46,XY disorders of sex development dengan penyebab defisiensi 5 alfa-reduktase tipe 2 di Indonesia: pola mutasi, hubungan genotipe-fenotipe, akurasi rasio testosteron/dihidrotestosteron dan rasio etiokolanolon/androsteron urin dalam diagnosis = 5 Alpha-Reductase deficiency type 2 as the etiology of 46,XY disorders of sex development in Indonesia: molecular characterization, genotype-phenotype correlations, the accuracy of testosterone/dihydrotestosterone ratio and urinary etiocholanolone/andros

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Abstrak

Defisiensi enzim 5 alfa-reduktase tipe 2 (D5AR2) merupakan penyakit genetik, yang disebabkan gangguan konversi testosteron (T) menjadi dihidrotestosteron (DHT). Diagnosis D5AR2 pada umumnya ditegakkan dengan rasio T/DHT dan analisis DNA gen SRD5A2, namun rasio T/DHT sering tidak konklusif, sehingga diperlukan pemeriksaan alternatif dalam diagnosis D5AR2, dalam hal ini dipilih rasio metabolit 5ÃÂÃÂ²/5ÃÂÃÂ±steroid urin yakni rasio etiokolanolon/androsteron (Et/An) urin karena telah dilaporkan oleh beberapa peneliti dan juga karena tersedia di Indonesia.

Penelitian ini bertujuan untukmembandingkan akurasi rasio T/DHT dan Et/An urin dalam diagnosis penyandang dan pembawa sifat D5AR2. Selain itu mengetahui pola mutasi gen SRD5A2 dan hubungan genotipe-fenotipe. Studi deskriptif potong-lintang dilakukan untuk mengetahui pola mutasi gen SRD5A2 dan hubungan genotipe-fenotipe dilakukandi Lembaga Eijkman, Jakarta sejak Juli 2016–September 2018. Studi komparatif dilakukan untuk membandingkan akurasi rasio T/DHT (dilakukan di Laboratorium Terpadu FKUI) dan rasio Et/An urin (dilakukan di Laboratorium Kesehatan Daerah DKI Jakarta) dalam mendiagnosis penyandang dan pembawa sifat D5AR2, dilanjutkan dengan analisis kurva ROC (receiver operating characteristics) masing-masing rasio, dengan baku emas analisis DNA gen SRD5A2.

Enam puluh enam subjek penyandang 46,XY DSD dan 95 subjek keluarga peyandang direkrut dalam penelitian ini. Hasil analisis DNA gen SRD5A2 membagi subjek penyandang 46,XY DSD menjadi 37 subjek penyandang D5AR2 dan 29 subjek bukan penyandang D5AR2, dan subjek keluarga menjadi 53 subjek pembawa sifat dan 42 subjek bukan pembawa sifat D5AR2. Rasio T/DHT tidak berbeda bermakna antara penyandang dan bukan penyandang D5AR2, dan juga tidak berbeda bermakna antara pembawa sifat dan bukan pembawa sifat D5AR2. Nilai AUC rasio Et/An urin dalam mendiagnosis penyandang D5AR2 adalah 79,7% (IK 95% 69,0–90,4%, p < 0,001). Dengan nilai titik potong >0,95 didapatkan sensitivitas rasio Et/An urin dalam diagnosis D5AR2 adalah 67,57% dan spesifisitas 86,21%. Dengan nilai titik potong >0,99 didapatkan sensitivitas rasio Et/An urin dalam mendiagnosis pembawa sifat D5AR2 adalah 67,92% dan spesifisitas73,81%. Kombinasi rasio Et/An urin subjek penyandang dan salah satu keluarga dekatnya meningkatkan perkiraan akurasi diagnostik menjadi sangat baik (AUC menjadi 84,1% (IK 95% 74,3–93,9%, p < 0,001), dengan sensitivitas sangat baik (89,19%), namun spesifisitas kurang baik (57,69%). Enam jenis mutasi baru dideteksi, yaitu c.34delGinsCCAGC, R50H, W136stop, G191R, F194I, I253V, dan 7 mutasi

lain. Tidak ada hubungan nyata genotipe dan fenotipe.

.....The 5 alpha-reductase type 2 deficiency (5ARD2) is a genetic condition associated with impairment in conversion of testosterone (T) to dihydrotestosterone (DHT), leading to undervirilization in 46,XY individuals. Diagnosis of 5ARD2 is mainly established by T/DHT ratio and molecular analysis. Yet, the T/DHT ratio often yielded in conflicting results, and the available urinary ratio of etiocholanolone/androsterone (Et/An) was selected as an alternative test.

This study aimedto compare the accuracy of T/DHT and urinary Et/An ratios in diagnosing 5ARD2 cases and carriers and to elaborate the molecular characteristics of SRD5A2 gene in 5ARD2 cases and the genotype and phenotype correlations.

Descriptive and comparative cross-sectional studies were conducted at the Eijkman Institute, Jakarta in year 2016–2018. The accuracy of T/DHT and Et/An ratios were compared using ROC (receiver operating characteristics) curve analysis in the cases and carriers group with molecular analysis of SRD5A2 gene as the gold standard. The molecular characterization of SRD5A2 gene and genotype-phenotype correlations were described.

Sixty six 46,XY DSD subjects and 95 subjects of their family members, who gave written consent or parental approval were recruited. Thirty seven 5ARD2 cases and 29 control were identified in 46,XY DSD subjects, and 53 carriers and 42 control of family members were confirmed by molecular analysis of SRD5A2 gene. The T/DHT ratios were not different significantly in cases and carriers group, while the AUC (area under the curve) of urinary Et/An showed 79.7% (95% CI 69.0–90.4%, $p < 0.001$). After determining cutoff values for diagnosing cases (>0.95) and carriers (>0.99), the sensitivity and specificity of urinary Et/An ratio in cases groups were 67.57% and 86.21%, respectively, and in carrier groups 67.92% and 73.81%, respectively. Simultaneous urinary Et/An ratios of cases and one of their closed family members increased the diagnostic accuracy with AUC of 84.1% (95% CI 74.3–93.9%, $p < 0,001$) and sensitivity 89.19%, yet the specificity of only 57.69%. Six novel mutations (c.34delGinsCCAGC, R50H, W136stop, G191R, F194I, and I253V), and 7 other mutations, which were G34Fs, c.699-1 G>T, V89L, Y128C, N193S, R227Q, and g.5352+15 T>C, in the SRD5A2 gene were detected. There were no clear genotype-phenotype correlations found.

Conclusion: The diagnostic accuracy of urinary Et/An ratio was good in 5ARD2 cases and carriers, and the accuracy was very good if the urinary Et/An ratios of cases and their closed family members were combined. The T/DHT ratio was inaccurate in diagnosing 5ARD2 cases. Six new mutations were detected.