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RESEARCH ARTICLE

Comparison of serum and salivary creatinine levels in preterm neonates

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ABSTRACT

Introduction: In recent years, saliva has been frequently tested as an alternative biological sample for diagnosing various diseases. Various substances reach the saliva through endogenous synthesis in acinar cells or from plasma. Passive diffusion, transudation, diffusion, or selective transport are how these substances cross from plasma to saliva. There are a small number of studies in children and adults that have examined renal function and the ratio of serum and salivary creatinine. To date, no study has been conducted that examined the existence of this correlation in neonates. Our study aimed to examine whether there is a correlation between serum and salivary creatinine values in preterm infants.

Methods: We conducted a prospective study that included 30 neonates, in whom serum and salivary creatinine levels were measured simultaneously in two-time spots.

Results: The mean value of salivary to serum creatinine (sCr) ratio was 0.700. Salivary to sCr ratio was statistically significantly higher in newborns of gestational age (GA)<28 gestational weeks (mean value 0.825), compared to children with GA≥28 gestational weeks (mean value 0.566), student t-test; p=0.003. Logistic regression showed that the correlation between serum and salivary creatinine levels was more coherent in newborns with GA<28 weeks.

Conclusions: In this study, it was examined for the first time whether there is a correlation between the values of serum and salivary creatinine in preterm infants. We found that the correlation between serum and salivary creatinine levels is strong in newborns with GA<28 weeks.

Keywords: creatinine, serum, saliva, premature, neonates

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INTRODUCTION

Acute kidney injury (AKI) is often diagnosed in neonatal patients who are treated at the department of neonatal intensive care (NICU). The mortality of those newborns is very high. Serum creatinine (sCr) levels are still used as the gold standard for diagnosing AKI. Determining the concentration of sCr requires repeated blood sampling. This procedure is invasive, and due to repeated blood sampling, it is often accompanied by anemia in this population of children. For these reasons, attempts are being made to find new, less traumatic, and less invasive ways to determine various laboratory parameters. In recent years, saliva has been frequently tested for this purpose, as an alternative biological sample for diagnosing many systemic diseases. Various substances reach the saliva through endogenous synthesis in acinar cells or from plasma. Passive diffusion, transudation, diffusion, or selective transport are how these substances mature from plasma to saliva. Saliva has been used the most so far in diagnosing endocrine, cardiovascular, autoimmune, and infectious diseases. There are also a small number of studies in children and adults that have examined renal function and the ratio of serum and salivary creatinine. To date, no study has been done that examined this correlation in preterm neonates.

Our study aimed to examine whether there is a correlation between serum and salivary creatinine values in preterm infants with and without AKI.

MATERIALS & METHODS

The study was prospective and included 30 premature infants treated at the department of neonatal intensive care and therapy of the Institute for Children and Youth Healthcare of Vojvodina in Novi Sad.

The study was prospective, and it included 42 premature newborns who were treated at the department of neonatal intensive care and therapy of the Institute for Children and Youth Healthcare of Vojvodina between 15.01.2022. and 10.05. 2022 in Novi Sad, Serbia.

Newborns in whom sCr analysis was not performed were excluded from the study, as well as those in whom it was not technically possible to collect enough saliva for analysis. Also, if there was an admixture of blood in the saliva samples due to mechanical injury of the mucous membranes of the oral cavity, no further analysis was done. The final number of the neonates in the study group was 30.

Gestational age (GA), Apgar scores (ASs) in the 1st and 5th minute, birth weight (BW), and birth height were analyzed in all patients.

From the second day of life, all subjects had their sCr values monitored - every day or as needed, depending on the child's clinical condition. Simultaneously with blood sampling to determine the value of sCr, a saliva sample was taken from the patient.

The saliva samples were taken, as follows: two holes were made in the test tube with the lid through which the tubes were passed (**Figure 1**). One tube was connected to the aspirator and the other was placed in the oral cavity of the newborn and saliva was aspirated (the volume of aspirated saliva in each sample was around one ml).

Both serum and saliva samples were frozen and kept at -20 °C. When all the samples were collected, they were thawed, and laboratory analyses were performed. Serum and saliva samples were de-frozen and mixed using a vortex mixer before testing and analyzed on Beckman Coulter AU 480 automatic analyzer using the reagents from the same manufacturer. The principle of the creatinine assay is a spectrophotometric assay with alkaline picrate, following Jaffe's modified method.

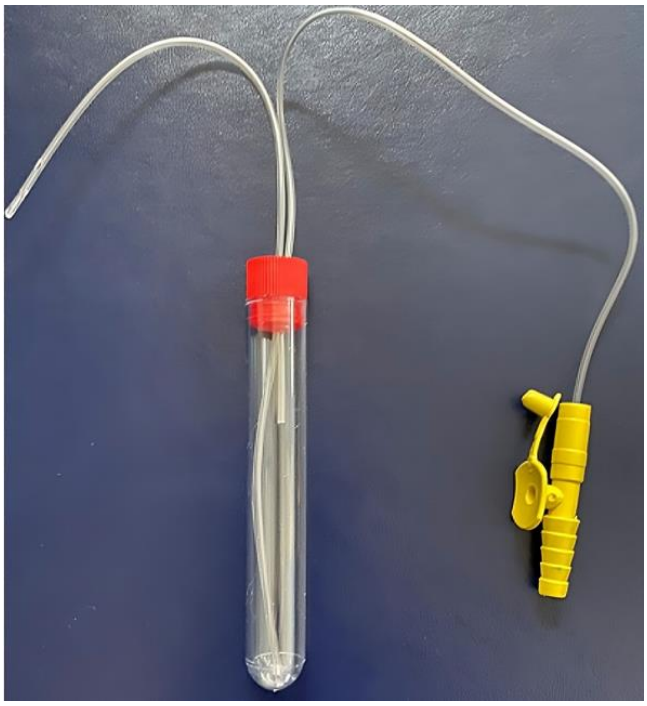

Figure 1. Equipment used for collection of saliva samples (Source: Authors)

At an alkaline pH, creatinine in the sample reacts with picrate to form a creatinine-picrate complex. The rate of increase in absorbance at 500 nm due to the formation of this orange-colored complex is directly proportional to concentration of creatinine in the sample.

RESULTS

The study included 30 patients, to whom serum and salivary creatinine levels were measured simultaneously in two-time spots. GA, ASs, BW, birth height, and mean values of serum and salivary creatinine levels from both measurements are shown in **Table 1**.

The correlation between serum and salivary creatinine levels was determined by Spearman's test of linear correlation. Correlation is considered strong at R=0.5 to 1.0, moderate at R=0.3 to 0.5, weak at R=0.1 to 0.3, very weak or no correlation at R<0.1.

Table 1. GA, ASs, BW, birth height, & mean values of serum & salivary creatinine levels from both measurements

	GA (weeks)	AS 1 minute	AS 5 minute	BW (g)	Height (cm)	sCr (µmol/l)	Saliva Cr (µmol/l)
Mean	29.271	5.088	6.235	1,283.333	38.611	92.872	60.117
Standard deviation	2.751	2.301	2.189	508.180	4.800	30.279	26.476
2SD	5.501	4.602	4.378	1,016.360	9.601	60.558	52.952
Minimum	24.560	1.000	1.000	630.000	31.000	44.800	12.000
Maximum	34.140	9.000	10.000	2,650.000	48.000	210.700	143.000

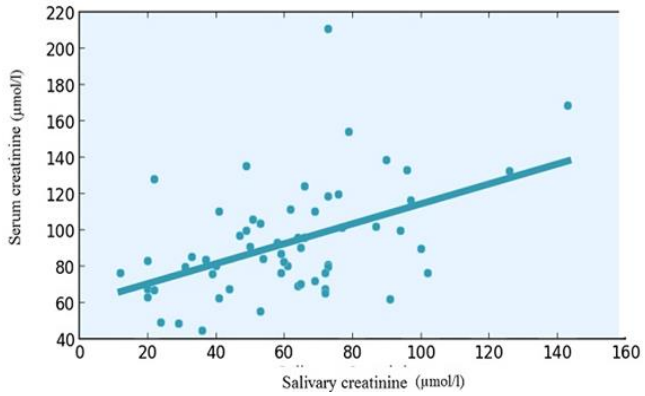

Figure 1. Correlation between serum & salivary creatinine levels (Source: Authors' own elaboration)

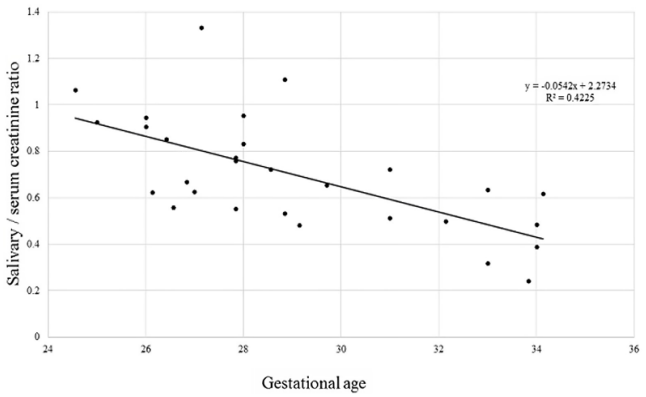

Figure 2. Correlation of salivary to sCr ratio with GA (Source: Authors' own elaboration)

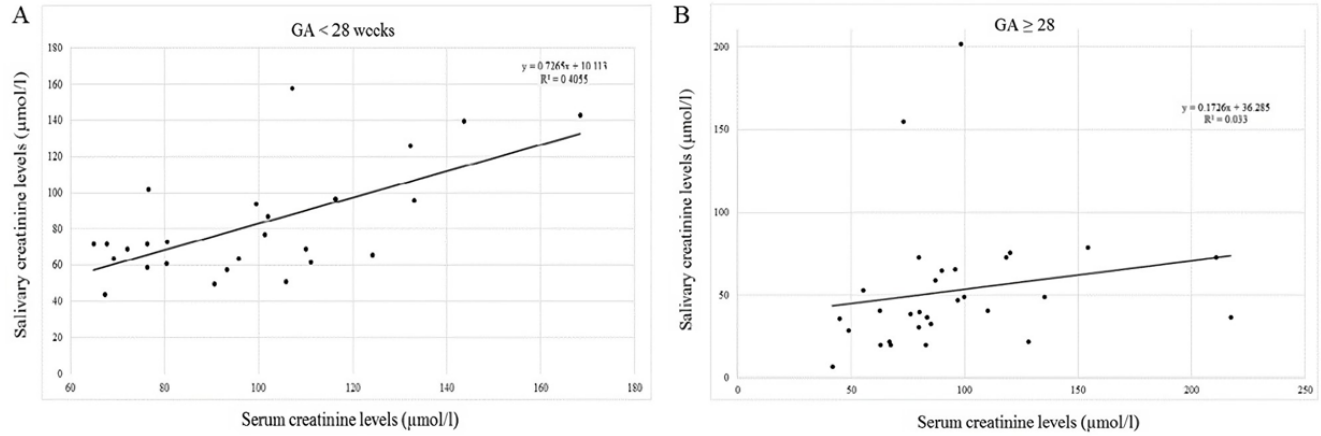

Figure 4. Correlation between sCr & salivary creatinine levels in newborns with GA<28 weeks (A) & GA≥28 weeks (B) (Source: Authors' own elaboration)

In our sample, Spearman's test of linear correlation showed moderate correlation between serum and salivary creatinine levels (p-value<0.001 [4.628e-4]; Spearman's R statistic: 0.438; degrees of freedom (df): 58; linear regression details: slope: 0.549; & intercept: 59.869) (**Figure 2**).

The mean value of salivary/sCr ratio was 0.700. This ratio was statistically significantly higher (student t-test; p=0.003) in newborns of GA<28 gestational weeks (mean value 0.825), compared to children with GA≥28 gestational weeks (mean value 0.566). Correlation between salivary and sCr ratio with GA is shown in **Figure 3**.

Logistic regression showed that correlation between serum and salivary creatinine levels was more coherent in newborns with GA<28 weeks (part A in **Figure 4**). On the other hand, correlation between serum and salivary creatinine levels in newborns with GA≥28 weeks, was very weak (part B in **Figure 4**).

DISCUSSION

Prematurely born newborns are a very vulnerable group of patients. Blood sampling is a very invasive method that can lead to additional complications in those already severely ill and fragile patients. AKI is frequently diagnosed in preterm infants treated at NICU. The diagnosis of AKI is confirmed based on monitoring the increase in sCr concentration, so for these purposes, blood must be sampled very often. The ability to noninvasively assess the clinical status of a neonate is a goal of modern medicine. So, nowadays, more and more research is being done to find less invasive ways to monitor and diagnose different diseases, including renal disease.

Saliva is a biofluid that is much easier to sample than blood. It is a multi-constituent biologic fluid secreted by the salivary glands. Although saliva mainly consists of water, it is a source of diverse proteins, genetic material (DNA and RNA), metabolites, and microorganisms. Through both extracellular and intracellular trafficking mechanisms, biomarkers enter the oral cavity and may be monitored to inform an investigator about overall health and systemic disease in a patient. Saliva has the benefits over other noninvasively obtained biofluids, such as urine or stool, in that it may be obtained on demand and repeatedly in a limited time frame. These advantages make it an ideal biofluid for non-invasive clinical assessment across a wide variety of healthcare settings, including in the very vulnerable newborn population. There are several studies

conducted in the adult population, with promising results, which show that saliva can be used to detect lung, pancreatic, or breast cancer, and type II diabetes. But to bring it to a clinical reality we need further scientific validation for each disease and required to benchmark the diagnostic capacity of saliva against other bodily fluids [1-9].

On the other hand, there have been very few, if any, studies conducted to examine the impact that the developing salivary glands may have on salivary constituents in the human newborn. It seems that the final structural and functional maturation of salivary glands happens late during fetal development. For example, terminal differentiation of the submandibular gland's end buds into secretory acini is apparent by 19-24 weeks in humans and this is followed by further growth and differentiation until the immature organ is capable of nerve-stimulated secretion forms (occurring at birth) [10, 11].

The saliva of a newborn differs from its adult counterpart. Filtration and diffusion mechanisms may be affected by the ongoing development of each gland, further altering salivary substrates in a neonate. These effects may influence data obtained on neonatal subjects and care should be made when extrapolating adult saliva studies to this population and/or combining samples obtained from neonates of different GA (i.e., different developmental stages of salivary glands).

In this study, it was examined for the first time whether there is a correlation between the values of serum and salivary creatinine in preterm infants.

We found that in preterm neonates' concentration of Cr in saliva is higher (mean value 60.12±52.95 μmol/l) than those reported in studies in adults. The values of salivary Cr levels represent around 2/3 of serum Cr levels. The mean value of the salivary to serum Cr ratio was 0.7. This ratio was statistically significantly higher (p=0.003) in newborns with GA<28 gestational weeks (mean value 0.825) compared to children with GA≥28 gestational weeks (mean value 0.566). In similar studies in adults, salivary Cr levels were much lower than serum concentrations, even in patients with advanced chronic kidney disease, like it is reported in [12] (3 μmol/L to 400 μmol/L, with a median of 11 μmol/L for salivary Cr vs 46 μmol/L to 1581 μmol/L, with a median value of 134 μmol/L for serum Cr).

This fact is usually explained by the characteristics of the Cr molecule itself. Creatinine is a large molecule, with a high molecular weight (113 Da) and molecular radius of 3.2 A, and it is maintained at constant blood levels by kidneys. Also, creatinine exhibits low lipid solubility. Thus, in a healthy state under normal conditions, owing to its physical properties, it is unable to diffuse easily across the cells and the tight intercellular junction of the salivary glands [13, 14]. In the literature, we did not find an exact explanation for our results. So, we hypothesized that altered salivary secretion of creatinine may be the consequence of structural or functional immaturity of the salivary glands of those neonates. Also, most of the subjects in the study were sick children and, as it is postulated in some literature sources - in the disease states (even in adults), possibly there is an alteration in the permeability of the salivary gland cells [15].

In most of the studies performed in adults, serum and salivary creatinine levels correlated well (r=0.82) [16]. In our study, we also found a moderately strong positive correlation between serum and salivary creatinine levels. Logistic regression showed that the correlation between serum and salivary creatinine levels was more coherent in newborns with GS<28 weeks. On the other hand, the correlation between serum and salivary creatinine levels in newborns with GA>28 weeks, was very weak. We think that a good correlation between salivary and sCr levels in less mature neonates is the reflection of "less controlled and more passive" diffusion of creatinine from the blood to saliva (due to salivary gland morphologic and functional immaturity) combined with low renal clients (due to renal immaturity, which is well described in literature), so the levels of salivary creatinine are directly and almost linearly dependent of serum concentrations of creatinine.

Poor correlation between salivary and sCr levels in more mature neonates (>28 GW) may be explained by the fact that salivary glands and kidneys are more mature in those neonates, which in turn results in less creatinine secretion in saliva and higher renal creatinine clients. Different speeds of maturation of the salivary glands and kidneys, i.e., different times of achieving full control of salivary creatinine excretion (what happens earlier) and achieving full levels of renal clearance of creatinine (happens later), may lead to discrepancies in serum and salivary creatinine levels and cause poor statistical correlation between these values. We assume that after achieving full control of salivary creatinine excretion and achieving full renal creatinine clearance (around two years of age), a balanced state is achieved between the mechanisms that control salivary creatinine secretion and renal creatinine excretion and that good correlation between serum and salivary creatinine levels is established after that age, as it is reported in studies in the adult population.

CONCLUSIONS

Based on the positive correlation between the serum and saliva creatinine levels observed in the present study, we think that saliva creatinine analysis may be considered as a non-invasive alternative to blood analysis in preterm neonates GA less than 28 GW, but not in more natural neonates. Salivary creatinine can only be accepted as an alternative diagnostic method if it is comparable to sCr in its ability to differentiate between those with and without kidney injury, so further research on this subject is needed.

Author contributions: VDS, NAB, MDJ, LZV, MDM, SLL, & JMD: study conception, design, material preparation, & data collection & data analysis; **VDS:** writing first draft; & **NAB, MDJ, LZV, MDM, SLL, & JMD:** comment on previous versions. All authors have agreed with the results and conclusions.

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Ethics statement: The authors stated that the study was approved by Ethics Committee of the Institute for Child and Youth Healthcare of Vojvodina on 22 February 2018 (Approval code: 611-11). Informed consents were obtained from the participants.

Declaration of interest: No conflict of interest is declared by the authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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