

## Dampak Progresi Diabetes terhadap Lien Tikus: Kajian Stereologi, Histopatologi, *Flow Cytometry* dan Ekspresi Ligan Kemokin

### Abstrak

**Latar Belakang:** Diabetes melitus diketahui mempengaruhi organ sistem imun, termasuk lien. Temuan terdahulu menunjukkan bahwa atrofi lien dan penurunan *pulpa alba* dapat terjadi dalam lima minggu setelah timbulnya hiperglikemia pada tikus diabetes yang diinduksi streptozotocin (STZ). Penelitian ini bertujuan melakukan evaluasi perubahan struktural dan imunologis lien pada dua tahap progresi diabetes.

**Metode:** Dua puluh enam tikus dibagi menjadi empat kelompok berdasarkan kondisi (kontrol normal [NC] atau model diabetes [DM]) dan waktu observasi pasca induksi (5 atau 10 minggu): NC5, DM5, NC10, dan DM10. Diabetes diinduksi menggunakan kombinasi streptozotocin dan nikotinamid (STZ-NA). Evaluasi mikroskopis dilakukan menggunakan teknik pewarnaan rutin pada sediaan histologis, sedangkan volume kompartemen lien diestimasi secara kuantitatif menggunakan metode hitung titik. Analisis imunohistokimia (IHK) dan *flow cytometry* digunakan untuk menentukan distribusi dan persentase *lymphocytus* T dan B. Analisis RT-qPCR digunakan untuk menilai ekspresi relatif gen ligan yang berperan dalam migrasi *lymphocytus* T dan B.

**Hasil:** Dibandingkan dengan kelompok NC5, kelompok DM5 menunjukkan adanya respon inflamasi, ditandai dengan infiltrasi *granulocytus neutrophilicus* (PMN); namun, tanpa adanya atrofi yang bermakna. Median skor imunohistokimia untuk *lymphocytus* T lebih tinggi secara bermakna ( $p < 0,01$ ) dan persentase *lymphocytus* B CD20<sup>+</sup>CXCR5<sup>+</sup> yang lebih tinggi ( $p < 0,05$ ), menunjukkan adanya aktivasi respons imun adaptif. Sebaliknya, dibandingkan dengan kelompok NC10, kelompok DM10 menunjukkan lien yang lebih kecil ( $p = 0,005$ ) namun proporsional dengan berat badan, disertai volume total *pulpa alba* ( $p = 0,015$ ) dan *zona marginalis* ( $p = 0,008$ ) yang lebih rendah, serta fraksi *textus connectivus* yang lebih tinggi ( $p < 0,001$ ). *Ccl19* lebih tinggi pada fase kronis awal, sedangkan *Ccl21* dan *Cxcl13* lebih tinggi pada fase kronis lanjut.

**Kesimpulan:** Lien pada diabetes awal menunjukkan respon inflamasi dan aktivasi imun adaptif tanpa atrofi. Pada diabetes lanjut terjadi atrofi lien proporsional terhadap berat badan, dengan penurunan volume pulpa alba dan zona marginalis disertai penurunan *lymphocytus* T dan B serta pergeseran ekspresi kemokin dari *Ccl19* ke *Ccl21* dan *Cxcl13*.

### Kata kunci:

Stereologi Berbasis Desain, Progresi Diabetes, Histopatologi, Atrofi Organ Lien, Volume *Pulpa alba*

***Impact of Diabetes Progression on the Rat Spleen: An Integrated Analysis using Stereology, Histopathology, Flow Cytometry, and Chemokine Ligand Expression.***

***Abstract***

**Background:** Diabetes mellitus is known to affect the immune system and lymphoid organs, including the spleen. Previous studies have reported that splenic atrophy and reduction of the white pulp may occur within five weeks after the onset of hyperglycemia in streptozotocin-induced (STZ) diabetic rats. This study aims to evaluate structural and immunological alterations in the spleen at two stages of diabetes progression.

**Methods:** Twenty-six rats were categorized into four groups based on condition (normal control [NC] or diabetic model [DM]) and observation period post-induction (5 or 10 weeks): NC5, DM5, NC10, and DM10. Diabetes was induced using a combination of streptozotocin and nicotinamide (STZ-NA). Histological evaluations were performed using standard staining techniques, while the volumes of spleen compartments were quantitatively assessed through point-counting methods on histological sections. Additionally, immunohistochemistry (IHC) and flow cytometry analyses were utilized to determine the distribution and percentages of T and B lymphocytes. Relative gene expression was used to determine the fluctuation of ligands responsible for lymphocyte migration.

**Results:** Compared to its NC5 control, the DM5 group exhibited inflammatory responses, as indicated by the infiltration of polymorphonuclear leukocytes (PMN); however, no significant atrophy was observed in this group. Notably, this group showed a significantly higher immunohistochemistry median score for T lymphocytes ( $p < 0.01$ ) and a higher percentage of CD20<sup>+</sup>CXCR5<sup>+</sup> B lymphocytes ( $p < 0.05$ ), compared to NC5, suggesting an active adaptive immune response. In contrast to the NC10 group, the DM10 group was significantly smaller ( $p = 0.005$ ) that remained proportional to body weight, with marked lower total white pulp volume ( $p = 0.015$ ) and marginal zone volume ( $p = 0.008$ ). Additionally, there was a higher connective tissue volume fraction ( $p < 0.001$ ). Ccl19 was higher in the early phase, whereas Ccl21 and Cxcl13 were higher in the advanced phase.

**Conclusions:** The spleen in early diabetes shows inflammation and adaptive immune activation without atrophy. In advanced diabetes, splenic atrophy occurs while organ size remains proportional to body weight, with reduced white pulp and marginal zone, increased connective tissue, decreased T and B lymphocytes, and a chemokine shift from Ccl19 to Ccl21 and Cxcl13.

**Keywords:**

*Design-Based Stereology, Diabetic progression, Histopathology, Spleen atrophy, White Pulp Volume.*