Analisis kecepatan pembentukan 4-hidroksisiklofosfamid dalam dried blood spot pasien kanker payudara Indonesia setelah pemberian siklofosfamid menggunakan KCKUT-SM/SM = analysis of the rate of formation of 4-hydroxycyclophosphamide in dried blood spot of breast cancer patients in Indonesia after administration of cyclophosphamide.

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Abstrak

Siklofosfamid (CP) merupakan salah satu obat kanker golongan agen pengalkilasi yang efektif digunakan untuk mengobati kanker payudara, limfoma non-Hodgkin, dan lain-lain. CP harus diubah menjadi metabolit aktifnya (4-hidroksisiklofosfamid/4-OHCP) untuk menghasilkan efek terapeutik. Siklofosfamid diubah menjadi 4-OHCP oleh beberapa enzim di hati, salah satunya sitokrom P450 2B6 (CYP2B6). CYP2B6 merupakan salah satu gen CYP yang paling bersifat polimorfik yang dapat memengaruhi regulasi transkripsional, ekspresi protein, dan kadar 4-OHCP dalam tubuh. Kadar 4-OHCP dapat menjadi parameter bahwa terapi yang diberikan efektif. Oleh karena itu, tujuan dari penelitian ini adalah untuk menentukan kecepatan hidroksilasi 4-OHCP dengan cara membandingkan konsentrasi 4-OHCP terhadap CP. Penelitian ini menggunakan 43 sampel Dried Blood Spot (DBS) pasien kanker payudara Indonesia yang terdapat CP dalam regimen terapinya. Darah pasien rata-rata diambil pada 2,23±0,38 jam (tmax CP) setelah pemberian kemoterapi. Sampel diekstraksi dengan pengendapan protein dan dianalisis menggunakan Kromatografi Cair Kinerja Ultra Tinggi Tandem Spektrometri Massa (KCKUT-SM/SM); kolom Acquity UPLC BEH C18 (2,1 x 100 mm; 1,7m); suhu kolom 50°C; fase gerak asam format 0,01% - metanol dengan elusi gradien; laju alir 0,15mL/menit; volume injeksi 10 L. Deteksi massa menggunakan ESI (+) dengan nilai m/z 260,65>140,03 untuk siklofosfamid, 33,65>221,04 untuk 4-OHCP-SCZ, dan 337,71>225,05 untuk 4-OHCP-d4-SCZ. Validasi parisal yang dilakukan memenuhi persyaratan FDA 2018. Metode ini linear pada rentang 5 – 60.000 ng/mL untuk CP dan 5 – 1000 ng/mL untuk 4-OHCP. Hasil Penelitian menunjukkan bahwa dari 43 pasien didapatkan rentang CP 2106,16 – 34386,90 ng/mL. dan 4-OHCP 24,85 – 995,071 ng/mL. Berdasarkan rasio 4-OHCP/CP, terdapat 53% (23 subjek) tergolong rapid metabolizer, dan 47% (20 subjek) tergolong poor metabolizer.

.....Cyclophosphamide (CP) is an alkylating agent for anticancer and effective in treating breast cancer, non-Hodgkin lymphoma, and others. CP must be converted to its active metabolite (4-hydroxycyclophosphamide/4-OHCP) to produce a therapeutic effect. CP is converted to 4-OHCP by several enzymes in the liver, cytochrome P450 2B6 (CYP2B) is one of them. CYP2B6 is one of the most polymorphic CYP genes that can affect transcriptional regulation, protein expression, and the level of 4-OHCP in the body. The level 4-OHCP can be a parameter of whether the therapy is effective. Therefore, the purpose of this study is to determine the hydroxylation rate of 4-OHCP by comparing the level of 4-OHCP to CP. This study used a sample of 43 breast cancer patients Dried Blood Spot who contained CP in their regiment therapy which was taken in average time 2.23± 0.38 hours (tmax CP) after CP’s administration. Samples were extracted by protein precipitation method and analysed using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS); Acquity UPLC BEH C18 column (2,1 x 100 mm; 1,7m); temperature was 50°C; 0,01% formic acid - methanol as mobile phase with gradient elution

for 6 minutes; flow rate was 0,15mL/minute; and injected volume 10 L. Mass detection using a triple quadrupole with ESI (+) and multiple reaction monitoring detection with m/z values 260,65>140,03 for cyclophosphamide, 33,65>221.04 for 4-OHCP-SCZ, dan 337.71>225.05 for 4-OHCP-d4-SCZ. The partial validation performed has successfully met the validation requirements that refer to FDA 2018. This method was linear in the range of 5 – 60.000 ng/mL for CP and 5 – 1000 ng/mL for 4-OHCP. The result showed that from 43 patients, the CP levels ranged from 2106,16 – 34386,90 ng/mL. and 24,85 – 995,071 ng/mL for 4-OHCP. Based on the 4-OHCP/CP ratio, 53% (23 subjects) were classified as rapid metabolizers, and 47% (20 subjects) were classified as poor metabolizers.