Pengembangan Uji Biologi Molekuler Untuk Mendeteksi Gen Penyandi Resistensi Amoksisilin Pada Pasien Terinfeksi Helicobacter pylori = Development of Molecular Biology Test as a Tool to Detect Gene Enconding Amoxicillin Resistance in Patients Infected with Helicobacter pylori

Evelyne Tiotantra, author

Abstrak

Latar Belakang: Eradikasi Helicobacter pylori menggunakan antimikroba klaritromisin, amoksisilin yang dikombinasi PPI selama 10-14 hari. Resistensi antimikroba menjadi penyebab utama kegagalan terapi. Amoksisilin sebagai salah satu rejimen terapi lini pertama H.pylori telah dilaporkan resistensi sebesar 5,2% di Indonesia tahun 2016. Beberapa penelitian menunjukkan multiple point mutation gen pbp1 hanya ditemukan pada strain H.pylori resisten amoksisilin. Kesulitan melakukan biakan Helicobacter pylori menyebabkan uji biologi molekuler sebagai pilihan alternatif.
Tujuan : Penelitian ini ditujukan untuk mengembangkan uji deteksi resistensi H.pylori dengan gen penyandi pbp 1.
Metode : Penelitian ini merupakan studi retrospektif 2017-2019. Sampel H.pylori positif histopatologis dari Departemen PA dikumpulkan sebanyak 54 sampel blok parafin dari pasien RSUPN Dr. Cipto Mangunkusumo antara tahun 2017-2019 , dilakukan uji Real Time PCR. Hasil positif Real Time PCR dilanjutkan uji nested PCR untuk mencari gen pbp 1 dan dilakukan sekuensing Hasil : Dari 34 sampel positif Real Time PCR didapatkan lima positif gen pbp 1, tetapi hanya empat gen pbp 1 yang dapat dianalisis setelah sekuensing. Hasil analisis dijumpai lima sekuen (sekuen 21, 1, 27, 32A, 32B), ditemukan juga tiga titik mutasi asam amino (Lys648Gln, Arg649Lys, Arg656Pro) yang berdasarkan penelitian sebelumnya hanya ditemukan pada strain H.pylori resisten amoksisilin. Empat sampel yang positif yaitu pada pasien tumor antrum lambung susp keganasan, ulkus gaster, gastritis kronis dan riwayat infeksi H.pylori dalam keluarga.
Kesimpulan : Uji biologi molekuler menggunakan gen pbp 1 sebagai gen penyandi resisten amoksisilin dapat digunakan sebagai uji alternatif untuk mendeteksi resistensi amoksisilin pada penderita infeksi H.pylori. Hasil analisis mutasi asam amino pada uji penelitian ini menunjukkan terdapatnya multiple point mutation yang sesuai dengan strain H.pylori resisten amoksisilin di Korea.
.....Background: To eradicate Helicobacter pylori infection, physician administered clarithromycin and amoxicillin plus proton pump inhibitor during 10-14 days.
Antimicrobial resistance is known as the major cause of treatment failure. The prevalence of Helicobacter pylori resistant strains against Amoxicillin in Indonesia was5,2% in 2016. Several studies in amoxicillin-resistant H.pylori strains had shown multiple point mutation in the pbp1 gene. Molecular method as an alternative tool was chosen due to the difficulties in cultivate H.pylori in a synthetic media.
Aim: This research was aimed to develop H.pylori resistance detection tests using pbp 1 gene.

Methods: The study was a retrospective study, with 54 histopathology of H.pylori positive samples obtained from patients at RSUPN dr. Cipto Mangunkusumo between 2017 to 2019. Real-time PCR tests were used to screen the samples before proceeded to nested PCR and obtained the pbp1 gene for sequencing.
Results: After the initial real-time PCR, 34 H.pylori positive samples were included in the study. From 34 samples of paraffin block only five specimens with positive pbp 1 genes, but only four samples can be analyzed after sequencing. The results found five sequences (sequences 21, 1, 27, 32A dan 32B), also found three amino acid mutation points (Lys648Gln, Arg649Lys, Arg656Pro) which were found only in amoxicillinresistant H.pylori strains. The four samples were collected from patients with antrum gastric tumor suspected as malignancy, gastric ulcer, chronic gastritis, and family history of H. pylori infection.
Conclusion: Molecular approach to detect pbp 1 gene encoding for amoxicillin resistance could be used as an alternative of antibiotic susceptibility test. The multiple point mutations found in this reseach were in accordance with the amoxicillin-resistant H.pylori strains in Korea.