Studi Ekspresi Kromatin Remodeling (Chromodomain Helicase Dna Binding Protein 5) Dan Status Modifikasi Histon (Histone 3 Lysine 9 Trimethylation Dan Histone 4 Lysine 12 Acetylation) Pada Testis Dengan Azoospermia Non Obstruktif = A Study On Chromatin Remodeling (Chromodomain Helicase Dna Binding Protein 5) And Histone Modification Status (Histone 3 Lysine 9 Trimethylation And Histone 4 Lysine 12 Acetylation) In Non-Obstructive Azoospermic Testis

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Abstrak

Latar Belakang: Infertilitas dialami oleh sekitar 15% pasangan di seluruh dunia, dengan kontribusi dari pihak laki-laki sekitar 50%. Salah satu penyebab infertilitas pada pria adalah azoospermia non obstruktif idiopatik, yang diduga melibatkan faktor epigenetik. Penelitian ini bertujuan menilai peran epigenetik, khususnya remodeling kromatin dan modifikasi histon, pada proses spermatogenesis pada testis dengan azoospermia non obstruktif.
Metode: Sampel BFPE dan TESE diperiksa menggunakan teknik HE lalu dikelompokkan berdasarkan tipe henti maturasi, yaitu SCO, STA, dan SDA. Sampel BFPE dilakukan pemeriksaan immunohistokimia menggunakan antibodi anti-CHD5, anti-H3K9me3, dan anti-H4K12ac. Proses pengolahan gambar immunohistokimia menggunakan ImageJ, IHC Profiler, dan StarDist. Sampel TESE dilakukan pemeriksaan qPCR untuk mengukur tingkat ekspresi gen CHD5 dan PHF7. Selain itu, pada sampel TESE dilakukan pemeriksaan ChIP untuk menilai kadar relatif gen WEE1 dan PRM1 yang berikatan dengan CHD5.
Hasil: Ekspresi CHD5 ditemukan pada spermatogonia dan spermatid bulat. Tidak ada perbedaan signifikan intensitas CHD5 pada spermatogonia antara kelompok STA dan SDA. Intensitas H3K9me3 dan H4K12ac pada spermatogonia, spermatosit, dan sel sertoli berdasarkan kelompok henti maturasi berbeda signifikan. Tingkat ekspresi gen CHD5 pada kelompok STA meningkat signifikan 67 kali lipat dibandingkan ekspresinya pada SCO, dan pada kelompok SDA meningkat signifikan 164 kali lipat dibandingkan ekspresi pada SCO. Tingkat ekspresi gen PHF7 pada kelompok STA meningkat signifikan 53 kali lipat dibandingkan ekspresinya pada SCO, dan pada kelompok SDA meningkat signifikan 192 kali lipat dibandingkan ekspresi pada SCO. Kadar DNA segmen promoter gen WEE1 pada ChiP-qPCR menggunakan antibodi anti-CHD5 ditemukan sebesar 1,19% untuk STA dan 1,87% untuk SDA, lebih tinggi dibandingkan kadar pada SCO yaitu 0,36%. Sedangkan kadar DNA segmen promoter gen PRM1 ditemukan sebesar 1,01% untuk STA dan 2,47% untuk SDA, lebih tinggi dibandingkan kadar pada SCO yaitu 0,29%.
Kesimpulan: CHD5 berperan pada spermatogenesis manusia, khususnya pada sel spermatogonia dan spermatid bulat. CHD5 terbukti meregulasi gen WEE1 dan PRM1 pada sel spermatogenik. H3K9me3 dan H4K12ac berperan pada kasus henti maturasi, dan berpotensi untuk menjadi marker kasus azoospermia non obstruktif.
.....Background: Infertility affect about 15% of couples worldwide, with male factors contributing to around 50% of cases. One of the causes of male infertility is idiopathic non-obstructive azoospermia, which is suspected to involve epigenetic factors. This study aims to assess the role of epigenetics, specifically

chromatin remodeling and histone modification, in the process of spermatogenesis in testes with non-obstructive azoospermia.
Method: The BFPE and TESE samples were examined using HE techniques and subsequently classified based on maturation arrest types, including SCO, STA, and SDA. Immunohistochemical analysis of the BFPE samples was conducted using anti-CHD5, anti-H3K9me3, and anti-H4K12ac antibodies. Image processing for immunohistochemistry was performed using ImageJ, IHC Profiler, and StarDist. The TESE samples underwent qPCR analysis to measure the expression levels of the CHD5 and PHF7 genes. Additionally, ChIP analysis was performed on the TESE samples to assess the relative levels of WEE1 and PRM1 genes bound to CHD5.
Result: The expression of CHD5 was found in spermatogonia and round spermatids. There was no significant difference in CHD5 intensity in spermatogonia between the STA and SDA groups. However, the intensities of H3K9me3 and H4K12ac in spermatogonia, spermatocytes, and Sertoli cells varied significantly among the maturation arrest groups. The expression level of the CHD5 gene in the STA group increased significantly by 67-fold compared to its expression in SCO, and in the SDA group, it increased significantly by 164-fold compared to its expression in SCO. The expression level of the PHF7 gene in the STA group increased significantly by 53-fold compared to its expression in SCO, and in the SDA group, it increased significantly by 192-fold compared to its expression in SCO. The DNA segment promoter level of the WEE1 gene in ChiP-qPCR using anti-CHD5 antibody was found to be 1.19% for STA and 1.87% for SDA, higher than the level in SCO, which was 0.36%. Meanwhile, the DNA segment promoter level of the PRM1 gene was found to be 1.01% for STA and 2.47% for SDA, higher than the level in SCO, which was 0.29%.
Conclusion: CHD5 plays a role in human spermatogenesis, particularly in spermatogonia and round spermatids. It has been shown to regulate the genes WEE1 and PRM1 in spermatogenic cells. H3K9me3 and H4K12ac are implicated in cases of maturation arrest and have potential as markers for azoospermia non obstructive cases.