Distal Renal Tubular Acidosis (dRTA) Among Southeast Asian Ovalocytosis (SAO) Patients in Malaria Endemic Area of Sekotong, Lombok Island

I Gusti Ayu Nyoman Danuyanti¹, Tasmini², Ahmad Hamim Sadewa²,*
¹Postgraduate program of Biomedical Science, Faculty of Medicine, Universitas Gadjah Mada.
²Departement of Biochemistry, Faculty of Medicine, Universitas Gadjah Mada

*Corresponding author: hamdewa@yahoo.com

ABSTRACT

Introduction: Southeast Asian Ovalocytosis (SAO) is caused by 27 bp deletion of the band 3 protein gene in erythrocyte membrane and characterized by oval erythrocyte. The erythroid band 3 (AE1) gene is expressed not only in erythrocyte membranes but also in the cell membrane of α-collecting renal tubular functions in the secretion of acid in renal tubules and HCO₃-/Cl⁻ anion exchange. An alteration of the band 3 (AE1) gene functions in cell of α-collecting renal tubules reduces HCO₃-/Cl⁻ ion exchange resulting in decreased secretion of H⁺ ions and disturbances in the process of pickling urine as an indicator of distal renal tubular acidosis (dRTA).

Objective: To determine the occurrence of dRTA among Southeast Asian Ovalocytosis (SAO) patients in malaria endemic area of Sekotong, Lombok Island by analyzing expression of erythroid band 3 (AE1) gene.

Methods: Ovalocytosis degree determined by oval erythrocytes morphology of 20%-100% in blood film. The 27 bp deletion of band 3 (AE1) gene was analyzed by polymerase chain reaction (PCR). An indicators of the dRTA was determined the pH of urine, levels of bicarbonate (HCO₃⁻), potassium (K⁺) and chloride (Cl⁻) in the blood.

Results: The degree of ovalocytosis more than 50% was found in 35,7% individuals and below 50% was found in 64,3% individuals. Percentage of 27 bp deletion of band 3 (AE1) gene among subject of ovalocytosis patients was 35,7% (n = 28) and frequency of the dRTA among SAO patients was 20% (n = 10). Individuals with SAO do not generally have dRTA. The presence of the two conditions in the same individuals suggests that there may be a common underlying molecular defect or genetic basis for SAO and dRTA.

Conclusion: There is a relationship between the 27 bp deletion protein band 3 (AE1) gene with distal renal tubular acidosis (dRTA) among Southeast Asian Ovalocytosis (SAO) patients in Sekotong, Lombok Island, even the percentage is low.

Keywords: distal renal tubular acidosis, Southeast Asian Ovalocytosis, Sekotong, (Lombok Island)

INTISARI

Pendahuluan: Southeast Asian Ovalocytosis (SAO) disebabkan oleh delesi 27 bp dari gen protein band 3 pada membran eritrosit yang ditandai dengan bentuk oval pada eritrosit. Gen protein band erythroid 3 (AE1) diekspresikan tidak hanya dalam membran eritrosit tetapi juga dalam membran sel α-collecting renal tubular yang berfungsi dalam sekresi asam dalam tubulus renal dan pertukaran anion HCO₃⁻/Cl⁻. Adanya perubahan gen band 3 (AE1) yang berfungsi dalam sel α-collecting renal tubules mengurangi HCO₃⁻/Cl⁻ pertukaran yang berakibat penurunan sekresi ion H⁺ dan gangguan dalam proses pengawetan urin sebagai indikator asidosis tubulus distal ginjal (dRTA).

Tujuan: Menentukan terjadinya dRTA pada pasien dengan SAO di daerah endemis malaria di Sekotong, Pulau Lombok dengan menganalisis ekspresi gen band erythroid 3 (AE1).

Metode: Derajat ovalocytosis ditentukan oleh morfologi eritrosit oval sebanyak 20%-100% dalam sediaan
Delesi 27 bp dari gen AE1 gen dianalisis dengan polymerase chain reaction (PCR). Indikator dRTA ditentukan oleh pH urin, kadar bikarbonat (HCO₃⁻), kalium (K⁺) dan klorida (Cl⁻) dalam darah.

Hasil: Tingkat ovalocytosis lebih dari 50% ditemukan pada 35,7% individu dan tingkat ovalocytosis di bawah 50% ditemukan pada sebanyak 64,3% individu. Pada pasien dengan ovalocytosis, 35,7% di antaranya mempunyai delesi 27 bp gen AE1 dan frekuensi dRTA pada pasien dengan SAO adalah 20% (n = 10). Individu dengan SAO biasanya tidak memiliki dRTA. Adanya dua kondisi dalam individu yang sama menunjukkan kemungkinan terjadinya defek molekuler.

Simpulan: Ada hubungan antara delesi 27 bp dari gen AE1 dengan terjadinya asidosis tubulus distal ginjal (dRTA) pada pasien (SAO) di Sekotong, Lombok Island, walaupun persentasenya rendah.

Kata kunci: distal renal tubular acidosis, Southeast Asian Ovalocytosis, Sekotong (Pulau Lombok)

INTRODUCTION

Ovalocytosis is a hereditary asymptomatic disease that is majority by oval form erythrocyte that are accompanied by irregular pale regions and stomatocys. An erythrocyte abnonormality caused by an imbalance of the structure and motion of cytoskeleton proteins because the mobility of the erythrocyte band 3 gene (AE1) membrane was decreased and the bond strength between the band 3 protein and other cytoskeleton protein erythrocyte membrane was increased.

Southeast Asian Ovalocytosis is an autosomal dominant erythrocytes abnormality that is widespread in Southeast Asian countries is caused by an 27 bp deletion of erythrocyte band 3 gene (AE1) in exon 11, resulting in deletion 9 amino acids at positions 400-408 the junction between the N-terminal domain and the first transmembrane span of the erythrocyte band 3 gene (AE1). SAO is characterized by increased erythrocyte rigidity and the deformability of erythrocyte membranes was decreased.

The erythrocyte band 3 gene (AE1) is not only expressed in erythrocyte membranes but also in the cell membrane of α-collecting renal tubular functions in the secretion of acid in renal tubules and HCO₃⁻/Cl⁻ anion exchange. An alteration of the erythrocyte band 3 gene (AE1) functions in cell of α-collecting renal tubules reduces HCO₃⁻/Cl⁻ ion exchange resulting in decreased secretion of H⁺ ions and disturbances in the process of pickling urine as an indicator of distal renal tubular acidosis (dRTA). dRTA is characterized by an inability to generate a normal minimum urinary pH (to pH below 5,5) even in the presence systemic metabolic acidosis due to failure of hydrogen ion secretion in the distal nephron. This results in hyperchloremic metabolic acidosis and usually accompanied by hypokalemia of varying severity.

Hospital-based study showed that more than 80% SAO was detected in dRTA patients. A community based-study was held in Sekotong, Lombok Island to clarify the relationship between SAO and dRTA among people live in that area.

MATERIALS AND METHODS

The records of patients diagnosed with ovalocytosis in Sekotong, Lombok Island were retrieved. A total of 28 ovalocytosis subject and 28 matched control with normal erythrocytes morphology. Ovalocytosis degree was determined by oval erythrocytes morphology in peripheral blood film. Venous blood (5ml) was collected from all subjects and controls in sterilis EDTA tubes. The parameter of the distal renal tubular acidosis is determined by measuring the pH of urine, levels HCO₃⁻, K⁺ and Cl⁻ in the blood.

The complete of dRTA syndrome was diagnosed in subject with a urinary pH over
5,5 (usually more than 6.0) despite systemic acidosis followed with the hypokalemic and hyperchloremic.

Genomic DNA was isolated from whole blood patients with ovalocytosis and control subject. The 27 bp deletion of erythrocyte band 3 gene (AE1) was analyzed by the method of Polymerase Chain Reaction (PCR) as previously described using the forward primer 5’-GGGCCCAGATGACCCTCTGC-3’ for bases 1098-1117 and the reverse primer 5’-GCCGAAGGTGATGGCGGGTG-3’ for bases 1272-1253. Initial denaturation was done at 94°C for 5 min and the amplification was performed for 30 cycles with a denaturation was done at 94°C for 1 min, annealing at 62°C for 1 min, extension at 72°C for 3 min with a final extension at 72°C for 7 min. The PCR products were size fractionated in 3% agarose gels and visualized by ethidium bromide. The expected sizes of the PCR products were 175 bp and 148 bp for the normal and mutant genes, respectively.

RESULTS AND DISCUSSION
Ovalocytosis degree was screened by peripheral blood film examination with Wright’s Stain. Among 28 ovalocytosis subjects, 10 (35.7%) individuals have ovalocyte morphology more than 50% and 18 (64.3%) individuals have 20-30%. In the control group showed normal morphology of erythrocytes (0% ovalocytes) on peripheral blood smear.

To confirm Southeast Asian Ovalocytosis was analyzed by method Polymerase Chain Reaction (PCR). Ten out of 28 DNA samples from ovalocytosis patients carry 27 bp deletion band 3 protein gene giving frequency deletion of 35.7%. The analysis of genomic DNA by polymerase chain reaction have two amplified products of different size from a region encompassing exon 11 of the band 3 gene. The sequence of the larger product matched perfectly with that of normal individuals. In the sequence of the smaller product, 27 nucleotides within exon 11 were deleted (Figure. 2).

Figure.1  Morphological finding of control subjects (a) and ovalocytosis patients (b) in peripheral blood smear. Arrow indicates a stomatosis and irregular pale region that may be accompanied ovalocytosis.
Figure 2 PCR amplification products of erythrocyte band 3 gene (AE1). Two amplified products corresponding to 175 bp and 148 bp were obtained from all samples of ovalocytosis patients diagnosed with SAO and had ovalocytosis degree more than 50% in peripheral blood smear (+), but only one products of 175 bp found in normal control (-). M refer to a size marker.

Figure 3 Incidence of distal renal tubular acidosis (dRTA) in patients with SAO and non SAO.

Therefore, it was concluded that SAO was present in ten out of 28 ovalocytosis patients giving frequency 35.7%. In contrast, none of the control subjects with normal erythrocyte morphology were confirmed to have SAO. The cut-off point to distinguish SAO and non SAO among ovalocytosis patients is ovalocytosis degree more than 50%.

The percentage of distal renal tubular acidosis (dRTA) among individual with SAO is 20% (2 out of 10). There is relationship between SAO and dRTA among ovalocytosis patients, even the percentage is low. In the non SAO subjects those not diagnosed with dRTA. There was a significant difference in the incidence of dRTA between two groups (p value < 0.05) giving result non dRTA have a higher risk compared with dRTA in SAO subjects (Figure. 3). Characteristics of SAO subjects an experiencing complete dRTA showes in Table 1.
This study screened SAO among ovalocytosis patients and control subjects with normal erythrocytes morphology in Sekotong, Lombok Island. The criteria of selected samples as subjects were patients who had ovalositosis degree 20% -100% in peripheral blood smear. It was conclude that ovalocytosis degree more than 50% giving frequency 35.7 % among ovalocytosis patients. The phenotype of SAO only presents among ovalocytosis patients with ovalocytosis degree more than 50%. Accordingly, we propose that the cut-off point to distinguish SAO and non SAO among ovalocytosis patients is ovalocytosis degree more than 50%.

The incidence of SAO among ovalocytosis patients in Sekotong, Lombok Island seemed exactly high, it is in line with previous data from the report from Indonesia where the incidence SAO is 12.6% 12. Other study reported that the incidence of SAO in Lombok Island from different population was very high13. The patient with SAO is less susceptible to cerebral malaria1,2. Remarkably, it was heterogenous incidence of SAO from different population.

The mutant protein seems to have an increased propensity to form oligomers, which appear as longitudinal strands of intramembrane particles and exhibit an increased association with the membrane skeleton11. An oligomerization underlies the increase in membrane rigidity by precluding membrane skeletal extention, leading to membrane deformation1,12,14. The 27 bp deletion erythrocyte band 3 gene (AE1) was not detected among patients with ovalocytosis degree less than 50%. This might be due to failure of heterodimer spektrin heterotetramer formation or dysfunction of the band 4.1 and glikoforin C protein which result in abnormalities of erythrocyte membrane into an oval form15,16.

The results in this study demonstrated that relationship between SAO and dRTA, even the percentage is low. The fact that only 2 out 10 SAO subjects had dRTA, indicated co-existence of SAO and dRTA is usually not seen in the same individual. Therefore, individuals with SAO not generally have dRTA. Several previous studies showed results that there is a relationship between the SAO and dRTA, but the subjects studied were patients with a diagnosis of dRTA and occured more than one mutation in band.

Table 1. Data for characteristics of SAO subjects an experiencing complete dRTA. Values of serum K⁺ (normal: 3.6–5.5), serum Cl⁻ (normal: 95–105) and serum HCO₃⁻ (normal: 24–31) are in mEq per liter. Normal range of urine pH is 7.36–7.44. SAO Southeast Asian ovalocytosis

<table>
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<th>Case</th>
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<th>Urine pH</th>
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<th>Cl⁻ (mEq/L)</th>
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Note : SAO and dRTA
3 protein gene accompanied or not with the deletion of 27 bp erythrocyte band 3 gene (AE1)\textsuperscript{5,7,17,18}. The presence of the two conditions in the same individuals suggests that there may be a common underlying molecular defect or genetic basis for SAO and dRTA \textsuperscript{7,14}. Other research showed that renal acidification was normal in the 20 individuals with SAO, it seemed that one mutation in erythrocyte band 3 gene (AE1) is not sufficient to cause dRTA\textsuperscript{14}.

The erythrocyte band 3 gene (AE1) is expressed not only in erythrocyte membranes but also in the cell membrane of α-collecting renal tubular using difference promoters and have difference the number of amino acids in the cytoplasmic domain (N-terminal) erythrocyte band 3 gene (AE1). The promoter for the band 3 protein expressed on the membrane of erythrocytes is located on the upstream exon 1 and has 911 amino acids while the α-collecting renal tubular promoter located within intron 3 and has a 65 amino acid in cytoplasmic domain (N-terminal)\textsuperscript{6,19,20}.

The mutation of erythrocyte band 3 gene (AE1) show pleiotropic effect resulting in two distinct signs which are an erythrocyte abnormality and distal renal tubular acidosis (dRTA). Heterozygote red cell membranes in SAO exhibit increase rigidity and cold-induced cation permeability. The stable mutant polypeptide is present at normal abundance in the membrane, where it heterodimerizes at apparent normal affinity with wild-type polypeptide\textsuperscript{10,19}. It was concluded the deletion of 27 bp erythrocyte band 3 gene (AE1) in SAO provides minimal effect on wild-type monomers so that the anion exchange mechanism Cl-/HCO3- is normal because it was compensated for by the presence of normal fragments within in SAO. The minimal impact of its dominant effects upon the wild type monomer within in SAO explain the lack of renal phenotype in the absence of a second mutant allele of erythrocyte band 3 gene (AE1)\textsuperscript{9,21}. Band 3 SAO is misfolded in the membrane and can no longer transport anions, so that the red cells of SAO individuals have only about half the normal anion transport activity and thus no evidence of dRTA, but require other mutations to cause of dRTA\textsuperscript{5,22}. Thus, the samples from the SAO subjects of this study will be analyzed further with the aim to identifying a second erythrocyte band 3 gene (AE1) mutation. A longitudinal follow-up study of a SAO or SAO with dRTA patients in Sekotong, Lombok Island population will be a fruitfull program.

CONCLUSIONS

There is a relationship between the 27 bp deletion protein band 3 (AE1) gene with distal renal tubular acidosis (dRTA) among Southeast Asian Ovalocytosis (SAO) patients in Sekotong, Lombok Island, even the percentage is low.

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REFERENCES

