

## HUBUNGAN METILASI PROMOTER GEN TOLL-LIKE RECEPTOR 4 (TLR4) DAN INTERLEUKIN-6 (IL6) DENGAN RESISTENSI INSULIN PADA SEL DARAH TEPI REMAJA DENGAN OBESITAS DI DAERAH ISTIMEWA YOGYAKARTA

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### INTISARI

**Latar Belakang.** Inflamasi kronik diketahui dapat memicu terjadinya resistensi insulin pada obesitas. Peningkatan ekspresi mRNA petanda inflamasi seperti *toll-like receptor 4 (TLR4)* dan *interleukin-6 (IL6)* dilaporkan meningkat baik pada jaringan maupun pada darah perifer individu dengan obesitas. Metilasi DNA disekitar *transcription start site (TSS)* diketahui berperan dalam regulasi tingkat ekspresi suatu gen.

**Tujuan.** Mengetahui status metilasi promotor *TLR4* dan *IL6* pada darah perifer remaja dengan obesitas dan korelasinya terhadap resistensi insulin (HOMA-IR).

**Metode.** Penelitian ini merupakan penelitian observational analitik dengan rancangan cross sectional. Sebanyak 50 remaja dengan obesitas dipilih sebagai subjek dalam penelitian ini dengan kriteria inklusi berusia 15-18 tahun, mengalami obesitas dengan z-score > 2 SD, tidak mengalami infeksi dalam 2 minggu terakhir dan menandatangani persetujuan sebagai subjek. Subjek yang mengalami diabetes melitus, data tidak lengkap dan konsentrasi DNA <20 µg/mL akan dikeluarkan sebagai subyek. Sampel yang digunakan adalah DNA yang diekstraksi dari darah tepi selanjutnya dikonversi menggunakan bisulfit. Resistensi insulin ditentukan berdasarkan nilai *Homeostatic Model Assasment Insulin Resistance (HOMA-IR)* yaitu > 3,2. Penentuan tingkat metilasi dilakukan menggunakan *Direct – Bisulfite PCR (Direct-BSP)* dengan metode sanger. Analisis statistik dilakukan dengan uji korelasi Spearman. Signifikansi ditandai dengan nilai  $p < 0,05$

**Hasil.** Seluruh subjek tidak menunjukkan adanya metilasi pada promotor target *TLR4*. Data tingkat metilasi *IL6* ditransformasi ke dalam bentuk kategorikal sehingga diperoleh kelompok subjek termetilasi terdiri atas 4 subjek (8%) mengalami resistensi insulin sedangkan 2 subjek (4%) tidak mengalami resistensi insulin dari total subjek. Tingkat metilasi *IL6* antara kelompok resisten insulin dengan non-resisten tidak berbeda signifikan ( $p=0,635$ ). Tidak terdapat korelasi signifikan ( $p > 0,05$ ) antara metilasi *IL6* dengan resistensi insulin (HOMA-IR) ( $r=0,051$ ). karakteristik subjek, mekanisme metilasi yang spesifik setiap sel, dan jenis sampel diduga menyebabkan rendahnya hasil yang diperoleh.

**Kesimpulan.** Tidak ditemukan korelasi antara tingkat metilasi promotor *TLR4* dan *IL6* dengan resistensi insulin pada sel darah tepi remaja dengan obesitas.

## CORRELATION OF METHYLATION OF TOLL-LIKE RECEPTOR 4 (TLR4) AND INTERLEUKIN-6 (IL6) PROMOTER WITH INSULIN RESISTANCE IN OBESE ADOLESCENTS PERIPHERAL BLOOD AT SPECIAL REGION OF YOGYAKARTA

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### ABSTRACT

**Background.** The chronic inflammation can leads to insulin resistance in obesity. Increased mRNA expression of inflammatory markers such as *toll-like receptor 4 (TLR4)* and *interleukin 6 (IL6)* were reported both in the tissue and the peripheral blood of obese adolescent and correlated with insulin resistance. DNA methylation surrounding TSS region was known to regulate the level of a gene expression.

**Objective.** To investigate the methylation status of *TLR4* and *IL6* promoter in peripheral blood of obese adolescent and its correlation to insulin resistance

**Methods.** This research was a cross sectional study with observational analytic approached. Fifty adolescent, aged 15-18 years, z-score value >2 SD, no experience an acute infection within 2 weeks and sign in informed concent, was chosen as a subject. Subject with diabetic mellitus, incomplete data and DNA concentration <20 µg/mL would be exclude. DNA sample was extracted from peripheral blood of 50 adolescent with obesity. The bisulfite converted DNA was analyzed for methylation level with direct sequencing. Correlation analysis was performed test with significancy  $p < 0.05$ .

**Result.** There was no methylation in *TLR4* promoter detected in all subject. The methylation level of *IL6* was transformed to categorical variabel. Four (8%) subject with insulin resistance and 2 (4%) without insulin resistance has methylation on a CpG site. Methylation of *IL6* was not statistically different between insulin resistance and non-resistance adolescent ( $p = 0.635$ ). The significant correlation was not found between methylation level in *IL6* promoter with insulin resistance (HOMA-IR) ( $r = 0.051$ ). Cell specific of methylation mechanism, characteristic of subjects and types of samples was suspected causing those results.

**Conclusion.** There was no correlation found between methylation levels of promoter *TLR4* and *IL6* with insulin resistance in obese adolescent.

**Keyword.** Obesity, methylation, TLR4, IL6, direct sequencing.