Original article

Interferon gamma: is it a co-player in the pathogenesis of idiopathic nephrotic syndrome

Introduction: Idiopathic nephrotic syndrome (INS), the most common form of NS in childhood, was considered 4 decades ago as a systemic disorder of T cells, mediated through its released cytokines. To date, the exact incriminated cytokine or immunological mediator is not properly defined. Interferon gamma (IFN-γ), a pro-inflammatory cytokine, is thought to have a role in the provocation of the T cell mediated INS relapse, through promotion of T helper1 (Th1) differentiation and suppression of regulatory T cells (Treg). Aim of the study: to evaluate the immunopathogenic role of IFN-γ in children with steroid sensitive idiopathic nephrotic syndrome (SSINS) through monitoring the changes in its levels with disease course.

Methods: This study included twenty-five newly diagnosed children with SSINS. They were all given full dose prednisolone, evaluated at initial diagnosis and at full remission as regards the serum level of IFN-γ. Results: Serum levels of IFN-γ were lowermost at time of diagnosis and increased with remission on corticosteroids. Conclusions: this study points to a role for the lower serum IFN-γ at diagnosis, in the immunopathogenesis of INS than at remission and the rise in its serum level might be a marker of remission induction, however this awaits confirmation in larger scale studies. Studies on renal biopsy specimens are needed to determine the exact renal in situ levels and effects of IFN-γ.

Keywords: Idiopathic nephrotic syndrome, Steroid sensitive nephrotic syndrome, IFN-γ.

INTRODUCTION

Idiopathic nephrotic syndrome (INS) is a common chronic illness characterized by massive proteinuria and hypoalbuminemia in children. The International Study of Kidney Disease in Childhood reported that 84.5% of children with INS had minimal change nephrotic syndrome (MCNS) and the pathological hallmark of MCNS is the effacement of foot processes in glomeruli, revealed by ultrastructural analysis, without any inflammatory injury or immune complex deposition. Up to 90% of children with INS respond to steroid treatment. However, 60%–80% of the initially steroid-responsive patients relapse.

Dyslipidemia, an important pathophysiological change in INS, was postulated that it directly stimulates mesangial cells’ proliferation and secretion of inflammatory factors including IFN-γ which is a proinflammatory cytokine produced by T-cells and natural killer (NK) cells. There is some controversy with respect to the levels and role of IFN-γ in nephrotic syndrome.

MCNS was considered as an exclusive systemic disorder of T cells and cell-mediated immunity 4 decades ago. A "two-hit" theory was proposed including the induction of CD80 (or B7-1) and Treg dysfunction, with or without impaired autoregulatory function of the podocytes. Recently, B-cell biology has been considered, since rituximab, a monoclonal antibody directed against CD20-bearing cells, showed good therapeutic potential in the treatment of both childhood and adulthood MCNS. However, the precise pathophysiology of MCNS still remains elusive.

It has been found that Tregs play a pivotal role in maintaining immune homeostasis and tolerogenecity. The Tregs dysregulation has been shown to cause imbalanced Th2/Th1 ratio with a predominant Th2 cytokine response and accordingly involved in the pathogenesis of many immune mediated diseases including MCNS.

Ahmed H. Hassan, Ihab Z. El-Hakim, Nermine H. Amr*, Dalia H. El-Ghoneimy**, Dina A. Soliman***, Dina E. Sallam.

Department of Pediatrics: Nephrology unit, Endocrinology unit*, and Immunology unit** and Department of Clinical Pathology***, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Correspondence: Ahmad Hussein Hassan, Pediatric Nephrology Unit, Children’s Hospital, Ain Shams University, Cairo, Egypt Email: ahmedkhattat@gmail.com
inducing a transient or persistent massive proteinuria, typically triggered by viral or bacterial infections, allergen- or T-cell-mediated release of cytokines (e.g., interleukin-13).\textsuperscript{10,11}

An association between various cytokines, circulatory factors and proteinuria have been shown to affect glomerular permeability,\textsuperscript{12} importantly, an increase in monocyte/macrophage cytokines, including interleukin IL1, IL12, IFN-\gamma and TNF-\alpha, were shown to be responsible for initiation and recurrence of INS.\textsuperscript{13}

IFN-\gamma is secreted from T-helper cells, cytotoxic T cells, natural killer cells, and macrophages. Interferons are well recognized for their strong antiviral properties, their ability to regulate cell growth and their immunomodulatory effects.\textsuperscript{13} IFN-\gamma promotes Th1 differentiation, eventually leading to cellular immunity and simultaneously inhibiting Th2 differentiation. It induces IL-12 production from antigen presenting cells (APCs), such as dendritic cells and macrophages in addition to the release of the vascular permeability factors by peripheral blood monocytes (PBMCs) that are all responsible for induction of glomerular injury and heavy proteinuria in INS.\textsuperscript{12,14}

This study is aimed to evaluate the immunopathogenic role of IFN-\gamma in children with steroid sensitive idiopathic nephrotic syndrome (SSNS), through monitoring the changes in its levels with disease course.

METHODS

An observational follow up pilot study was conducted on newly diagnosed 25 pediatric SSNS patients, presenting to children’s Hospital, Ain-Shams University, Cairo, Egypt, from June 2014 till the end of June 2016. An informed consent was obtained from each participant’s parents/caregivers after approval by the local Research Ethics Committee with approval number FMASU 1913/2014.

Patients enrolled in the study aged 2 to 8 years. They first presented with urinary protein/creatinine ratio > 2 g/mg, with hypoproteinemia and normal kidney function, before starting steroid therapy. Patients with secondary nephrotic syndrome, steroid resistant nephrotic syndrome or associated with systemic illness were excluded from the study. All patients received oral prednisolone at a dose of 60 mg/m\textsuperscript{2}/day.

Children were followed up clinically for 28 days until full remission guided clinically and laboratory with normalization of serum albumin and disappearance of proteinuria according to KDIGO 2012\textsuperscript{15}. Laboratory and clinical assessments were done at the time of diagnosis and at time of full remission and included serum total proteins and albumin, triglycerides (TG) and total cholesterol, complete blood count (CBC), complete urine analysis, protein creatinine ratio and serum level of IFN-\gamma by ELISA (the Quantikine Human IFN-\gamma Immunoassay, USA and Canada | R and D Systems, Inc. 614 McKinley Place NE, Minneapolis, MN 55413, USA).

Data Management and Statistical Analysis:
The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 13.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

RESULTS

The demographic and laboratory data are presented in table 1. There was improvement of serum albumin and protein/creatinine with remission.
Table 1. Sociodemographic and laboratory parameters of the studied patients

| Variable                  | Timing          | Mean ± SD       | Median (IQR)  | Range |
|---------------------------|-----------------|-----------------|---------------|-------|
| Age (years)               |                 | 3.86 ± 1.68     | 3.1 (2.8 - 5.08) | 2 - 7.5 |
| Weight (kg)               |                 | 17.22 ± 4.84    | 15 (14.5 - 20) | 11.5 - 30 |
| Systolic BP (mmHg)        |                 | 95.84 ± 7.45    | 95 (90 - 100)  | 85 - 110 |
| Diastolic BP (mmHg)       |                 | 63.8 ± 5.82     | 60 (60 - 70)   | 60 - 80 |
| S. IFN-γ (pg/ml)          | initial         | 27.12 ± 16.95   | 21 (16 - 30)   | 10 - 70 |
|                           | at full remission | 37.16 ± 14.03  | 35 (30 - 40)   | 20 - 80 |
| U. Prot./creat. ratio (g/mg) | initial         | 7.77 ± 4.8      | 6.8 (3.6 - 11) | 2.6 - 20 |
|                           | at full remission | 0.15 ± 0.02     | 0.12 (0.08 – 0.16) | 0.03 – 0.2 |
| S. albumin (g/dl)         | initial         | 1.4 ± 0.29      | 1.4 (1.1 - 1.5) | 1 - 2.2 |
|                           | at full remission | 3.86 ± 0.6      | 3.8 (3.5 - 4.2) | 2.5 - 5 |
| S. creatinine (mg/dl)     | initial         | 0.29 ± 0.12     | 0.2 (0.2 - 0.3) | 0.2 - 0.6 |
|                           | at full remission | 0.26 ± 0.07     | 0.3 (0.2 - 0.3) | 0.2 - 0.5 |
| Blood urea (mg/dl)        | initial         | 17.72 ± 7.62    | 17 (12 - 22)   | 7 - 37 |
|                           | at full remission | 19.92 ± 6.2     | 20 (16 - 22)   | 10 - 35 |
| TLC (*10^3)/µl            | initial         | 10.03 ± 2.62    | 9.5 (8 - 12.2) | 5.4 - 14 |
|                           | at full remission | 13.27 ± 2.47    | 14 (11 - 15)   | 7.9 - 18 |
| Hemoglobin (gm/dl)        | initial         | 12.23 ± 1.15    | 12.4 (11.5 - 13) | 10 - 15 |
|                           | at full remission | 12.73 ± 1.04    | 12.5 (12 - 13.6) | 10.8 - 14.5 |
| Platelets (*10^3)/µl      | initial         | 393.64 ± 81.76  | 400 (350 - 453) | 233 - 500 |
|                           | at full remission | 362 ± 95.25     | 350 (280 - 450) | 230 - 552 |
| S. total cholesterol (mg/dl) | initial         | 488 ± 63.4      | 470 (331 - 527) | 400-600 |
|                           | at full remission | 279 ± 67.7      | 256 (214 - 357) | 185-437 |
| S. triglycerides (mg/dl)  | initial         | 240.6 ± 74      | 204.8 (160 - 297) | 144-438 |
|                           | at full remission | 89.5 ± 33       | 67 (55 - 94)   | 34-150 |

IFN-γ: interferon gamma; BP: blood pressure; prot./creat. ratio: protein/creatinine ratio, TLC: total leucocytic count; S.: serum; U.: urinary; SD: standard deviation; IQR: interquartile range.

Table 2. Serum level of IFN-γ at initial diagnosis and at time of full remission

| IFN-γ (pg/ml) | Mean  | SD      | Paired t test | t  | p     | significance |
|---------------|-------|---------|---------------|----|-------|--------------|
| Initial       | 27.12 | 16.95   | 3.35          | 0.003 | S     |
| Remission     | 37.16 | 14.03   |               |     |       |              |

IFN-γ: interferon gamma, SD: standard deviation, S: significant

The serum level of IFN-γ showed significant increase at time of full remission with 88% of patients showing a rise in the levels from baseline.

Table 3. The frequency and average percentage of change in serum IFN-γ from initial values.

| Frequency of IFN-γ change | N   | %    |
|----------------------------|-----|------|
| Increased                  | 22  | 88%  |
| Decreased                  | 3   | 12%  |

Average percentage of change 25 60.8%

Table 4. Correlation of IFN-γ at initial diagnosis with the age of the patients and other laboratory investigations

| IFN-γ initial (pg/ml) | r    | p    |
|-----------------------|------|------|
| Age (years)           | -0.071 | 0.735 |
| U. Prot./creat. ratio (g/mg) | 0.104 | 0.621 |
| Serum albumin (g/dl)  | -0.297 | 0.149 |
| Total proteins (g/dl) | -0.227 | 0.276 |
| S. triglyceride (mg/dl) | -0.036 | 0.864 |
| S. total cholesterol (mg/dl) | 0.045 | 0.832 |
| Total leucocytic count (10^3)/µL | 0.202 | 0.333 |
| Absolute neutrophil count (10^3)/µL | 0.405 | 0.045 |
| Absolute lymphocytic count (10^3)/µL | 0.372 | 0.067 |

S.: serum; U.: urinary; IFN-γ: interferon gamma, prot./creat. ratio: protein creatinine ratio.
A moderate correlation was found between serum levels of IFN-γ and the absolute neutrophil count and a weak one with absolute lymphocytic count at initial diagnosis. Otherwise, there were no significant correlations.

**Table 5. Correlation of IFN-γ at remission with other variables**

| Variables                        | IFN-γ remission (pg/ml) | r      | p     |
|----------------------------------|-------------------------|--------|-------|
| S. albumin (g/dl)                | -0.283                  | 0.170  |       |
| Total leucocytic count (10³/µL) | -0.042                  | 0.843  |       |
| Absolute neutrophil count (10³/µL) | 0.020                  | 0.923  |       |
| Absolute lymphocytic count (10³/µL) | 0.095                  | 0.651  |       |
| S. triglyceride (mg/dL)          | -0.059                  | 0.779  |       |
| S. total cholesterol (mg/dL)     | -0.140                  | 0.504  |       |
| Cumulative dose of prednisolone (mg) | 0.4                    | 0.21   |       |

*S.: serum; IFN-γ: interferon gamma.

**DISCUSSION**

In this study, serum levels of IFN-γ of the studied patients were noted to be non-significantly lower at diagnosis of nephrotic syndrome compared to remission. With disease remission, serum IFN-γ increased in most of the patients (22 out of 25) with percentage change ranging from 11% to 200% and only 3 had decreased levels, with percentage change ranging from -17% to -57%. The predominant rise in level suggests the recovery of some balance between Th1 and Th2 with subsequent normalization of Th1 cytokine profile including IFN-γ with consequent remission of NS. Concerning the 3 patients who showed a drop in IFN-γ at remission, their TLC was elevated at that time suggesting the occurrence of concomitant infection that might explain the initially high IFN-γ levels.

Prior studies reported that the serum level of IFN-γ was significantly low at the diagnosis of nephrotic syndrome in comparison to their respective values at remission and controls, supporting a role for Tregs dysregulation with a predominant effect of T helper 2 (Th2) cells in the pathogenesis of INS and suppressed effect of T helper 1 (Th1) cells and its secreted cytokines. In contradiction to our finding, IFN-γ was found to be significantly higher in patients with active nephrotic syndrome at diagnosis, whereas remissions are characterized by downregulation of that cytokine. The authors related their finding to the different cells of origin of IFN-γ (dendritic cells, macrophages and other antigen presenting cells) other than Th1 cells which are not the master cells in the immunopathogenesis of nephrotic syndrome. Although the present study confirmed that IFN-γ had no significant correlation with urinary protein creatinine ratio at initial diagnosis, their study demonstrated that serum IFN-γ correlated positively with 24-hour urinary proteins.

Serum IFN-γ in our study had negative, though insignificant correlations with serum triglycerides and total cholesterol at full remission, lending support for the absence of a role for IFN-γ in promoting the inflammation mediated by dyslipidemia in patients with INS.

Limitations of the study were absence of control group, small sample size being a pilot study, with a relatively short duration and narrow scale with exclusion of other types of INS (resistant and dependent).

This study points to a role for the relatively lower serum IFN-γ at diagnosis than at remission, in the immunopathogenesis of INS and the rise in its serum level might be a marker of remission induction. Further studies on renal biopsy specimens are needed to determine the renal in situ effect of IFN-γ.

**REFERENCES**

1. **DH J, Kemper MU.** Minimal change (steroid sensitive) nephrotic syndrome in children: new aspects on pathogenesis and treatment. Minerva Pediatr 2012;64:197-204

2. **Eddy AA, Symonds JM.** Nephrotic syndrome in childhood. Lancet 2003;362:629-39

3. **Kim SH, Han KH, Kronbichler A, Saleem MA, Oh J, Lim BJ, et al.** Pathogenesis of minimal change nephrotic syndrome: an immunological concept. Korean J Pediatr 2016;59(5):205-11.

4. **Wu T, Xie G, Wang H, Zhou XJ, Schwartz N, Calixto S, et al.** Elevated urinary VCAM-1. P-selectin, soluble TNF receptor-1, and CXC chemokine ligand 16 in multiple murine lupus strains and human lupus nephritis. J Immunol 2007;179:7166-75.
5. Stevenson F, Shearer G, Atkinson D. Lipoprotein-stimulated mesangial cell proliferation and gene expression are regulated by lipoprotein lipase. Kidney International 2001;59:2062-8.

6. Zhen, J, Li, Q, Zhu, Y, Yao X, Wang L, Zhou A, et al. Increased serum CXCL16 is highly correlated with blood lipids, urine protein and immune reaction in children with active nephrotic syndrome. Diagn Pathol 2014;9:23.

7. Shalhoub RJ. Pathogenesis of lipoid nephrosis: a disorder of T-cell function. Lancet 1974;2:556-60.

8. Zhao Z, Liao G, Li Y, Zhou S, Zou H. The efficacy and safety of rituximab in treating childhood refractory nephrotic syndrome: a meta-analysis. Sci Rep 2015;5:8219.

9. Zou, W. Regulatory T cells, tumour immunity and immunotherapy. Nat Rev Immunol 2006;6(4):295-307.

10. Grant, CR, Liberal, R, Mieli-Vergani, G, Vergani D, Longhi MS. Regulatory T-cells in autoimmune diseases challenges, controversies and--yet-- unanswered questions. Autoimmun Rev 2015;14(2):105-16.

11. Prasad, N, Jaiswal, AK, Abarwal, V, Yadav B, Sharma RK, Rai M, et al. Differential alteration in peripheral T-regulatory and T-effector cells with change in P-glycoprotein expression in Childhood Nephrotic Syndrome A longitudinal study. Cytokine 2015;72(2):190-6.

12. Pereira W de F, Brito-Melo, GEA, Guimaraes FTL, Carvalho TGR, Matedo EC, Silva ACS. The role of the immune system in idiopathic nephrotic syndrome a review of clinical and experimental studies. Inflamm Res 2014;63(1):1-12.

13. Le Berre L, Bruneau S, Renaudin K, Naulet J, Usal G, Smit H, et al. Development of initial idiopathic nephrotic syndrome and post-transplantation recurrence evidence of the same biological entity. Nephrol Dial Transplant 2011;26(5):1523-32.

14. Shalaby SA, Al-Edreessi HM, El-Tarnhouny SA, Fath El-Bab M, Zolaly MA. Type 1/type 2 cytokine serum levels and role of interleukin-18 in children with steroid-sensitive nephrotic syndrome. Arab J Nephrol Transplant 2013;6(2):83-8.

15. KDIGO Clinical Practice Guideline for Glomerulonephritis: Steroid-sensitive nephrotic syndrome in children. Kidney Inter Suppl 2012; 2: 139-274 Available from: https://kdigo.org/wp-content/uploads/2017/02/KDIGO-2012-GN-Guideline-English.pdf.

16. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma an overview of signals, mechanisms and functions. J Leukoc Biol 2004; 75(2):163-89.

17. Park, BS, Hahn, WH, Kim, SD, Cho BS. Remission of refractory minimal change nephrotic syndrome after basiliximab therapy. Pediatr Nephro 2009, 24(7):1403-7.

18. Printza N, Papachristou F, Tzimiouli V. IL-18 is correlated with type-2 immune response in children with steroid sensitive nephrotic syndrome. Cytokine 2008;44(2):262-8.