. In this reaction two moles of NADH are oxidized into NAD for each molecule of urea hydrolyzed. The rate of decrease in the concentration of NADH is directly proportional to the concentration of urea in the sample. Absorbance was measured at 340 nm.

Creatinine in whole unstimulated saliva. Creatinine levels in whole unstimulated saliva were measured using Jaffe reaction colorimetric method. I*n an alkaline mediumcreatinine forms a yellow–orange complex* with *picrate* anions. The rate of formation of color is proportional to the creatinine in the sample. Since bilirubin causes negative interference in creatinine value measurement, the assay used speed blocking to avoid bilirubin interference. Due to unspecific reactions with pseudo-creatinine chromogens, such as proteins and ketones, the results were corrected by specific indices.

alkaline pH

Creatinine + picric acid ————————> yellow-orange complex

he statistical analysis was performed using SPSS Statistics Software package for epidemiological and clinical research (IBM SPSS Statistics 20.0, 2011). The following statistical methods were used: descriptive statistics for qualitative data (frequency and percentage tables) and output graphs; cross tabulation for qualitative data; Phi and Cramer's V nominal associations for correlation between qualitative variables; Pearson's chi-square test for linking qualitative variables and output graphs.

Results

Urea levels in blood serum. The data from the blood urea assay are presented in Table 1.

	Values taken prior to CD (mmol/L)	Values taken after CD (mmol/L)	Statistical difference	t	P
Urea	21.24	14.71	6.53	21.274	0.000

Table 1. Results from the T-test for statistically significant differences in blood serum urea levels before and after hemodialysis in CKD patients

The mean arithmetic values (21,24 ± 6.49 mmol/L) and the median (21.10 (14.60 – 29.10 mmol/L)) were very close and were approximately seven times higher than the reference range (3.2 – 8.2 mmol/L) prior to the start of the chronic hemodialysis (CD). Following the CD, the mean arithmetic values and median of the urea in blood serum were reduced by nearly 1.5 times and were 4.4 times higher than the normal range, respectively.

A T-test for paired samples was then carried out in order to determine the statistically significant difference in the blood urea levels established after the renal replacement therapy. The results matched the expectations that there was a statistically significant reduction in blood urea levels (P = 0.000) following a CD (Table 1).

Creatinine levels in blood serum. The data obtained from the dependent T-test for paired samples confirmed the expectations that there was a statistically significant reduction in blood creatinine levels (P = 0.000) following a CD (Table 3).

Cut-off value	Sensitivity	1-specificity
12.9550	0.929	0.069
13.5450	0.929	0.034
13.7800	**0.929**	**0.000**
15.1550	0.914	0.000
17.6150	0.900	0.000

Table 3. ROC Curve coordinates for Surea

	Values taken prior to CD (mmol/L)	Values taken after CD (mmol/L)	Statistical difference	t	P
Creatinine	734.99	355.86	379.13	22.076	0.000

Table 2. Results from the T-test for statistically significant differences in blood creatinine levels before and after chronic hemodialysis (CD)

Evaluation of the diagnostic potential of urea and creatinine levels in saliva. Receiver operating characteristic (ROC) analysis was performed to determine the diagnostic potential of salivary urea (Surea) and salivary creatinine (Screat), as compared to their blood level (Figure 1).

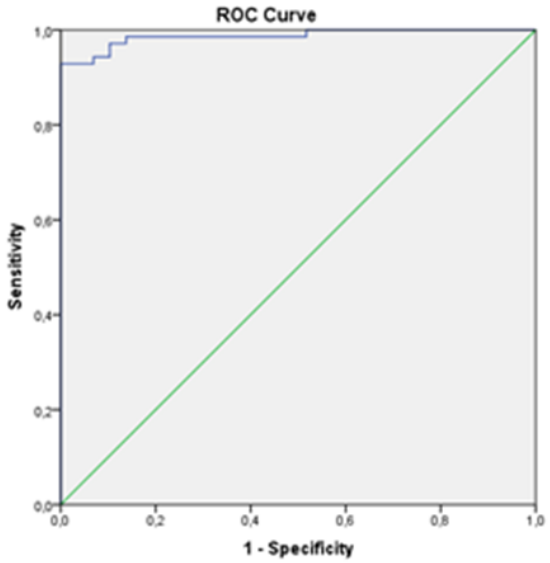

Fig.1 ROC Curve for Surea

The sensitivity and specificity of the salivary urea and creatinine test can be determined by how accurately it separates CKD patients from healthy controls. Accuracy is estimated by the area under the ROC curve (AUC). It can be seen in Figure 1 that the area under the curve for salivary urea was 0.99 (Standard Error = 0.009, p <0.001, 95% confidence interval = 0.97-1.00). The large AUC showed that saliva as a medium was a reliable alternative diagnostic tool for distinguishing CKD patients from healthy controls.

The sensitivity and specificity for different levels of salivary urea were measured and a cut-off value was estimated (Table 3).
As seen in Table 3, the bolded cut-off value of 13.7800 mg/dl for salivary urea was the best compromise between sensitivity and specificity. This cut-off point accurately separated 93% of the clinical group from 7%, who had values below this threshold, and were classified as healthy controls. The cut-off value classified 0% of the control group as CKD patients.
Similarly, ROC analysis was also applied in order to assess the diagnostic potential of salivary creatinine (Figure 2).

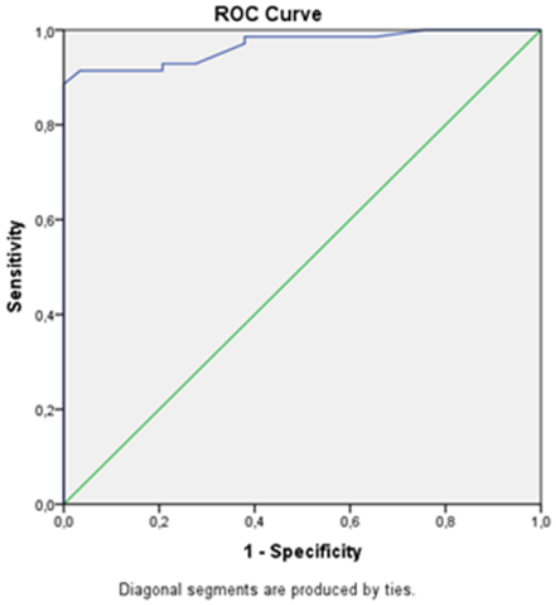

Fig.2 ROC Curve for Screat

The AUC was 0.97 (Standard Error = 0.016, p <0.001, 95% confidence interval = 0.94 – 1.00), confirming that as a medium saliva was a reliable alternative diagnostic tool for distinguishing CKD patients from healthy controls.
The sensitivity and specificity for different levels of salivary creatinine were measured and a bolded cut-off value was estimated (Table 4).

Cut-off value	Sensitivity	1-specificity
16.6100	0.914	0.103
17.9650	0.914	0.069
18.9800	**0.914**	**0.034**
19.3200	0.886	0.000
20.6750	0.871	0.000

Table 4. ROC Curve coordinates for Screat

The cut-off value of 18.9800 mg/dl for salivary creatinine correctly classified 91% of the clinical group as CKD patients, and 9%, who had creatinine values below this threshold, were classified as healthy controls. The cut-off value incorrectly classified 3% of the control group as CKD patients. Urea in the salivary secretion was not a normally distributed variable; therefore it was regarded as a regular variable. Three groups were identified (Table 5).

Target	Low levels	Mean levels	High levels
Surea	0.31 - 3.19 mmol/L	3,20 - 7.62 mmol/L	7.63 - 13.78 mmol/L
Screat	2.71 - 6.10 mcmol/L	6.11 - 10.17 mcmol/L	10.18 - 18.98 mcmol/L

Table 5. Values taken for Surea and Screat according to groups

Urea levels in whole unstimulated saliva. The analysis of the data obtained from the study of urea levels in whole unstimulated saliva revealed that the mean arithmetic for CKD patients was 46.78 ± 23.47 mmol/L, while the median value was 43.45 (6.50-103.40) mmol/L. A dependent T-test for paired samples was performed to establish a statistically significant difference in urea levels in unstimulated saliva in CKD patients after renal replacement therapy. (Table 6) The results revealed that there was no statistically significant dependence between the levels obtained prior to and after a CD (p = 0.240).

Table 6*. T-test results for urea levels in whole saliva before and after chronic dialysis (CD)*

Creatinine levels in whole unstimulated saliva. Following the dialysis procedure, a significant reduction in residual nitrogen fractions in blood serum was detected, whereas the creatinine levels in whole unstimulated saliva remained statistically significant in CKD patients undergoing CD. The results can be seen in Table 7.

Table 7. *T-test results for the statistically significant differences in creatinine levels in whole saliva before and after chronic dialysis (CD)* A dependent T-test for paired samples was performed on the creatinine levels in unstimulated saliva in CKD patients prior to and after CD. No statistically significant dependence was detected (p=0.065). (Table 7)

Comparison between creatinine and urea levels in blood serum versus salivary medium. The data analysis indicated that 98.8% of patients had blood urea levels higher than the reference range, as compared to 71.4% of patients who had higher than normal urea levels in whole unstimulated saliva. Comparing subjects with elevated levels of urea in bothblood serum and whole unstimulated saliva, parallel high levels were found in 71.4% of patients (50 persons).

The comparison between blood creatinine and salivary creatinine in patients with elevated levels revealed parallel increased levels in 68.5% of patients (48 subjects). The results indicated blood serum values higher than the reference range in 100% of patients and salivary values higher than the normal level in 68.5% of patients.

Discussion

The data obtained from the present study demonstrated that following an end-stage CKD replacement therapy, aiming at excess fluid management with ultrafiltration and removal of accumulated waste nitrogenous products, such as urea and creatinine, their values were significantly reduced but still remained higher than the reference values. Similar results are observed by Baria D. et al. in their study (2013)[13]. Suresh G. et al. established a statistically significant correlation between blood urea and salivary urea levels in CKD patients undergoing hemodialysis (2014) [11]. The authors concluded that salivary urea tests could be used in place of blood tests (especially with children on renal replacement therapy). Lasisi TJ. et al. conducted a similar study (2016), examining urea and creatinine levels in blood serum and in whole unstimulated saliva for patients with end-stage CKD and healthy controls. The researchers established a positive correlation between blood and salivary creatinine as well as urea levels in patients undergoing dialysis treatment [14]. The positive correlation between urea and creatinine levels, tested in blood serum as well salivary medium, was equally reflected in studies carried out by Zuniga MEt al. (2012), Peng CH. et al. (2013) and Venkatapathy R. et al. (2014) [15–17]. The ROC analysis revealed that urea and creatinine tests in salivary medium had limited use in patients with renal failure. The present study indicated a positive correlation between urea and creatinine levels in whole unstimulated saliva as compared to the corresponding levels in blood serum. However, while after the dialysis procedure a significant reduction in residual nitrogen fractions in blood serum were greatly reduced, urea and creatinine values in whole unstimulated saliva were found to be without change and remained statistically significant. The data obtained from precise T-tests for paired samples between urea and creatinine levels taken prior to and after the hemodialysis procedure, for the purposes of the present study, indicated that despite the parallel increase of either levels in blood serum (P= 0.000) and in whole unstimulated saliva (P=0.240 for urea levels, P = 0.065 for creatinine levels), saliva as a medium could not be used as sufficient evidence for the effectiveness of the dialysis treatment.

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