SERUM LEVELS OF INTERLEUKINS 13 AND 17A IN IRAQI CHILDREN WITH NEPHROTIC SYNDROME

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ABSTRACT

Childhood nephrotic syndrome is a collection of symptoms results from kidney damage that leads to massive protein loss in the urine, low plasma albumin levels, and edema. This study aims to determine serum Levels of Interleukins 13 and 17A in Iraqi children with nephrotic syndrome. The results of serum IL-13 and IL-17A levels showed a significant increase in NDNS children (8.25 ± 0.12 pg/ml) (713.28 ± 7.31 pg/ml) respectively compared to the healthy control group (4.13 ± 0.11 pg/ml) (214.03 ± 3.41 pg/ml), respectively. Furthermore, there was a significant correlation between biochemical markers and immunological parameters in children with nephrotic syndrome.

Keywords: Il-13, Il-17A, idiopathic nephrotic syndrome, podocin

I. INTRODUCTION

Nephrotic syndrome (NS) disease outcomes from a significant loss of protein into the urine decreased plasma albumin level and edema. In spite of the apparent heterogeneity, the primary disorder is the loss of the natural selective properties of the glomerular filtration barrier (GFB). The evidence on the molecular basis hail from the detection of autosomal inheritance (dominant or recessive), and the discovery of genetic polymorphisms in Dennis-Drash syndrome (DDS), NS, and Wilms' tumor (Bierzynska et al., 2015).

Because nephrin, podocin, and dystroglycan are important protein molecules in maintaining the integrity of the slit diaphragm and attachment of podocyte foot processes to the glomerular basement membrane (GBM), IL-13 may play an important role in the development of proteinuria in NS by exerting a direct effect on podocytes, acting through the IL-13 receptors on the podocyte cell surface, initiating certain signaling pathways that eventually lead to changes in the expression of these podocyte related proteins. IL-13 gene expression was upregulated in both CD4 and CD8 T cells in children with steroid-sensitive nephrotic syndrome in relapse. This was associated with increased intracytoplasmic IL-13 production by CD3 cells, as well as downregulation of gene expression of the monocyte proinflammatory cytokines IL-8 and IL-12 (Lai et al., 2007).

Th17 cells may participate in the onset of the inflammatory process and chronic progression in primary nephrotic syndrome by mediating proteinuria emergence and progressive renal damage. IL-17A is a pleiotropic cytokine that participates in tissue inflammation by inducing the expression of chemokines, proinflammatory cytokines and matrix metalloproteases. More specifically, IL-17A decreases the concentration of podocalyxin and induces podocyte apoptosis. A dynamic equilibrium between Th17 cells and T regulatory cells has been demonstrated in children after the development of a primary nephrotic syndrome with apparent renal tubular epithelial cells and interstitial lesions (Cortvrindt et al., 2017).

Most subjects of NS are steroid-sensitive nephrotic syndrome (SSNS). About 50% of the latter recur frequently and require relapse prevention by non-steroidal drugs. Steroid-resistant nephrotic syndrome (SRNS) mostly leads to end-stage renal failure. While, 30-40% of the latter are associated with mutations in the genes that encode podocin. The rest is due to one or several different common factors (Davin and Rutjes, 2011).
This study aims to evaluate determine serum Levels of Interleukins 13 and 17A in Iraqi children with nephrotic syndrome.

II. SUBJECTS, MATERIALS AND METHODS

2.1 Studied Cases

According to the steroid therapy responses, 75 cases (100%) newly diagnosed nephrotic syndrome children (NDNS 52M:23F) were divided into two general groups: 50 cases (67%) steroid sensitive group (SSNS 35M:15F) and 25 cases (33%) steroid resistance group (SRNS 17M:8F). Steroid-sensitive group was divided into two sub-groups depending on relapse number within 6 months of the initial response: 27 cases (36%) frequent relapse (SSFR 18M:9F) and 23 cases (31%) infrequent relapse (SIFR 17M:6F) while steroid resistance group which was divided into two subgroups depending on genetic screening: 17 cases (23%) genetic screen negative steroid resistance (GNSR 11M:6F) and 8 cases (10%) genetic screen positive steroid resistance (GPSR 6M:2F).

2.2 Evaluation of human interleukin – 13 and 17A by ELISA technique

The RayBio® Human IL-13 and IL-17A ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IL-13 and IL-17A in serum, plasma, and cell culture supernatants. This assay employs an antibody specific for human interleukins was pre-coated on a 96-well plate. Standards and samples were pipetted into the wells and interleukins present in a sample is bound to the wells by the immobilized antibody. The wells were washed and biotinylated antihuman IL-13 and IL-17A antibodies were added. After washing away unbound biotinylated antibody, HRP conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution was added to the wells and color develops in proportion to the number of interleukins bound. The stop solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm.

III. RESULTS

The statistical analysis for results of some immunological markers levels (interleukin 13 (IL-13) and interleukin-17A (IL-17A)) in children newly diagnosed with nephrotic syndrome, treated groups (SSNS and SRNS)/ subgroups (SSFR, SIFR, GPSR and GNSR), and healthy control were shown below in Tables (1, 2).

Table (1): Comparison between different groups (control, newly diagnosed, general treated group) in immunological parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Immunological parameters (Mean ± SEM)</th>
<th>IL- 13 (pg/mL)</th>
<th>IL- 17A (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.13 ± 0.11 c</td>
<td>214.03 ± 3.41 c</td>
<td></td>
</tr>
<tr>
<td>NDNS</td>
<td>8.25 ± 0.12 a</td>
<td>713.28 ± 7.31 a</td>
<td></td>
</tr>
<tr>
<td>Treated NS</td>
<td>6.52 ± 0.07 b</td>
<td>372.93 ± 5.58 b</td>
<td></td>
</tr>
<tr>
<td>LSD value</td>
<td>0.330 **</td>
<td>20.545 **</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

NDNS: Newly diagnosed nephrotic syndrome group; Means having with the different letters in same column differed significantly; ** (P≤0.01)

Table (2): Comparison between different groups (control, newly diagnosed, steroid sensitive and resistance groups) in immunological parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Immunological parameters (Mean ± SEM)</th>
<th>IL- 13 (pg/mL)</th>
<th>IL- 17A (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.13 ±0.11 c</td>
<td>214.03 ±3.41 d</td>
<td></td>
</tr>
<tr>
<td>NDNS</td>
<td>8.25 ±0.12 a</td>
<td>713.28 ±7.31 a</td>
<td></td>
</tr>
<tr>
<td>Treated NS</td>
<td>6.48 ±0.08 b</td>
<td>392.62 ±5.03 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.61 ±0.13 b</td>
<td>333.53 ±9.33 c</td>
<td></td>
</tr>
<tr>
<td>LSD value</td>
<td>0.381 **</td>
<td>22.302 **</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>
3.1 Interleukin-13 (IL-13)

Interlukine-13 is an anti-inflammatory cytokine that is secreted by various T-cell subsets and dendritic cells. It composites from 132 amino acids and its molecular weight is 12 kd. (Vries, 1998). IL-13 shows anti-inflammatory activities by suppressing the production of inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and IL-8, by human peripheral blood monocytes promoted by lipopolysaccharide (Shaikh, 2011).

Table (1) showed a significant increase in serum IL-13 mean levels in NDNS children (8.25 ± 0.12 pg/ml) compared to the treated NS group (6.52 ± 0.07 pg/ml) and healthy control group (4.13 ± 0.11 pg/ml). While there were no significant differences among different treated NS groups (SSNS and SRNS) in serum IL-13 mean levels as shown previously in Table (2). These results agreed with Mishra et al. (2014) who suggested the raise serum IL-13 in active phase and normalization in remission.

Figure (1): Comparison between difference groups in serum Interlukine-13 levels

NDNS: Newly diagnosed children with typical nephrotic syndrome; SSFR: Steroid sensitive Infrequent relapse group; SSIFR: Steroid sensitive frequent relapse group; GPSR: Genetic screen positive steroid resistance group; GNSR: Genetic screen negative steroid resistance group; Means having with the different letters differed significantly; ** (P≤0.01)

In addition, among the different NS-treated subgroups (SSFR, SSIFR, GPSR, and GNSR), Figure (1) showed that there were no significant differences between mean serum IL-13 levels. These results are in agreement with Mamizadeh et al. (2017) who reported that Level of serum IL-13 does not significantly drop after steroid therapy. Higher concentration of serum IL-13 in children with acute minimal change disease (MCD) in comparison with a healthy control group. They raised expression of IL-13 by T-helper 2 cells in patients with acute nephrotic syndrome. Steroid can repress the IL-13 production by CD4+ cells rather than by CD8+ T-lymphocyte. Another study reported by Mishra et al. (2014) demonstrated a greater concentration of urinary IL-13 in untreated nephrotic syndrome patients in comparison to healthy controls and the percentage of CD3+ IL-13-producing cells was significantly greater in relapsing nephrotic and reduced during remission.

IL-13 correlated with the induction of podocyte structural alteration that are capable of changing the filtration selectivity and causing nephrotic syndrome (Medrano et al. 2012). Overexpression of IL-13 gives rise to the
downregulation of podocin, nephrin, and dystroglycan. These proteins are decisive molecules in maintaining the integrity of slit diaphragm, and the synchronous upregulation of CD80, IL-13Rα2 and IL-4Rα in IL-13 transfected rats (Kim et al. 2016).

IL-13 prompt CD80 expression in podocytes, and CD80 is the key mediator for prompting proteinuria. It is assumed that NS has two steps: the first step is raising serum IL-13 concentration and then CD80 in podocytes cytoplasm and the second step is having inadequate CD80 silencing by deficient release of soluble CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) (Mamizadeh et al. 2017). Nephrotic syndrome can begin with a raise in production of IL-13 but its remission may concern with CD80/CTLA-4 ratio not on the serum IL-13 concentration (Mamizadeh et al. 2017).

3.2 Interleukin-17A (IL-17A)

Interleukin-17 (IL-17) is a potent pro-inflammatory cytokine that is produced by activated T lymphocytes (Nakae et al. 2003). IL-17A is the founding member of the IL-17 family of cytokines produced by a subset of T helper (Th) cells, termed Th17 cells, which also secrete a number of other cytokines, including IL-6, IL-17F, IL-21, IL-22, granulocyte macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor (TNF) (Korn et al., 2009). Although there is a 50% homology at protein level between IL-17A and IL-17F, the strength of IL-17A signaling is 10–30-fold stronger than IL-17F (Gaffen, 2009).

Table (1) showed a significant increase in serum IL-17A mean levels in NDNS children (713.28 ± 7.31 pg/ml) compared to the treated NS group (372.93 ± 5.58 pg/ml) and healthy control group (214.03 ± 3.41 pg/ml).

Among the different treated NS groups (SSNS and SRNS), Table (2) showed that the mean serum levels of IL-17A in SRNS (333.53 ± 9.33 pg/ml) were significantly lower than those of SSNS (392.62 ± 5.03 pg/ml). These results agreed with Shao et al., (2009) who suggested that elevated serum IL-17A may play a role in the pathogenesis of neuropsychiatric and renal manifestations.

In addition, among the different NS-treated subgroups (SSFR, SSIFR, GPSR, and GNSR), Figure (2) below showed that SSFR and SSIFR children had mean serum IL-17A levels (386.27 ± 7.80 pg/ml) and (400.09 ± 5.77 pg/ml) respectively, while the GPSR and GNSR children had serum levels of IL-17A (325.36 ± 13.54 pg/ml) and (337.37 ± 12.31 pg/ml) respectively. These results are in agreement with Wang et al., (2017).

Figure (2): Comparison between difference groups in serum Interlukine-17A levels
NDNS: Newly diagnosed children with typical nephrotic syndrome; SSIFR: Steroid sensitive Infrequent relapse group; SSFR: Steroid sensitive frequent relapse group; GNSR: Genetic screen negative steroid resistance group; GPSR: Genetic screen positive steroid resistance group; Means having with the different letters differed significantly: ** (P≤0.01)

Th17 cells may participate in the onset of the inflammatory process and chronic progression in primary nephrotic syndrome by mediating proteinuria emergence and progressive renal damage (Shao et al. 2009). More specifically, IL-17A decreases the concentration of podocalyxin and induces podocyte apoptosis (Wang et al., 2017). A dynamic equilibrium between Th17 cells and Treg cells has been demonstrated in children after the development of a primary nephrotic syndrome with apparent renal tubular epithelial cells and interstitial lesions. A significant increase in the frequency of Th17 cells, Th17-related cytokines (IL-1b, IL-6, IL-17A and IL-23) and retinoid orphan nuclear receptor (RORc) levels has been detected in primary nephrotic syndrome patients, compared with normal subjects and patients with isolated hematuria (Shao et al. 2009).

Higher circulating frequencies of Th17 cells have been reported in children with non-minimal change nephrotic syndrome (including focal segmental glomerulosclerosis and mesangial proliferative glomerulonephritis) in comparison with minimal change nephrotic syndrome patients (Wang et al., 2017).

Effective corticosteroid treatment reversed the Th17/Treg functional imbalance and resulted in a normalization of the urinary IL-17A excretion (Matsumoto et al., 2002). Also, the anti-CD20 antibody rituximab reduced the Th17 cell response in patients with a minimal change nephrotic syndrome (van de Veerdonk et al., 2009).

REFERENCES


