American Journal of Clinical Medicine Research, 2018, Vol. 6, No. 2, 20-23
Available online at http://pubs.sciepub.com/ajcmr/6/2/1

DOI:10.12691/ajcmr-6-2-1

Detection of Urinary Epithelial Sodium Channel (ENaC) Protein

Yulia Sofiatin[1,*], Rully MA Roesli[2]

[1]Department of Public Health, Faculty of Medicine, Universitas Padjadjaran Bandung, Indonesia
[2]Department of Internal Medicine, Faculty of Medicine, Universitas Padjadjaran Bandung, Indonesia
*Corresponding author: y.sofiatin@unpad.ac.id

Abstract Epithelial sodium Channel (ENaC) protein is an important substance in maintaining plasma sodium level. Its DNA sequences are similar in rat model with and without salt sensitivity, but the mRNA number increase in salt sensitive rat model with high salt diet. This condition is assumed to be similar in human. ENaC protein is abundant in lung, gut and kidney, and plays a similar physiological process but different diseases. ENaC protein levels have to be measured from specific locations; kidney will be the good source for hypertension related sodium excretion. The possibility of ENaC protein as a marker to screen salt-sensitivity is needed to be explored. The aim of this research is to explore the possibility of ENaC protein detection in urine. This is an observational descriptive study. Enzyme-linked immunosorbent assay (ELISA), using Cloud-Clone reagent catalog number SED337Hu, was used to detect and measure urinary and plasma ENaC protein level. For the first step of study, ELISA was conducted toward various dilution of healthy individual spot urine; in the 2nd step, several locations of repeated centrifuged spot urine and 24 hour collected urine were explored for the presence of ENaC protein; on the next step, 3 subjects, non-hypertensive, and hypertensive with and without family history of hypertension were recruited; for the fourth step, 13 (6 male and 7 female) non-hypertensive subjects were recruited; all steps are aimed to explore the detection of urinary EnaC protein level. ENaC proteins can be detected in both supernatant and sediment of centrifuged urine. In plasma of non-hypertensive, hypertensive with, and without family history of hypertension are 1.12 ng/ml, 2.7 ng/ml and 4.0 ng/mL respectively. ENaC protein levels from centrifuged urine at lower part of supernatant are lower but consistent with serum level. Mean ENaC protein level in non-hypertensive men are lower than women. Mean ENaC protein level in those with family history of hypertension are lower both in men and women. ENaC protein is detectable in spot urine; the levels differ by hypertension status, family history of hypertension and also by gender.

Keywords: *ELISA, ENaC protein, urine*

Cite This Article: Yulia Sofiatin, and Rully MA Roesli, "Detection of Urinary Epithelial Sodium Channel (ENaC) Protein." *American Journal of Clinical Medicine Research*, vol. 6, no. 2 (2018): 20-23. doi: 10.12691/ajcmr-6-2-1.

1. Introduction

ENaC is a transmembrane protein which regulates sodium exchange in several tissues, namely lung, gut and kidney [1,2,3]. Studies showed that ENaC structures are similar among species; it consists of 3 subunits, α, β, and γ [4]. Studies in animal and human kidney showed that there are 3 parts of tubules that involved in sodium regulation in the urine, they are distal convolute tubule, connecting tubule and collecting duct with similar ENaC protein expressions and its subcellular location differ as a response toward different sodium intake [2]. In salt sensitive and salt resistant rat models, there are no genetic differences in their DNA sequences [5], but their mRNA levels are differ [6,7]. mRNA levels of salt resistant rat are significantly higher; the transcript levels after 4 week high salt diet in salt sensitive rat are significantly higher than normal salt diet in salt resistant rat [6].

ENaC protein is abundant in lung, gut and kidney, and plays a similar physiological process but resulting different diseases [4,8,9]. The possibility of ENaC protein as a marker to screen salt-sensitivity is needed to be explored. ENaC protein levels have to be measured from specific locations; kidney will be the good source for hypertension related sodium excretion [10], but kidney biopsy will be too invasive for screening purposes. Urine is the closest source to kidney, but there is no publication about ENaC protein detection in urine yet. In conventional Elisa, substrate detection usually conducts toward supernatant, but is it high enough to be detected? Should the urine be concentrated? ENaC protein is heavy; logically it will be centrifuged near the sediment, should the supernatant near the sediment be used? Physiologically, ENaC protein will be destructed after its role in sodium excretion in the tubules, is this reagent able to detect fragmented ENaC protein?

The aim of this research is to explore the possibility of ENaC protein detection in urine.

2. Methods

This is a descriptive explorative study. Enzyme-linked immunosorbent assay (ELISA), using Cloud-Clone reagent

catalog number SED337Hu, were conducted following the enclosed instruction. There are 4 steps of studies, exploring the urinary epithelial sodium channel protein presence in:

1. Different urinary dilution

First step of the study was measuring the level of urinary ENaC protein with several dilution scenarios. Urine was taken from a normal male subject and diluted in phosphate buffer saline (PBS) solution, then centrifuged. Elisa was performed towards supernatant.

2. Different part of centrifuged urine.

Spot urine from a healthy person collected together with his serum and 24 hour collected urine from other healthy person collected for other purpose were treated similarly to explore the presence of ENaC protein in repeated centrifugation. A 100 ml, 50 ml, 40 ml, 30 ml, 20 ml and 10 ml urine were centrifuged; 10 ml of the lower part with the sediment were taken and re-centrifuged. Elisa was performed towards upper part of supernatant, which was taken around the surface; and the lower part of supernatant, which was taken about 1 cm above the sediment. Sediments were collected, manually grinded, diluted with PBS solution in several concentrations and then the ENaC protein level was measured by Elisa. Sediment is not a conventional sample for Elisa, but in the case of urinary ENaC protein, its possibility should be explored since the intact ENaC protein is pooled in the cytoplasmic vesicle of tubule cells.

3. Different subject conditions, which are normotensive subject and hypertensive subjects with and without family history of hypertension.

In the third step, serum and spot urines samples from those subjects were collected, the urines were centrifuged and ENaC protein was measured from different places of the centrifuged urine. Based on the result of step 1 study, urines were not diluted anymore.

4. Random subjects.

In this step, 13 adult subjects were randomly recruited; spot urine samples were collected, centrifuged, and ENaC protein level was measured from lower supernatant. Demographic characteristics, anthropometric measures and blood pressures were collected through interview, anthropometric measurement and digital blood pressure measurement respectively, following the standard protocol.

3. Results

Table 1 showed the result of urinary ENaC protein detection in several urine dilutions. ENaC protein is 0.2 ng/mL in non-diluted urine and is not detectable in diluted urine.

Table 2 showed the result of ENaC protein detection in serum and urine of healthy persons; urines were taken as spot urine and 24 hours urine collection, with multiple centrifugations.

Table 1. Urinary ENaC level with dilution, without centrifugation

Urine Dilution With PBS	ENaC Level (ng/mL)
No dilution	0.2
1:2	0.0
1:4	0.0
1:8	0.0
1:16	0.0
1:32	0.0
1:64	0.0
1:128	0.0

Table 2. Serum and Urinary ENaC protein level, with repeated urinary centrifugations

	ENaC Protein Level (ng/mL)	
serum	1.12	
	Morning Urine	24 hour Urine
100 ml	1.4	0.1
50 ml	0.6	0.2
40 ml	2.9	0.2
30 ml	0.8	0.2
20 ml	0.2	0.2
10 ml	0.4	0.3

ENaC protein level form multiple centrifugations are not consistent in spot urine, but relatively consistent in 24 hours urine. Moreover, single centrifugation urines, both spot and 24 hours urines reveal the similar ENaC protein levels.

Table 3. Urinary ENaC protein detection of grinded sediments with several PBS solution dilutions

Sediment Dilution	ENaC Protein Level (ng/mL)
1:2	5.8
1:4	15.6
1:8	25.6
1:16	65.6
1:32	99.2
1:64	345.6
1:128	332.8
1:256	68.3

The urinary sediment's ENaC protein detection reveals inconsistent high concentration levels.

Table 4.Urinary ENaC protein level in different subjects

Source Of Sample	ENaC Protein Level (ng/mL)		
	Non-hypertensive Subject	Hypertensive Subjects	
		With Family History Of Hypertension	Without Family History Of Hypertension
serum	1.12	2.7	4.0
upper supernatant	0.4	2.8	2.7
Lower supernatant	0.3	2.7	3.7
Sediment	2.8	2.5	2.3

Serum ENaC protein level is lowest in non-hypertensive subject and highest in hypertensive subject without family history of hypertension. Its level pattern in lower supernatant is similar with the serum level, but opposite to ENaC protein levels in sediment.

Table 5. Characteristics and urinary ENaC protein level in healthy subjects

Characteristics	Min	Max	Average
Age (years)	22	50	33.64
BMI (kg/m^2)	21.2	31	25.89
ENaC level (ng/mL)	0.2	2.7	1.75
• female	1.5	2.7	2.1
○ with family history of hypertension	1.5	2.5	1.9
○ without family history of hypertension	2.5	2.7	2.6
• male	0.2	2.0	1.35
○ with family history of hypertension	0.2	2.0	1.13
○ without family history of hypertension	1.4	1.7	1.6

Family history of hypertension is positive in 3 male and 5 female subjects. The range of ENaC protein level is similar with the previous results. Urinary ENaC protein level is higher in female compare to male; higher in subjects without family history of hypertension.

4. Discussion

The result of step 1 study showed that urinary ENaC protein level is low, no dilution is needed. It is low but still in the range that is detectable by the reagent (0.156-10 ng/mL) [11]. Lesson from this step is urinary ENaC protein detection should be conducted without urine dilution.

Since urinary ENaC protein is so low, we considered the possibility of using concentrated urine. Concentrating urine was conducted by assuming that a big molecule of ENaC protein (85-95kDa) [4] will be settled near the sediment during centrifugation; we tried to concentrate the urine by double centrifugation by collecting 10 ml of near sediment supernatant of 20-100 ml spot urine as well as 24 hour urine to be re-centrifuged. We also measured the presence of serum ENaC protein to ensure the correctness to the process. Serum ENaC protein level is 1.12ng/mL. Table 2 showed us that single centrifuged urine; both spot and 24 hour collection reveal a similar level of ENaC protein. Multiple centrifuged spot urine reveal non consistent level of ENaC protein, and 24 hour collection urine gave the same level of ENaC protein, lower than those in spot urine; it is understandable because of the presence of urinary protease which could cut any protein in the urine [12] if it left without treatment. ENaC protein detection from upper supernatant and lower supernatant reveal a similar result. Lesson learnt from this step is that spot urine seems to be as good as 24 hour collected urine; since 24 hours urine collection is difficult to achieve [13], then a spot urine will be better. Concentrating the urine by double centrifugation is not likely to be necessary.

ENaC activity is regulated by intra and extracellular protease which cut αENaC and loop γENaC to activate the channel. It means that soluble ENaC will be cut ENaC protein; as consequences, the level of urinary ENaC will be lower [4]. The intact ENaC will be stay in the cytoplasmic vesicle, within the cells [3]. With that consideration, sediment which will contain cells was also used to detect the presence of ENaC protein. Table 3 showed us that the ENaC protein level is detectable but the results are not consistent; the higher the dilution, the higher the ENaC protein level. It could be due to the interference of the big particles within the sediment. Cell lysis should be done properly; sonicated with an ultrasonic cell disrupter or subjected the sediment into two freeze-thaw cycles as mentioned in the protocol [11]. We also have to consider the cell number in the sediment, should it be counted first, or should we limit the cell number before ENaC protein detection? The use of sediment to detect urinary ENaC protein level needs a further study.

The third step of the study aimed to explore the difference of urinary ENaC protein level in subject with and without hypertension, with and without family history of hypertension. It is interesting that the level of serum and lower supernatant ENaC protein are consistent, the lowest on non-hypertensive subject and the highest on hypertensive subject without family history of hypertension. Putting it into theory, individual with normal blood pressure despite their salt intake will need fewer ENaC protein to allow sodium to be excreted into the urine; while hypertensive individual will tend to retain sodium and use more ENaC protein to enable the process [3]. Based on the result on step 2 where dilution of sediment gave inconsistent results, in this step sediment was used without any dilution. The levels of ENaC protein in sediment are the opposite of lower supernatant results; it can also be explained by the same theory, in normotensive individual ENaC protein is utilized in a smaller number, it leaves the pooled ENaC protein within the cytoplasmic vesicle and detected in the sediment where cells lie after centrifugation. This result raise a question, does it mean that sediment, if properly treated will be a better source for ENaC protein detection?

Learning from step 3 of the study, in the 4th step we only use lower supernatant. From Table 5 we learn that the level of urinary ENaC protein in non-hypertensive subjects is affected by gender and the presence of family history of hypertension, it is confirmed that, in mice model, female hormones protect them from hypertension, one of the mechanisms is through the reduction of salt sensitivity [14]. A bigger sample size is needed to define the normal range of urinary ENaC protein level.

It can be concluded that Cloud-Clone reagent catalog number SED337Hu can be used to detect urinary ENaC protein. Spot urine is better than collected urine and lower part of supernatant yielding a consistent level of ENaC protein with serum levels in subjects with various condition of hypertension.

Acknowledgements

The authors would like to thank Anna Tjandrawati MD, clinical pathologist and Ms Yanti Herawan at Clinical Pathology Department, Hasan Sadikin General Hospital, Bandung, for very valuable supports during this research.

References

[1] Garty H, Benos DJ. Characteristics and regulatory mechanisms of the amiloride-blockable Na+ channel. Physiological Reviews. 1988;68(2):309-73. PubMed PMID: 2451832.

[2] Loffing J, Schild L. Functional Domains of the Epithelial Sodium Channel. J Am Soc Nephrol. 2005 November 1, 2005; 16(11): 3175-81.

[3] Rossier BC, Schild L. Epithelial Sodium Channel: Mendelian Versus Essential Hypertension. Hypertension 2008;52(4):595-600.

[4] Bhalla V, Hallows KR. Mechanisms of ENaC Regulation and Clinical Implications. J Am Soc Nephrol. 2008 October 1, 2008; 19(10): 1845-54.

[5] Shehata MF, Leenen FH, Tesson F. Sequence analysis of coding and 3' and 5' flanking regions of the epithelial sodium channel α, β, and γ genes in Dahl S versus R rats. BMC Genetics. 2007;8:35.

[6] Shehata MF. Characterization of the epithelial sodium channel alpha subunit coding and non-coding transcripts and their corresponding mRNA expression levels in Dahl R versus S rat kidney cortex on normal and high salt diet. Int Arch Med. 2009; 2(1): 5. PubMed PMID: 19284664. Pubmed Central PMCID: 2669474. Epub 2009/03/17. eng.

[7] Shehata M, Youssef F, Pater A. Genetic Determinants of Salt- Sensitive Hypertension, Editorial J Genet Disor Genet Rep 2012; 1: 1.

[8] Kostakis ID, Cholidou KG, Perrea D. Syndrome of impaired ion handling in the distal nephron peudohypoaldosteronism and familial hyperkalemic hypertension. Hormones. 2012;11(1):31-53.

[9] Knight KK, Olson DR, Zhou R, Snyder PM. Liddle's syndrome mutations increase Na+ transport through dual effects on epithelial Na+ channel surface expression and proteolytic cleavage PNAS. 2006; 103(8): 2805-8.

[10] Kim S, Wang W, Kwon T, Knepper M, Frokiaer J, Nielsen S. Increased expression of ENaC subunits and increased apical targeting of AQP2 in the kidneys of spontaneously hypertensive rats. Am J Physiol Renal Physiol. 2005;289:F957 - F68. PubMed PMID: doi:10.1152/ajprenal.00413.2004.

[11] Cloud-Clone Corp. Enzyme-linked Immunosorbent Assay Kit For Amiloride Sensitive Sodium Channel Subunit Alpha (SCNN1a) Organism Species: Homo sapiens (Human). Instruction manual. In: Life Science Inc., editor. 11th ed. Houston, Texas. 2013.

[12] Kania K, Byrnes EA, Beilby JP, Webb SA, Strong KJ. Urinary proteases degrade albumin: implications for measurement of albuminuria in stored samples. Annals of clinical biochemistry. 2010 Mar;47(Pt 2):151-7. PubMed PMID: 20150213. Epub 2010/02/13. eng.

[13] Côté AM, Firoz T, Mattman A, Lam EM, von Dadelszen P, Magee LA. The 24-hour urine collection: gold standard or historical practice? Am J Obstet Gynecol. 2008;199(6):625.e1-.e6.

[14] Brinson KN, Rafikova O, Sullivan JC. Female sex hormones protect against salt-sensitive hypertension but not essential hypertension. Am J Physiol Regul Integr Comp Physiol. May 14. PubMed PMID: 24829498. Epub 2014/05/16. Eng.