RINGKASAN

Profil TNF-α, GDF-9 dan Hyaluronan pada Gangguan Folikulogenesis sebagai Gambaran Penurunan Kualitas Oosit Pasien Infertil dengan Endometriosis

Endometriosis adalah terdapatnya jaringan seperti endometrium yang berada di luar kavum uteri dan dapat menginduksi terjadinya reaksi inflamasi kronis. Gangguan folikulogenesis berperan pada terjadinya penurunan kualitas oosit pada pasien infertil dengan endometriosis. Telah diketahui beberapa senyawa terkait dengan proses folikulogenesis pada petengahan siklus menstruasi, seperti GDF-9 dan Hyaluronan, sedanglan TNF-α berhubungan patogenesis dan makin beratnya endometriosis. Profil TNF-α, GDF-9 dan Hyaluronan pada gangguan folikulogenesis yang berhubungan dengan penurunan kualitas oosit pasien infertil dengan endometriosis belum jelas.

Penelitian ini bertujuan menjelaskan gangguan folikulogenesis yang berhubungan dengan penurunan kualitas oosit pada pasien infertil dengan endometriosis.

Penelitian ini merupakan suatu studi observasional analitik, potong lintang pada 50 pasien infertil dengan melakukan aspirasi zalir peritoneum dan folikel dengan cara laparoskopi di Klinik Fertilitas Graha Amerta RSU Dr Soetomo Surabaya. Terdapat dua tahap penelitian. Tahap pertama karakterisasi protein GDF-9 dilakukan dengan metode *Dot Blot*, SDS PAGE, *Western Blot* dan deglikosilasi. Tahap kedua dilakukan pengukuran kadar TNF-α, GDF-9 dan Hyaluronan dengan metode ELISA.

Rancangan penelitian ini disusun untuk membuktikan bahwa terdapat perbedaan kadar TNF-α, GDF-9 dan Hyaluronan pada kelompok kontrol, endometriosis ringan dan endometriosis berat serta korelasi antara TNF-α dan

Hyaluronan; GDF-9 dan Hyaluronan; TNF-α dan GDF-9. Uji beda dari data hasil penelitian dianalisis dengan Anova (kadar GDF-9) dan Kruskal Wallis (kadar TNF-α dan kadar Hyaluronan). Sedangkan uji korelasi *Spearman* digunakan untuk menilai korelasi antara kadar TNF-α dengan GDF-9, Kadar GDF-9 dengan Hyaluronan, dan Kadar TNF-α dengan Hyaluronan.

Hasil penelitian ini adalah keberadaan protein GDF-9 yang berasal dari folikel pra ovulasi ditunjukkan dengan adanya pita pada 53 kDa pada pemeriksaan *Western Blot*. Gradasi intensitas pita tersebut sesuai dengan stadium endometriosis. Karbohidrat pada glikoprotein GDF-9 dipotong oleh enzim N-Glycosidase-F, hal ini ditunjukkan dengan perubahan pita dari 53 kDa (GDF-9) menjadi 48 kDa (GDF-9 dG).

Terdapat perbedaan bermakna antara kadar TNF-α, GDF-9 dan Hyaluronan pada kelompok kontrol, endometriosis ringan dan endometriosis berat ($p<0.05$). Selain itu terdapat korelasi bermakna antara TNF-α dan Hyaluronan serta GDF-9 dengan Hyaluronan ($p<0.05$). Tidak didapatkan korelasi bermakna antara kadar TNF-α dengan GDF-9 ($p>0.05$). TNF-α berperan terhadap terjadinya peningkatan Hyaluronan. Dominasi jalur TNF-α. ke peningkatan Hyaluronan dapat melalui peran NF-kB dan TSG-6.

Kesimpulan penelitian ini adalah peningkatan kadar TNF-α dan Hyaluronan serta penurunan kadar GDF-9 berperan pada gangguan folikulogenesis yang berhubungan dengan penurunan kualitas oosit pada pasien infertil dengan endometriosis.

Penelitian ini merupakan laporan penelitian *in vivo* pertama yang mengeksplorasi ekspresi GDF-9 di zalir folikel pasien endometriosis. Diperlukan penelitian lanjutan untuk mengeksplorasi peran Kit *ligand*, NF-kB, HAS-2 dan TSG-6

pada gangguan folikulogenesis, serta pemanfaatan TNF-α sebagai dasar diagnostik dan terapi pada endometriosis dan infertilitas. Diperlukan pula eksplorasi lebih lanjut Hyaluronan sebagai petanda keberhasilan fertilisasi dan pemanfaatan jangka panjang GDF-9 untuk terapi gangguan folikulogenesis.

SUMMARY

The profile of TNF-α, GDF-9 and Hyaluronan in folliculogenesis disturbance as a marker of reduced oocyte quality in infertile patients with endometriosis

Endometriosis is defined as the presence of endometrial-like tissue outside the uterus, which induces a chronic inflammatory reaction. Folliculogenesis disturbance has an important role in reduced oocyte quality of infertile patients with endometriosis. Number of molecules have been identified to correlate with folliculogenesis in mid cycle such as GDF-9 and Hyaluronan, whereas TNF-α has been correlated with the pathogenesis and severity of endometriosis. The profile of TNF-α, GDF-9 and Hyaluronan in folliculogenesis disturbance that cause reduced of oocyte quality in infertile patients with endometriosis remains controversial.

The purpose of this study was to clarify the folliculogenesis disturbance that cause reduced oocyte quality in infertile patients with endometriosis.

Using a cross sectional design study, peritoneal and follicular fluid were aspirated laparospically from 50 infertile patients in Fertility Clinic, Graha Amerta, Dr Soetomo Teaching Hospital Surabaya. Two research steps were conducted. Step 1 was characterization of GDF-9 potein assessed by using Dot Blot, SDS PAGE, Western Blot and deglycosylation methods. Step 2 was detection of TNF-α, GDF-9 and Hyaluronan concentrations to all samples performed by using ELISA laboratory technique.

The data was analysed using Anova for the difference of GDF-9 and Kruskal Wallis test for the difference of TNF-α and Hyaluronan concentrations between 3 groups: control, mild and severe endometriosis. Spearman test has been performed to

assess the correlation between TNF-α and GDF-9, GDF-9 and Hyaluronan and also TNF-α and Hyaluronan.

Result showed that the presence of GDF-9 protein in pre-ovulatory follicles was confirmed by Western blot method of follicular fluid in the band of 53 kDa. The intensity of GDF-9 band depends on the severity of endometriosis. The carbohydrate of glycoprotein GDF-9 has been cut successfully by deglycosilated N-Glycosidase-F, showed by the alteration of the band from 53 kDa (GDF-9) to 48 kDa (GDF-9 dG).

There was a significant difference concentration of TNFα, GDF-9 and Hyaluronan between 3 groups ($p<0.05$). There were significant correlations were found between TNF-α and Hyaluronan and between GDF-9 and Hyaluronan ($p<0.05$) but there was no significant correlation between TNF-α and GDF-9 ($p>0.05$). TNFα plays a role in the increase of Hyaluronan. The domination of this pathway was supposed due to NF-kB and TSG-6.

As a conclusion in infertile patients with endometriosis, the increase of TNF-α and Hyaluronan, and also the decrease of GDF-9 play an important role in folliculogenesis disturbance as a marker of reduced oocyte quality.

This study is the first in vivo study report for exploring GDF-9 expression in follicular fluid of infertile patients with endometriosis. A further study is required to explore the role of Kit ligand, NF-kB, HAS-2 and TSG-6 in the disturbance of folliculogenesis and the development of TNF-α as a tool of diagnostic and therapy for infertile patients with endometriosis. An exploration of Hyaluronan is also required as a marker of fertilization and the development of GDF-9 for folliculogenesis disturbance therapy.

ABSTRACT

The profile of TNF-α, GDF-9 and Hyaluronan in folliculogenesis disturbance as a marker of reduced oocyte quality in infertile patients with endometriosis

Endometriosis is defined as the presence of endometrial-like tissue outside the uterus, which induces a chronic inflammatory reaction. Folliculogenesis disturbance has a role in reduced oocyte quality of infertile patients with endometriosis.

The purpose of this study was to clarify the folliculogenesis disturbance that cause reduced oocyte quality in infertile patients with endometriosis.

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The presence of GDF-9 protein in pre-ovulatory follicles was confirmed by Western blot method of follicular fluid in the band of 53 kDa. The intensity of GDF-9 band depends on the severity of endometriosis. The carbohydrate of glycoprotein GDF-9 has been cut successfully by enzimatically deglycosilated N-Glycosidase-F, showed by the alteration of the band from 53 kDa (GDF-9) to 48 kDa (GDF-9 dG).

There was a significant difference concentration of TNF-α, GDF-9 and Hyaluronan between 3 groups: control, mild and severe endometriosis ($p<0.05$) and significant correlations were found between TNF-α and Hyaluronan and between GDF-9 and Hyaluronan ($p<0.05$) but there was no significant correlation between TNF-α and GDF-9 ($p>0.05$).

As a conclusion in infertile patients with endometriosis, the increase of TNFα and Hyaluronan and also the decrease of GDF-9 concentrations play an important role in folliculogenesis disturbance as a marker of reduced oocyte quality.

Keywords: TNF-α, GDF-9, Hyaluronan, folliculogenesis, infertile, endometriosis