Karakterisasi molekuler gen rdxA Helicobacter pylori penyebab resistensi metronidazol = Molecular characterization of the Helicobacter pylori rdxA gene which causes metronidazole resistance

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Abstrak

Latar belakang : Asosiasi Gastroenterologi Indonesia melaporkan bahwa infeksi H. pylori di Indonesia telah mencapai 22,1% dari pasien dengan gejala dispepsia. Salah satu masalah dalam pengobatan infeksi H.pylori yaitu terdapatnya resistensi H.pylori terhadap antibiotik. Metronidazol telah dilaporkan menunjukkan resistensi terbesar 46,7% di Indonesia, dan sampai saat ini metronidazol masih digunakan sebagai terapi lini pertama. Peneliti sebelumnya telah melaporkan bahwa terdapatnya mutasi gen rdxA H. pylori dapat digunakan sebagai penanda resistensi metronidazol. Bentuk kokoid dari H. pylori sulit dideteksi oleh biakan. Alternatif uji lain yaitu menggunakan uji biologi molekuler yaitu deteksi mutasi menggunakan uji PCR diikuti dengan sekuensing DNA. Tujuan : Penelitian ini diharapkan dapat memberikan informasi baru tentang pengembangan uji resistensi H. pylori, menambah literatur sekuens unik gen rdxA H.pylori dan untuk menentukan mutasi gen rdxA H. pylori yang diprediksi berperan dalam resistensi H. pylori terhadap metronidazol. Metode : Penelitian ini bersifat eksploratif menggunakan 34 sampel blok parafin biopsi lambung yang telah dikonfirmasi mengandung DNA H.pylori pada uji real time PCR. Penelitian ini menggunakan uji nested PCR dan diikuti uji sekuensing DNA, kemudian dilanjutkan analisis bioinformatika yang terdiri dari analisis perubahan asam amino, homologi, filogenetik, konformasi protein dan penambatan molekuler (docking). Hasil : Berdasarkan hasil penelitian, dijumpai terdapatnya mutasi pada gen rdxA akibat insersi dua asam amino, substitusi, frameshift, dan ditemukan premature stop codon. Hasil analisis docking menunjukkan bahwa senyawa metronidazol kurang efektif terhadap H.pylori yang memiliki mutasi insersi dua asam amino, sedangkan H.pylori yang memiliki mutasi substitusi (tanpa insersi) menunjukkan afinitas yang lemah antara metronidazol dan gen rdxA H.pylori. Kesimpulan : Metode molekuler dapat menjadi uji alternatif untuk menguji resistensi H. pylori terhadap antibiotik. Telah ditemukannya sekuens unik berupa insersi dua asam amino yang belum ditemukan pada literatur lain, dapat menambah ilmu pengetahuan bagi para ilmuwan dibidang sains kedokteran. Keberadaan gen rdxA H.pylori yang bermutasi telah dibuktikan dapat menyebabkan resistensi terhadap metronidazol melalui analisa docking.
.....Background : The Indonesian Gastroenterology Association reports that H. pylori infections has reached 22.1% of patients with dyspeptic symptoms in Indonesia. One of the problems in the prevention and treatment of H. pylori infection is H. pylori resistance to some antibiotics as first-line therapy. Metronidazole has been reported to show the greatest resistance of 46.7% in Indonesia, and to date metronidazole is still used as first-line therapy. The presence of rdxA gene mutations in H. pylori isolates can be used as a marker of metronidazole resistance. The cocoid form of H. pylori is difficult to detect by culture. Another alternative test is to use molecular biology tests, namely the detection of mutations using the PCR test followed by DNA sequencing. Aim : This research is expected to provide new information about the development of H. pylori resistance tests, add to the unique sequences H.pylori rdxA gene literatur and to determine the H. pylori rdxA gene mutation plays a role in H. pylori resistance to metronidazole. Methods : This explorative study used 34 samples of gastric biopsy paraffin blocks that were confirmed that

were confirmed to contain H. pylori DNA Indonesian strain in a real time PCR test. The sample was analyzed using a nested PCR test and followed by DNA sequencing test, and bioinformatics analysis consisting of amino acid changes, homology, phylogenetics, protein conformation and molecular docking. Result : In this study, the results showed that mutations were found in the rdxA gene sequence due to the insertion of two amino acids, substitution, frameshift, and found premature stop codon. The results of the docking analysis showed that the metronidazole compound was less effective against H. pylori which had insertion of two amino acids, whereas H. pylori which had substitution (without insertion) showed a weak affinity between metronidazole and the rdxA gene. Conclusion : Molecular method can be an alternative to test H. pylori resistance to antibiotics. The discovery of a unique sequence in the form of the insertion of two amino acids that have not been found in other literature, can increase knowledge for scientists in the field of medical science. The presence of the mutated H. pylori rdxA gene has been shown to cause resistance to metronidazole through docking analysis.