Assessment of IL23/Th17 Axis in Egyptian Patients with Grade 2 Hypertension
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ABSTRACT
Background: Accumulating evidence indicates that the immune system plays a critical role in the pathogenesis of cardiovascular diseases including hypertension. T helper 17 (Th17) is a recently discovered subgroup of helper T cell characterized by the secretion of interleukin 17 (IL17). It is believed that Th17 may play a role in the pathogenesis of hypertension which is probably caused by the increased plasma IL-23.

Objective: The aim of the work was to assess Th17 cells and plasma IL23 level in patients with uncontrolled grade 2 hypertension.

Patients and method: This study was conducted on 45 subjects divided into 30 primary hypertensive patients (subdivided into Group A: 15 recently diagnosed/uncontrolled patients with grade 2 hypertensive episode, group B: 15 patients with controlled blood pressure) and 15 healthy volunteers.

Result: We found that the mean±SD of IL23 level showed a highly significant difference between the three groups being higher in uncontrolled grade 2 hypertension patients (group A) (3355.1±216.9 pg/ml) than the controlled hypertension group B (2207.5±135.8 pg/ml), and group B was higher than the normal healthy controls (1852.7±73.2 pg/ml) (p<0.0001). The mean±SD of Th17 cells% was highly significantly different between the three groups being higher in uncontrolled grade 2 hypertension patients (group A) (1.27±0.09 %) than the controlled hypertension group B (0.38±0.09%), which in turn was higher than the normal healthy controls (0.15±0.05 %) (p<0.0001).

Conclusion: We conclude that IL23/Th17 axis may be involved in the pathogenesis of hypertension, especially in patients with unexplained grade 2 hypertensive patients experiencing an acute episodes, suggesting a state of ongoing subclinical inflammation.

Keywords: Grade 2 hypertension, Interleukin 23, Th17.

INTRODUCTION
All inflammatory-related mechanisms are enhanced in hypertension, such as adhesion molecules and chemokine expression, immune cell activation, cytokine release and oxidative stress (1).

Th17 cells develop from naive CD4+ T cells, Th17 differentiation, Th17 polarization in humans requires IL1 beta, IL6, IL21, and IL23. Signaling through IL23 activates the STAT3-dependent expression of IL21, IL23 R, and the transcription factor, ROR gamma t. IL21 and IL23 regulate the establishment and clonal expansion of Th17 cells. So, IL23 is an important factor in Th17 cell survival and proliferation. This pathway is described in literature as IL23/Th17 axis (2).

Cytokines secreted by Th17 cells stimulate chemokine secretion by resident cells, leading to the recruitment of neutrophils and macrophages to sites of inflammation. These cells, in turn, produce additional cytokines and proteases that further exacerbate the immune response (3). Persistent secretion of Th17 cytokines promotes chronic inflammation and may be involved in the pathogenesis of inflammatory and autoimmune diseases, including rheumatoid arthritis (4), psoriasis (5), multiple sclerosis (6), and inflammatory bowel disorders (7).

Several studies have demonstrated the role of immune system in the pathogenesis of hypertension, both innate and adaptive immune system work in a very coordinated manner and play a pivotal role in vascular remodeling, and development of hypertension (8, 9).

Therefore, the question whether both systems communicate during hypertension development has come to light (10). Evidence from experimental and observational studies indicates that alterations in immune cell populations, as activation of different lymphocyte subtypes play an important role in the pathogenesis of hypertension (11).

To our knowledge, studies focusing on the role of IL17 in hypertension are relatively more plentiful compared to those addressing the role of IL23. Moreover, the number of studies that assess both Th17 and IL23 in hypertension is inadequate (12). Therefore, despite its potential importance, we are only at the very beginning of studies on the IL-23/Th17 axis in essential hypertension.

The aim of the work was to assess Th17 cells and plasma IL23 level in patients with uncontrolled grade 2 hypertension.

PATIENTS AND METHODS
This cross-sectional case-control study was carried out on forty five adult individuals that were recruited from the Department of Internal Medicine. Subjects were further divided into three groups according to the blood pressure as follow:

Group A: 15 hypertensive patients with recently diagnosed/uncontrolled patients with grade 2 hypertensive episode.
**Group B:** 15 hypertensive patients with controlled blood pressure.

**Group C:** 15 healthy control subjects with normal blood pressure.

**Inclusion criteria:**

All patients included were hypertensive diagnosed according to the Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) guidelines (10). Inclusion criteria for hypertensive patients with grade 2 hypertension: (1) incident hypertension, SBP ≥ 160 mmHg and/or DBP ≥ 100 mmHg, no previous history of hypertension found; (2) Blood pressure controlled in the normal course but are experiencing a sudden unexplained rise of SBP ≥ 160 mmHg and/or DBP ≥ 90 mmHg.

**Exclusion criteria:**

- Acute coronary syndrome; acute and chronic heart failure; autoimmune diseases; acute severe infection; bronchial asthma; tumor; diabetes; hyperlipidemia; acute cephalic vascular accident; liver and kidney dysfunction; use of steroids or immunosuppressive drugs; patient experiencing acute rise in blood pressure due to non-compliance to antihypertensive treatment.

All members of the study were subjected to full medical history taking, clinical examination and laboratory investigation including complete blood count, kidney function tests (serum urea and creatinine by ELISA), liver function tests (SGOT and SGPT) and lipid profile. Also, estimation of plasma IL23 level by ELISA, and detection of Th17 percentage and absolute count in peripheral blood by Flow cytometry.

Determination of human (IL-23) concentrations in plasma: 5 ml venous blood was drawn from every patient after over 8 h fasting. Blood was collected in heparin anticoagulation tube. The blood samples of group A were collected within 24 h of acute blood pressure increase. The plasma was separated from peripheral blood by centrifuging at 2000 rpm for 20 min. The levels of IL 23 in plasma were detected by corresponding Quantitative Human IL-23 ELISA Kit (eBioscience, USA) as instructed by the manufacturer.

Detection of T helper17 cells (Th 17) by measurement of the combined expression of CD3, IL23R and intracellular IL22, IL17 using four colors flow cytometer Beckman Coulter Navios with incorporated Navios software (Version1.2). Peripheral Blood Mononuclear Cells (PBMCs) were isolated by Ficoll density according to manufacturer’s instructions (RandD Systems® multicolor flow cytometry Kit, Catalog # FMC007, USA). PBMCs were then resuspended in 0.5 mL of Fixation/Permeabilization Buffer and incubated at 2-8 °C for 30 minutes. Cells were vortexed intermittently to maintain a single cell suspension. The cells were centrifuged, and the pellet was resuspended in 100-200 μL of the Permeabilization/Wash Buffer. Cells were then collected and stained simultaneously with the addition of 10 μL of each antibody (anti-CD3, anti-IL17, anti-IL22 and anti-IL23R antibodies).

The mixture was incubated for 30-45 minutes at 2-8 °C in the dark. Following the incubation, any excess antibody was removed by washing the cells in 2 mL of Permeabilization/Wash Buffer. The final cell pellet was resuspended in 200-400 μL of PBS for flow cytometric analysis. Cells were analyzed by flow cytometer. Lymphocytes were gated according to their forward and side scatter properties and CD3+ve (T cells) were gated out of total lymphocytes. Th17 were identified by combined expression of CD3, IL23R and intracellular IL22, IL17 on peripheral blood lymphocytes. Isotype-matched controls were used to rule out the effect of autofluorescence and nonspecific binding of monoclonal antibodies.

**Ethical consent:**

An approval of the study was obtained from Ain Shams University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

**Statistical analysis**

Data were analyzed using STATA intercooled version 14.2. (StataCorp LLC, College Station, TX, USA). Quantitative data were represented as mean and standard deviation. Data were analyzed using one-way ANOVA for comparison of the means of three groups with post hoc Bonferroni test. Correlation analysis was accomplished by using Pearson test. Graphs were produced by using Excel or STATA program. P value was considered significant if it was less than 0.05 and highly significant if less than 0.01.

**RESULTS**

Demographic and clinical characteristics of all participants:

Thirty patients with primary hypertension and 15 healthy individuals were enrolled in the study. As regard age or gender, no statistically significant difference between studied groups was detected. Systolic and diastolic blood pressure of studied populations were significantly higher in patients compared to the control group (Table 1).
Table (1): Comparison between studied groups regarding systolic and diastolic blood pressure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group (A) (N=15)</th>
<th>Group (B) (N=15)</th>
<th>Group (C) (N=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>167±7.27</td>
<td>127±4.92</td>
<td>115.33±5.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>DBP (mmHg)</td>
<td>99±5.07</td>
<td>83±2.53</td>
<td>74.33±5.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
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</table>

Laboratory investigations all participants:
As regards routine laboratory investigations (urea, creatinine, SGPT, SGOT, HB) there was non-significant difference between the three groups.

Comparison between plasma IL23 among all participants:
Regarding IL23 level in plasma, there was a highly significant difference between the three studied groups, being highest in Group A, and higher in group B than the normal controls (Table 2).

Detection of Th17 in all participants:
As regards Th17 flow cytometry of our studied populations, we found that mean of Th17% were statistically highly significant in group A in comparison to group B, and in group B in comparison to group C (Table 2).

Table (2): Comparison between studied groups regarding laboratory investigations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group (A) (N=15)</th>
<th>Group (B) (N=15)</th>
<th>Group (C) (N=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL23 (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>3355.1±216.9</td>
<td>2207.5±135.8</td>
<td>1852.7±73.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CD4 (Mean ± SD)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>43.87±9.52</td>
<td>1.27±7.51</td>
<td>45.4±7.41</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Th17 (10^3/ml) %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.27±0.09</td>
<td>0.38±0.09</td>
<td>0.15±0.05</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Correlation between Th17 and IL23 was highly significant with both groups A and B and significantly correlated in group C (Table 3 and figures 1, 2, 3).

Table (3): Correlation between Th17% and IL23 level among the different groups

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient (r)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (A)</td>
<td>0.86</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Group (B)</td>
<td>0.87</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Group (C)</td>
<td>0.57</td>
<td>0.03</td>
</tr>
<tr>
<td>All subjects</td>
<td>0.96</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Figure (1): Simple scatter graph showing the correlation between Th17% and IL23 level among group A subjects
Figure (2): Simple scatter graph showing the correlation between Th17% and IL23 level among group B subjects

Figure (3): Simple scatter graph showing the correlation between Th17% and IL23 level among all subjects

Also we found that Th17% showed highly significant correlation with each SBP, DBP and HDL, it was also significantly correlated with each LDL, TG and SGPT. On the other hand it showed non-significant correlation with each of TC, HB, SGOT, CD4, Urea, creatinine and age of the subjects. IL23 was highly significantly correlated with each SBP, DBP and HDL, it also significantly correlated to TG, SGOT and SGPT. On the other hand it showed a non-significant correlation with each TC, LDL, HB, CD4, Urea, creatinine and age of the subjects (Table 4).

Table (4): Correlation between IL23 and variable data

<table>
<thead>
<tr>
<th></th>
<th>Correlation co efficient (r)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>0.163</td>
<td>0.31</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>0.862</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>0.820</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG (ng/mL)</td>
<td>0.431</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>0.261</td>
<td>0.08</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>0.530</td>
<td>&lt;0.001</td>
</tr>
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</table>
DISCUSSION
Although hypertension is considered one of the most prevalent chronic medical conditions, its pathophysiology is complicated and is not fully understood (14). Recent studies suggest that hypertension is in part mediated by immune mechanisms. Both interleukin IL23 and IL17 are up-regulated in several experimental hypertensive rodent models, as well as in hypertensive humans in observational studies. Recent preclinical studies have shown that either IL23 or IL17R treatment induce blood pressure elevation and associated end organ damage. However, the IL23/IL17 axis has not been studied thoroughly in human studies, unlike in other autoimmune diseases (12). The mechanisms through which T cells undergo activation in the setting of hypertension remain an intense area of investigation.

In our study, we found that mean of Th17 cells % was higher in group A, followed by group B then group C (1.27±0.09, 0.38±0.09, 0.15±0.05 respectively). This goes in agreement with a previous study by Wang et al. (15) who demonstrated an increased level of Th17 cells expressing IL23R in hypertensive patients with acute increases of blood pressure. Also, Itani and colleagues (16) reported that circulating Th17 lymphocytes were increased in hypertensive Humanized Mice and Hypertensive Humans compared with normotensive controls. Our study goes in concordance with a study done by Ji et al. (17) who investigated the circulating T helper subsets in patients with primary hypertension. Their results showed that the circulating Th17 levels especially in non-dipper patients and patients with carotid atherosclerotic plaque, were markedly higher than those in the control group.

We found also that mean of plasma IL23 level was higher in group A, followed by group B then group C (3355.1±216.9, 2207.5±135.8, 1852.7±73.2 pg/ml respectively), all these differences were highly significant. Ye and coworkers (18) reported that serum IL23 level was higher in patients with hypertension compared to healthy controls and was positively correlated with blood pressure. This result is also consistent with a previous study reporting higher serum IL23 levels in a young obese hypertensive population (19). Interestingly, this was supported by another study that links high salt intake with higher plasma levels of IL23 and IL17 in healthy human subjects (20). Although blood pressure was not assessed in that study, this is reminiscent of the effects that salt intake has inducing IL23R expression and Th17 differentiation via SGK1 activation (21, 22).

It is interesting to note that many of the currently used drugs in the therapy of hypertension, including angiotensin II receptor blockers (ARBs) and statins, have the ability to decrease IL23 levels and ameliorating Th17/Treg functional imbalance in hypertensive patients with carotid atherosclerosis (23).

However, some studies showed that IL23, although not required for the differentiation, was shown to play a role in the survival/expansion of Th17 cells (24, 25). We found that IL23 show a highly significant correlation with Th17 % in both groups A and B and a significant correlation in group C, our results go in agreement with Wang et al. (15) as in their study, they also found a positive correlation between IL23 and IL17 expression, suggesting that IL23 might participate in the increase of blood pressure through promoting the differentiation of Th17.

Therefore, inflammation axis of IL23/Th17/IL17 is closely associated with the change of blood pressure and may be involved in the reoccurrence of hypertension on the other hand. Results of Simunidi et al. (26) suggest that hypertension stimulates the immune system regardless of blood pressure control status, and that prolonged hypertension influences peripheral monocyte TLR4 expression and IL17A serum levels. Toll-like Receptor (TLR) activation via damage-associated molecular patterns (DAMPs) is believed to link inflammation to hypertension. An increasing body of evidence suggests that oxidative stress is also associated with accelerated vascular aging, which contributes to vascular stiffening, perivascular inflammation and vascular calcification in angiotensin II induced hypertension (27).

According to our study, Th17 was highly significantly correlated with each SBP, DBP and HDL, it also significantly correlated with each LDL, TG and SGPT. We also found that IL23 was highly significantly correlated with each SBP, DBP and HDL, it also significantly correlated with each TG, SGOT and SGPT, while Manti et al. (28) stated that IL17 and IL23 levels positively correlated with total cholesterol, LDL, and triglycerides values and although not statistically significant, an inverse correlation has been noted between serum IL17, IL23 and HDL levels in children with dyslipidemia. In patients with atherosclerosis, IL23 and IL23R were increased in atherosclerotic plaques, compared with non-affected vessels and higher levels of IL23 that were observed in patients with more recent symptoms. Moreover, long-term outcomes showed an adjusted association between higher IL23 plasma levels and mortality (29).

CONCLUSION
We demonstrated that IL23/Th17 axis may be involved in the pathogenesis of acute blood pressure increase in hypertensive patients, suggesting a state of ongoing subclinical inflammation that may be exacerbated by salt intake or by a yet unidentified trigger. Understanding its inflammatory characterization might be of fundamental importance for the prevention and targeted treatment of acute hypertensive episodes. Therefore, an overall comprehension of how the adaptive immune system triggers hypertension is needed to tailor pharmacological inhibition of this process with minimal immunosuppressive effects in hypertensive patients.
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Conflict of interest: Nil.

REFERENCES


