Deteksi dan analisis filogenetik gen 16S rRNA helicobacter pylori pada jaringan biopsi lambung penderita dispepsia = Detection and phylogenetic analysis of helicobacter pylori 16S rRNA gene in gastric biopsy from patients with dyspepsia

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Abstrak

ABSTRAK
Helicobacter pylori diperkirakan menginfeksi lebih dari setengah populasi orang dewasa. Deteksi dini H.pylori sangat diperlukan untuk mencegah berkembangnya infeksi menjadi keganasan lambung. Bentuk coccoid dari H. pylori sulit dideteksi dengan kultur dan histopatologi, namun dapat terdeteksi dengan metode molekuler seperti real-time PCR. Salah satu gen yang dapat digunakan sebagai gen target real-time PCR yaitu 16S rRNA, yang diketahui spesifik dan juga digunakan untuk menganalisis kekerabatan antar strain. Analisis ini bermanfaat untuk melihat penyebaran infeksi H.pylori di dunia. Penelitian ini merupakan penelitian eksperimental laboratorium. Metode pengambilan sampel yang digunakan yaitu, metode consecutive sampling. Biopsi diambil dari 2 antrum dan 2 korpus pada 42 penderita dispepsia untuk pemeriksaan real-time PCR dan histopatologi. Optimasi kondisi real-time PCR meliputi uji volume cetakan DNA, sensitifitas dan spesifisitas teknik, kemudian dilanjutkan aplikasi pada sampel klinis dari biopsi lambung. Delapan dari 11 sampel yang positif dilakukan sekuensing dan analisis filogenetik. Hasil optimasi diperoleh suhu annealing 64?C, konsentrasi primer 0,8 ?M dan konsentrasi probe 0,6 ?M. Ambang batas deteksi real-time PCR untuk mendeteksi jumlah DNA minimal H.pylori yaitu 46 bakteri. Spesifisitas uji reaksi silang real-time PCR ini tidak menunjukkan adanya reaksi silang dengan mikroorganisme lain. Proporsi positif hasil pemeriksaan real-time PCR sebesar 26,2 , sedangkan histopatologi sebesar 11,9 . Pemeriksaan real-time PCR mampu meningkatkan diagnosis sebesar 14,3 dibandingkan pemeriksaan histopatologi. Hasil sekuensing dan analisis filogenetik menunjukkan bahwa strain H.pylori dari sampel memiliki kekerabatan dengan strain Taiwan, India, dan Australia. Kata kunci : H.pylori, histopatologi, real-time PCR, analisis filogenetik
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ABSTRACT
Helicobacter pylori infection is estimated infect almost half of the adult population in the world. Early detection of H.pylori is needed to prevent the development of infections into gastric malignancies. The coccoid form of H. pylori is difficult to detect using culture and histopathology but it can be detected by molecular methods such as real time PCR. One of the genes that can be used as a real time PCR target gene is 16S rRNA, which is known to be specific and also used to analyze closely related strain. This analysis were useful to showed the spread of H.pylori infection in the world.This study is an experimental laboratory. The sampling method used is the consecutive sampling method. Biopsy was taken from 2 antrum and 2 corpus in 42 patients with dyspepsia for real time PCR and histopathology examination. Optimization real time PCR conditions include DNA template volume testing, sensitivity and specificity of the technique, followed by application of clinical samples from gastric biopsy. Eight of the 11 positive samples were sequenced and analyzed for phylogenetics pattern. The optimization result obtained annealing temperature

64 C, primer concentration was 0,8 M and probe concentration was 0,6 M. Limit detection of the DNA was 46 bacteria. The specificity of the PCR 39 s real time indicate that there was no cross reaction with other microorganisms. The positive proportion of PCR real time examination was 26.2 , while histopathology was 11.9 . A real time PCR examination was able to improve the diagnosis by 14,3 compared to histopathology examination. Sequencing and phylogenetic analysis results showed that our strain were closely related to Taiwan, India and Australia strains. Keywords H.pylori, histopathology, real time PCR, phylogenetic analysis