THE PROFILE OF URINARY TH-1 AND TH-2 CYTOKINES IN PATIENTS WITH LUPUS NEPHRITIS

By

DR. NORHAYATI YAHAYA

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ABSTRACT.

Introduction.

Systemic lupus erythematosus (SLE), considered as the prototype of human systemic autoimmune disease, is a chronic life-long disease. It occur secondary to a dysregulated immune system, which, via various cytokine productions, leads to the production of pathogenic autoantibodies and immune complex deposition in multiple organs. Renal involvement, or lupus nephritis, can be one of the most serious complications seen in SLE. Th cells (CD4 cell) can be divided into few subsets, whose action is mediated by cytokine production. Abnormality in Th cell activity, mainly Th1 and Th2, is postulated to play an essential role in the pathogenesis of LN.

Objective

The study was conducted to determine the level of four urinary cytokines, IL-2 and IFN-γ representing Th 1; and IL-6 and IL-13 representing Th2, in SLE patients and healthy control. The study also wants to compare the level of the 4 cytokines with the activity of LN. In addition, this study also wants to assess the association between degree of proteinuria and the 4 urinary cytokines level in active LN.

Methodology.

In a cross-sectional study, 80 SLE patients attending HUSM and HKB were selected and they were divided active and inactive LN, plus non-renal SLE. Twenty eight controls were included in the study. Urine cytokine levels for each group, plus control, were

determined using ELISA method. Subsequently, the level of urinary cytokines between each groups were compared. Clinical data obtained from the SLE patients included renal function, amount of 24-hour urine protein, WHO class of LN for those with renal biopsy, and treatment record.

Results.

The majority of the patients were Malays (91%) and female (85%) in gender, with mean age of 31.2 years. Proteinuria was highest in the active LN group (2.95 +/- 2.8gm/day) compared to 0.2 +/- 0.2 gm/day in inactive LN and 0.1 +/- 0.1 gm/day in non renal SLE group. Calculated creatinine clearance was comparable among the 3 SLE groups.

Marginal difference in detectable IL-2 level was found in the different groups, although still highest in active LN (median value in active LN 13.3 pg/ml, inactive LN 11.6pg/ml, non-renal SLE 11.9pg/ml, control 10.2pg/ml). This was similar to the finding of previous studies. IL-6 level was highest in active LN (median value active LN 10.5pg/ml, inactive LN and non-renal SLE 0pg/ml, control 1.0pg/ml). This was also comparable to previous studies. However, the result for IFN-γ was the opposite of previous results, being slightly higher in the control group instead of the SLE groups (median value 0pg/ml in active LN, inactive LN, non-renal SLE and 1.3pg/ml in control group). As for IL-13, median values for all groups were similarly low (median value in active LN 0.3 pg/ml, inactive LN and non-renal 0.7 pg/ml, control 1.2 pg/ml). No previous study looking at urinary IL-13 has been done.

Comparison between groups revealed an association between the activity of LN and urinary levels of IL-6 and IL-2 (Active LN compared to inactive LN: for IL-6, p= 0.001; for IL-2, p=0.003. Active LN compared to control: for IL-6, p=0.001; for IL-2, p=0.001. P is significant if <0.008) However, the present study showed significant but opposite findings for IFN-γ with higher level in control when compared to both active and inactive groups (p=0.001 for both comparison). The result for IL-13 was not significant for all group comparison. The study also did not find statistically significant association between degree of proteinuria with the 4 urinary cytokine levels in active LN group.

Conclusion

Cytokines from both Th subsets were found to be significantly elevated in the active LN group ie IL-6 from Th2 subset, IL-2 from Th1 subset. This indicates the importance of both cytokines in the pathogenesis of LN and may provide another method in monitoring disease progression and response to treatment in LN.

ABSTRAK.

Pengenalan

Sistemik lupus eritematosus (SLE), adalah penyakit 'autoimmune' kronik yang dihidapi sepanjang hayat. Ia berlaku kerana kepincangan system ketahanan badan, yang melalui penghasilan beberapa sitokin, menyebabkan terhasilnya antibodi-antibodi berlebihan yang menyebabkan kerosakan pelbagai organ. Penglibatan buah pinggang, atau ' lupus nephritis' (LN) adalah salah satu komplikasi serius yang dilihat dalam SLE. Sel T penolong (Tp) boleh dibahagikan kepada beberapa kumpulan yang bertindak melalui sitokin yang dihasilkan. Aktiviti Tp yang tak normal, terutama yang melibatkan Tp1 dan Tp2, dijangka memainkan peranan penting dalam patogenesis SLE.

Objektif

Satu kajian telah dijalankan untuk menentukan tahap 4 sitokin dalam air kencing, IL-2 dan IFN-γ sebagai wakil Tp1, dan IL-6 serta IL-13 sebagai wakil Tp2. Selain dari itu, kajian ini juga ingin melihat hubungan antara aktiviti LN dan tahap sitokin-sitokin tersebut. Satu lagi tujuan kajian ialah untuk menentukan samada ada hubung antara tahap protin dalam air kencing dan jenis rawatan yang diambil oleh pesakit, dengan tahap sitokin IL-6 untuk pesakit yang mengalami LN secara aktif.

Metodologi

Dalam satu kajian keratan lintang, lapan puluh pesakit SLE yang menerima rawatan di HUSM dan HKB telah diambil untuk memasuki kajian ini. Mereka dibahagi kepada LN aktif, LN tidak aktif dan SLE yang tak menglibatkan buah pinggang. Dua puluh lapan

kontrol juga diambil untuk kajian ini. Tahap sitokin untuk setiap kumpulan, dan kontrol, ditentukan menggunakan cara ELISA. Selepas itu, tahap sitokin antara kumpulan telah dibandingkan. Data-data klinikal melibatkan pesakit SLE seperti fungsi buah pinggang, jumlah protin dalam air kencing yang dikumpul selama 24 jam, kelas WHO untuk pesakit yang ada 'renal biopsy', dan rekod rawatan juga diperolehi.

Keputusan

Kebanyakan pesakit adalah dari golongan Melayu (91%) dan perempuan (85%) dengan purata umur 31.19 tahun. Protin dalam air kencing terbanyak didapati dalam kumpulan LN aktif (2.95 +/- 2.72 gm/sehari) dibandingkan dengan 0.2 +/- 0.16 gm/sehari untuk kumpulan LN tidak aktif, dan 0.12 +/- 0.09 gm/sehari untuk SLE yang tak menglibatkan buah pinggang. 'Calculated cretinine clearance' adalah lebih kurang sama antara ketiga-tiga kumpulan

Perbandingan tahap IL-2 antara kumpulan menunjukkan perbezaan yang kecil. Walaubagaimanapun, tahap tertinggi adalah dalam kumpulan LN aktif (LN aktif 13.3 pg/ml, LN tidak aktif 11.60pg/ml, SLE tidak melibatkan buah pinggang 11.85pg/ml, kontrol 10.20pg/ml). Ini adalah sama dengan keputusan kajian sebelumnya. Tahap IL-6, juga tertinggi dalam kumpulan LN aktif (LN aktif 10.5pg/ml, LN tidak aktif dan SLE tidak melibatkan buah pinggang 0pg/ml, kontrol 1.0pg/ml). Ini juga sama dengan keputusan kajian sebelumnya. Tetapi, keputusan tahap IFN-γ adalah berbeza dibandingkan kajian sebelumnya, dimana tahapnya tinggi sedikit dalam kumpulan kontrol dibandingkan kumpulan lain (0pg/ml untuk LN aktif, LN tidak aktif, SLE tidak melibatkan buah pinggang, dan 1.25pg/ml dalam kontrol). Untuk IL-13, kajian ini

ABSTRACT OF DISSERTATION - NOT SUBMITTED YET

Th1/Th2 Cytokines in Patients with Systemic Lupus Erythematosus:

Understanding the mechanism of Systemic Lupus Erythematosus – The role Of Th1 and Th2 cytokines

Objectives:

To determine the circulating levels of two Th1 (IL-2,IFN-γ) and twoTh2 (IL-6,IL 13) cytokines in patients with systemic lupus erythematosus (SLE) , to elucidate their association with disease activity and to assess the relationship between these cytokines and organ involvement in SLE patients.

Methods:

90 SLE patients and 31 healthy controls was included in this comparative cross sectional study carried out in Hospital Universiti Sains Malaysia (USM) and General Hospital Kota Bharu from October 2003 till september 2005 by selecting SLE patients in Medical wards and Outpatient clinics. Serum levels of interleukin (IL)-2, interferon (IFN) γ, IL-6 and IL-13, as well as anti-dsDNA, ANA (Immunofluorescence IF) and C3,C4complement levels and CRP (Immunoturbidometrytechniques), were determined. Disease activity was recorded according to the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and classified as active (SLEDAI > 8), moderately active (SLEDAI: 4-7), or inactive (SLEDAI < 8).

Results:

The median age of the patients was 29and IQR of 16 years, Eighty one female patients (90%) and Nine male patients (10%), majority were Malay (92.2%) and the rest were Chinese (7.8%) there was no other ethnic groups. Fifty one patients (57.8%) Inactive SLE with SLEDAI(score 0-3 N = 32, score 4-7 N=20), and 39 patients (42.2%) had active SLE. Levels of IFN-γ, IL-2, IL-6 and IL-12 were significantly higher in patients than in healthy controls , there is no significant difference in the level of IL-2 in the active and inactive SLE nor in the 3 subgroups (p< 0.2). However there are higher level of IL-6, IL-13 and IFN-γ in the active SLE groups and in between the 3 subgroups with and increasing trend (P<0.01, P<0.02, P<0.01 respectively), suggesting major participation of Th1 over Th2 cytokines. Nevertheless, a direct correlation between Th1 (IFN- γ) and Th2 (IL-6 and IL-13) cytokines is observed in patients, indicating a mutual Th1-Th2 participation. No

menunjukkan tahap yang sama rendah untuk semua kumpulan(LN aktif 0.3 pg/ml, LN tidak aktif and SLE tidak melibatkan buah pinggang 0.7 pg/ml, kontrol 1.2 pg/ml).

Perbandingan antara kumpulan menunjukkan adanya hubungkait antara tahap keaktifan LN dan tahap IL-2 dan IL-6 dalam air kencing ie. tahap IL-6 dan IL2 adalah lebih tinggi dalam pesakit yang ada LN aktif. Walaubagaimanapun, kajian ini menunjukkan keputusan yang sebaliknya untuk IFN-γ, iaitu lebih tinggi dalam kontrol bila dibandingkan dengan LN aktif dan LN tidak aktif. Untuk IL-13 pula, keputusan kajian menunjukkan tiada hubungkait yang dapat dilihat apabila perbandingan antara kumpulan dibuat. Kajian ini tidak menunjukkan adanya hubungkait antara tahap protin dalam air kencing dan jenis rawatan yang diambil oleh pesakit, dengan tahap sitokin IL-6 untuk pesakit yang mengalami LN secara aktif.

Rumusan

Keputusan kajian ini menunjukkan tahap IL-2 dan IL-6 dalam air kencing yang tinggi dalam kumpulan LN aktif. Ini menunjukkan pentingnya kedua-dua sitokin ini dalam patogenesis LN. Selain dari itu, tahap sitokin dalam air kencing mungkin boleh dijadikan satu lagi cara untuk memantau penyakit SLE dan keberkesanan perawatan.

significant correlation between the level of these cytokines and the various organs involved .