Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

IJBMS

Evaluation of urinary enzymes in newborns treated with gentamicin

Ali Mohammadi Karakani [1, 2], Solmaz Asgharzadeh Haghighi [1], Mahmoud Ghazi-Khansari [3*], Afsaneh Seyed-Ebrahimi [1], Sharareh Zeighami-Mohammadi [4]

[1] Alborz Hospital, Social Security Organization, Karaj, Iran
[2] Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[3] Departments of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[4] Nursing and Midwifery, Islamic Azad University of Karaj, Iran

ARTICLE INFO

Article type:
Short communication

Article history:
Received: Jun 29, 2013
Accepted: Dec 31, 2013

Keywords:
Biomarker
Gentamicin
Newborn
Urine

ABSTRACT

Objective(s): In recent years, there has been an increasing amount of study on early diagnosis of kidney injury through sensitive and specific biomarkers. We examined the practical applicability of the urinary levels of NAG (N-acetyl-β-D-glucosaminidase), AP (alkaline phosphatase), and LDH (lactate dehydrogenase) as renal dysfunction screening biomarkers in full and pre-term newborns treated with gentamicin.

Materials and Methods: Fourteen pre-term and fifteen full-term newborns who received gentamicin for suspected infections were enrolled. Serum and urine specimens were obtained before the zero days and after gentamicin infusion on the 1st, 3rd, and 5th days of treatment.

Results: In full-term newborns a significant increase in urinary NAG, LDH, AP after 5 days of gentamicin administration compared with control group was noted (P<0.05, P<0.001 and P<0.01; respectively).

Conclusion: Our findings indicate that urinary enzymes may be useful in full-term newborns as a non-invasive method for evaluation of tubular function.

► *Please cite this paper as:*
Mohammadi Karakani A, Asgharzadeh Haghighi S, Ghazi-Khansari M, Seyed-Ebrahimi A, Zeighami-Mohammadi Sh. Evaluation of urinary enzymes in newborns treated with gentamicin. Iran J Basic Med Sci 2014; 17:391-395.

Introduction

Gentamicin is commonly used in newborn infants with suspected or proven bacterial infections in neonatal units throughout the world despite its risk of nephrotoxicity on renal tubular cells (1). Based on evidence available in the current literature, main approaches toward reduction of renal toxicity of gentamicin are once-daily administration, administration time, usage of antioxidant substances, antagonists of megalin, and early detection of renal damage biomarkers (1, 2). The traditional clinical biomarker indicators, serum creatinine, and blood urea nitrogen, are used for evaluation of drug-induced nephrotoxicity. Unfortunately, these parameters are non-specific, insensitive, unreliable, and have time delay between renal injury and detection (2, 3). On the other hand, delay in diagnosis and monitoring of kidney injury may adversely impact clinical decision and clinical outcome; therefore, various parameters in urine/plasma (i.e. enzymes, antigens, cytokines) have been examined to detect minor changes in proximal tubular function (3, 4).

Urinary excretion of renal tubular enzymes, especially N-acetyl-β- D-glucosaminidase (NAG) and alkaline phosphatase (AP) are recommended as useful parameters for renal tubular impairment (2). In addition, the urinary enzymes are considered a relatively simple, cheap, fast, and non-invasive laboratory technique in the detection and follow-up of renal disorders (3). Some previous studies have reported on the evaluation of renal function such as urinary electrolyte excretion (5), while other studies have shown the benefits of other biomarkers such as urinary enzymes for the early detection of drug-induced renal injury in infants (6, 7). In this study, we examined the changes in urinary levels of NAG, AP, and LDH (lactate dehydrogenase) in full and pre-term newborns treated with gentamicin to assess their usefulness as renal biomarkers. Therefore, we selected biochemical parameters to evaluate renal function including urinary levels of microalbumin as well as serum creatinine and urea levels for assessment of the glomerular filtration, fractional

*Corresponding author: Mahmoud Ghazi-Khansari. Departments of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. Tel/Fax: +98-21-66402569; email: ghazikha@sina.tums.ac.ir

Table 1. Biochemical parameters of pre and full-term newborns treated with gentamicin and control group (healthy infants) (mean ± SD)

Parameter	Days	Pre-term infants (n=14)	Full-term infants (n=15)	Healthy infants (n=15)
Serum creatinine (mg/dl)	0	0.76 ± 0.3	0.75 ± 0.22	0.74 ± 0.25
	1	0.77 ± 0.25	0.78 ± 0.28	
	3	0.8 ± 0.22	0.75 ± 0.2	
	5	0.85 ± 0.25	0.77 ± 0.18	
Serum urea (mg/dl)	0	25.2 ± 4.8	25.9 ± 4.1	25.5 ± 3.7
	1	26.8 ± 5.4	26.1 ± 3.5	
	3	26.5 ± 6.0	25.5 ± 4.0	
	5	27.1 ± 5.5	25.1 ± 3.0	
FE (Na) %	0	1.1 ± 0.5	1.2 ± 0.4	1.5 ± 0.8
	1	1.4 ± 0.7	1.3 ± 0.4	
	3	2.8 ± 1.5 **(b)	1.7 ± 1.0	
	5	3.0 ± 1.4 **(c)	2.2 ± 0.9 (b)	
Urinary LDH (U/ g Cr)	0	220 ± 15	195 ± 25	205 ± 35
	1	215 ± 25	220 ± 25	
	3	235 ± 50	195 ± 15	
	5	370 ± 35 ***(c)	320 ± 45 ***(c)	
Urinary AP (U/ g Cr)	0	230 ± 85	200 ± 40	225 ± 55
	1	250 ± 70	250 ± 45	
	3	290 ± 120	270 ± 50(b)	
	5	345 ± 75 ***(b)	330 ± 70 **(c)	
Urinary albumin (mg/g Cr)	0	15.4 ± 4.6	15.1 ± 1.4	17.5 ± 5.1
	1	17.9 ± 2.4	15.9 ± 1.3	
	3	18.9 ± 3.4	17.3 ± 2.1(b)	
	5	20.1 ± 6.9	16.7 ± 1.5	

Significant difference between control group (healthy infants) and pre and full-term newborns treated with gentamicin groups * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Significant difference between zero day and other days of gentamicin administration of pre and full-term newborn groups [a] $P<0.05$; [b] $P<0.01$; [c] $P<0.001$ Abbreviations: FE Na% (fractional excretion of sodium), LDH (lactate dehydrogenase) and AP (alkaline phosphatase)

excretion of sodium (FE Na%) for investigation of the tubular re-absorption capacity, urinary activities of NAG and AP for survey of the structural integrity of renal proximal tubules, and LDH for its distribution over most parts of nephron.

Materials and Methods

Patients and healthy subjects

We identified 29 participants of a total of two hundred participants admitted between February 2009 and September 2011 to the Pediatric Ward of Alborz Hospital, Karaj, Iran. Using the below criteria (5), fourteen pre-term newborns (8 males and 6 females) with gestational age of 32–36 weeks and fifteen full-term newborns (8 males and 7 females) with gestational age of 38–40 weeks who were treated for suspected infections with gentamicin (including neonatal sepsis, pneumonia, prolonged rupture of membrane, or meconium aspiration syndrome) in the first week of life, were enrolled. As the control group, fifteen healthy infants (in the first week of life with gestational age 38–42 weeks) were included in this study. Briefly, inclusion criteria were infants with normal respiratory and metabolic stability through blood gas analysis (pH: 7.25–7.45, pO_2:50–70 mm Hg, pCO_2:≤55 mmHg) and exclusion criteria were history of prenatal asphyxia or shock, renal impairment [Normal Glomerular Filtration Rate (GFR) is 17–60 ml/min/1.73 m^2], hypotension, use of other potentially nephrotoxic drugs, and polycythemia (hematocrit level of greater than 65%). The administered gentamicin dose was 1.5 mg/kg/8 hr intravenously over a 30 min period via an intravenous drip infusion in a total volume of 5 ml of 5% dextrose solution over a period of 5 days. The parental informed consent was obtained before study and the study protocol was approved by the Research Committee of Social Security Organization Hospital, Karaj, Iran.

Collection of urine and blood samples

Urine and blood specimens were obtained from the subjects before and after gentamicin infusion on the 1st, 3rd, and 5th days of treatment. Urine specimens were obtained over a 2 to 3 hr period using adhesive bags. To obtain serum, venous blood samples were

collected into test tubes without any anticoagulant. Urine and blood samples were centrifuged (2000×g for 10 min) immediately after collection.

Biochemical laboratory parameters

All chemicals used were of analytical grade. PNP (ρ-nitrophenol) & PNP-NAG (ρ- nitrophenyl -N-acetyl-β-D-glucosaminide) were obtained from Sigma Chemical co., St. Louis, Mo, USA. All other required chemicals were purchased from Merck, GmbH, Germany. NAG activity in urine was measured at 37°C, according to the described method (7). Briefly, the reaction mixture consisted of ρ- nitrophenyl-N-acetyl-β-D-glucosaminde (PNP) as substrate in 25 mmol of acetate, or 100 mmol of citrate (pH 4.8) incubated with 0.025 ml of urine and a photometer (Model ECOM-E 6125, Eppendorf Inc. Germany) at 405 nm.

Serum and urinary creatinine and urinary urea, AP, LDH, and microalbuminuria were measured by using commercially available assay kits (Pars Azmun Co. Ltd, Iran) by Jaffe, Urease-GLDH, DGKG, DGKG, and the immunoturbidometry method respectively. All these tests were measured with the Hitachi 902 autoanalyser (Hitachi Ltd., Japan). Sodium was determined using flame photometry (Model EFOX 5054, Eppendorf Inc. Germany). FE Na (%) expresses the fractional excretion of the sodium, which is calculated as follows:

FE (Na) = Urine Na × Serum Cr / Serum Na × Urine Cr.

To correct for variations in urine flow, the urinary enzyme activities (U/l) were normalized to urinary creatinine concentration (g/l) and given as U/g creatinine.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD). The difference between different days was analyzed using one way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. Statistical significance was accepted at $P<0.05$ for all comparisons and in all correlations.

Results

Table 1 depicts the routine biochemical parameters of newborns treated with gentamicin and the control group (Healthy Infants). For serum creatinine, blood urea nitrogen, and urinary albumin no differences between the control group and treated groups were detected. In pre-term newborns, a statistically significant increase in FE Na (%) was noted on the 3rd and 5th days of therapy compared with control group ($P<0.01$ and $P<0.01$; respectively). Moreover, in full-term newborns a statistically significant increase was observed in FE Na (%) the 5th day of therapy compared with before gentamicin administration ($P<0.01$). During gentamicin treatment, a statistically significant increase in urinary LDH was noted in both pre-term and full-term newborns on the 5th day compared with the control group ($P<0.001$ and $P<0.001$; respectively) as well as before gentamicin administration in pre-term and full-term newborns compared with 5th day of therapy ($P<0.001$ and $P<0.001$; respectively). Additionally, a statistically significant increase in urinary AP was noted in both pre-term and full-term newborns on the 5th day compared with the control group (P<0.001 and $P<0.01$; respectively). Finally, in pre-term newborns a statistically significant increase was observed in urinary AP on the 5th days compared with before gentamicin administration ($P<0.01$). Furthermore, in full-term newborns a statistically significant increase was observed in urinary NAG on 3rd and 5th days compared with before gentamicin administration ($P<0.05$, $P<0.001$ and $P<0.001$; respectively).

As shown in Figure 1, a statistically significant decrease was observed in urinary NAG on first (185±60), 3rd (75±30), and 5th (43±5) days compared with before gentamicin administration in pre-term newborns (250 ± 55; $P<0.01$, $P<0.001$, and $P<0.001$; respectively). In contrast, in full-term newborns a statistically significant increase was observed in urinary NAG on first (29±2), 3rd (35±3), and 5th (61±4) days compared with before gentamicin administration (25±4; $P<0.05$, $P<0.001$, and $P<0.001$; respectively). During gentamicin treatment, a statistically significant increase in urinary NAG was observed in the full-term newborns on the 5th day of therapy (61±4) compared with the control group (26.5±2.5; $P<0.05$). Additionally, in the pre-term newborns a statistically significant increase in urinary NAG was observed before (250±55) and on first (185± 60) and third (75±30) days after gentamicin administration compared with control group (26.5±2.5; $P<0.001$, $P<0.001$, and $P<0.001$; respectively).

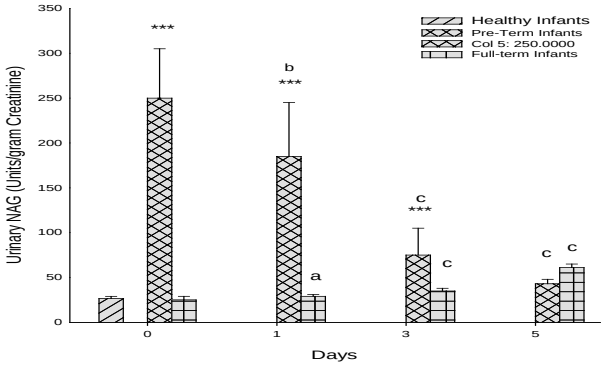

Figure 1. Mean values ± SD of urinary NAG (Units per gram Creatinine) in pre and full-term infants as well as control groups (Healthy Infants) on days 1, 3, and 5 after gentamicin administration. Time zero is defined as the time of gentamicin administration

Significant difference between control group (Healthy Infants) and pre and full-term newborns treated with gentamicin groups * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Significant difference between zero day and other days of gentamicin administration of pre and full-term newborn groups [a] $P<0.05$; [b] $P<0.01$; [c] $P<0.001$

Discussion

In pediatric clinical practice, the most commonly used conventional blood parameters of kidney injury assessment remain serum creatinine and urea (3). Our study, however, found no significant differences in these markers between neonatal patients treated with gentamicin and control group. On the other hand, a previous study reported that aminoglycosides nephrotoxicity caused non-oliguric renal failure with a slow rise in serum creatinine (8). This contradicts other studies which speculate that gentamicin-induced nephrotoxicity, which occurred in proximal tubules and serum creatinine, is a marker of glomerular filtration in renal function (7, 9). Moreover, Tugay *et al* (10) showed that gentamicin can cause a significant increase in the urine albumin ratio during treatment, which is contrary to our data. We attribute this difference between results to study design and the sample size.

As shown in Figure 1, urinary NAG in the full-term newborns may be useful for evaluation of minor effects on tubular function. These results are consistent with other studies showing that urinary NAG can act as indicator of renal damage in early stages (7, 11). In contrast, a previous study demonstrated a 10-day gentamicin treatment in neonates without any changes in NAG activity (12). The difference between our results and the mentioned study may be due to the study design and differences in laboratory methods used for measurement of biomarkers. Furthermore, clinical studies have demonstrated that gentamicin treatment in pre-term neonates results in increased urinary NAG and claim that it could be used for the assessment of antibiotic-induced renal injury in these patients (13, 14). In the present study, however, we found that urinary NAG was decreased during gentamicin administration. Possibly, activity of urinary NAG in pre-term neonates reflects the maturity of renal tubular epithelium (15), therefore, urinary NAG may be used for evaluation of kidney maturity after birth. Overall, the difference between above studies may partly be due to the differences in study design, sample size, dose administration, length of hospitalization, and other confounding factors.

Urinary electrolyte excretion in sick newborns depends on nutrition, electrolyte balance, and the rate of diuresis as well as the arterial blood pressure; and since sick newborns are usually salt-losers and at risk of developing a negative Na balance (5), adjustment of normal electrolyte balance is difficult. Since evaluation of renal function through urinary electrolyte excretion has the previously mentioned limitations, the increase in FE Na% during treatment with gentamicin in both groups, was not considered a definite biomarker of renal dysfunction in our study.

As a final remark, the results of our study also showed that in both full and pre-term newborns, urinary LDH and AP increased on the 5th day of gentamicin administration. Since AP is a brush–border membrane enzyme with high sensitivity for tubular damage while LDH is found in most parts of the nephron, it can be assumed that these urinary enzymes may be helpful in renal injury detection in the early stages.

Limitations of this study are due to the small patient sample size, the untreated pre-term newborn group, and ROC curve analysis. Thus, future studies with larger sample size and ROC curve analysis with sensitivity and specificity of all urinary enzymes are mandatory to confirm the gentamicin induced renal injury in this population.

Conclusion

In conclusion, the most obvious finding to emerge from this study is that urinary NAG as well as urinary LDH and AP may be useful particularly in full-term newborns for evaluation of tubular function when monitoring of serum gentamicin is not possible. In addition, in pre-term infants urinary LDH and AP may be helpful as biomarkers for evaluation of renal insufficiency.

Acknowledgment

We are grateful to the Hospital Manger as well as Pediatric Unit staff at Alborz Hospital, Karaj, Iran, the participants, and their families.

References

1. Chattopadhyay B. Newborns and gentamicin--how much and how often? J Antimicrob Chemother 2002; 49:13–16.
2. Vaidya VS, Bonventre JV. Mechanistic biomarkers for cytotoxic acute kidney injury. Expert Opin Drug Metab Toxicol 2006; 2:697–713.
3. Skalova S. The diagnostic role of urinary N-acetyl-beta-D-glucosaminidase (NAG) activity in the detection of renal tubular impairment. Acta Medica 2005; 48:75–80.
4. Sharifipour F, Zeraati A, Sahebari M, Hatef M, Naghibi M, Rezaieyazdi Z, *et al.* Association of urinary lipocalin-2 with lupus nephritis. Iran J Basic Med Sci 2013; 16:1011-1015.
5. Giapros VI, Cholevas VI, Andronikou SK. Acute effects of gentamicin on urinary electrolyte excretion in neonates. Pediatr Nephrol 2004; 19:322–325.
6. Colding H, Brygge K, Brendstrup L, Bentzon MW, Andersen GE. Enzymuria in neonates receiving continuous intravenous infusion of gentamicin. APMIS 1992; 100:119–124.
7. Mohammadi-Karakani A, Asgharzadeh-Haghighi S, Ghazi-Khansari M, Seyed-Ebrahimi A, Ghasemi A, Jabari E. Enzymuria determination in children treated with aminoglycosides drugs. Hum Exp Toxicol 2008; 27; 879–882.
8. Espandiari P, Zhang J, Rosenzweig BA, Vaidya VS, Sun J, Schnackenberg L, *et al.* The utility of a rodent model in detecting pediatric drug-induced nephrotoxicity. Toxicol Sci 2007; 99:637-648.

9. Mingeot-Leclercq MP, Tulkens PM. Amino-glycosides: nephrotoxicity. Antimicrob Agents Chemother 1999; 43:1003–1012.
10. Tugay S, Bircan Z, Caglayan C, Arisoy AE, Gökalp AS. Acute effects of gentamicin on glomerular and tubular functions in preterm neonates. Pediatr Nephrol 2006; 21:1389–1392.
11. Skalova S, Rejtar P, Kutilek S. Urinary N-acetyl-beta-D-glucosaminidase (U-NAG) activity in children with vesicoureteral reflux. Bratisl Lek Listy 2009; 110:69-72
12. Davidovic-Plavsic B, Vujic T, Uletilovic S, Predojevic-Samardzic J, Malcic D, Sanicanin Z. Urinary Activities of Proximal Tubule Enzymes in Neonates Treated with Gentamicin. J Med Biochem 2010; 29:44–47.
13. Watanabe K, Kojima T, Fukuda Y, Ohbayashi K, Kobayashi T, Iwase S. Reliability of urinary N-acetyl-beta-D-glucosaminidase as an indicator of renal tubular damage in neonates. Biol Neonate 1987; 52:16–21.
14. McWilliam SJ, Antoine DJ, Sabbisetti V, Turner MA, Farragher T, Bonventre JV, *et al*. Mechanism-based urinary biomarkers to identify the potential for aminoglycoside-induced nephrotoxicity in premature neonates: a proof-of-concept study. PLoS One 2012; 7:e43809.
15. Tsukahara H, Hori C, Tsuchida S, Hiraoka M, Sudo M, Haruki S. Urinary N-acetyl-beta-D-glucosaminidase excretion in term and preterm neonates. J Paediatr Child Health 1994; 30:536–538.