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# Decreased serum interleukin-17 and increased transforming growth factor–$\beta$ levels in subjects with metabolic syndrome (Chennai Urban Rural Epidemiology Study–95)

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**Abstract**

The term *metabolic syndrome* (MS) refers to a conglomeration of many metabolic disorders. Recent studies suggest that inflammation plays a vital role in MS. There are however no data available on the recently characterized novel T-cell–derived cytokine interleukin (IL)-17 in MS; studies on the anti-inflammatory cytokine transforming growth factor (TGF)–$\beta$ are also limited. The aim of the study was to look at IL-17 and TGF-$\beta$ levels in subjects with and without MS. The study subjects were recruited from the Chennai Urban Rural Epidemiology Study (CURES), a population-based study in Chennai (formerly Madras) in southern India. Group 1 consisted of subjects without MS (non-MS) (n = 98) and group 2 consisted of subjects with MS (n = 156). MS was defined using the National Cholesterol Education Program Adult Treatment Panel III criteria modified for waist, according to the World Health Organization Asia Pacific guidelines. Serum IL-17 and TGF-$\beta$ levels were estimated by enzyme-linked immunosorbent assay. Interleukin-17 levels were decreased ($P < .001$) and TGF-$\beta$ levels ($P < .001$) were increased in subjects with MS compared to those without. With an increase in the number of metabolic risk factors, the IL-17 levels showed a decline, whereas the TGF-$\beta$ levels showed an increase ($P < .001$). With respect to individual components of MS, TGF-$\beta$ and IL-17 showed a significant association with blood pressure and blood glucose even after adjusting for age and sex. We report that IL-17 levels are decreased, whereas TGF-$\beta$ levels are increased, among Asian Indians with MS.


## 1. Introduction

*Metabolic syndrome* (MS) is a term used to refer to a conglomeration of metabolic disorders including abdominal obesity, hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C), hypertension, and hyperglycemia. Metabolic syndrome is associated with a 2-fold risk for cardiovascular disease and a 5-fold risk for diabetes [1]. Because of sedentary lifestyle and escalation of obesity rates, the prevalence of the MS is increasing worldwide. In India, the prevalence rate of MS is 30%, which is higher than that reported for other Asian and European countries [2]. Recently, inflammation has been identified as one of the major etiologic factors for MS. The inflammation in MS is chronic, low grade, and non–antigen specific in nature and is associated with insulin resistance [3]. The organ-specific (adipose) inflammation, seen during early stages of the disease, becomes more systemic with disease progression and starts affecting the blood vessels leading to vasculopathies [4] like kidney and heart failures, which are commonly seen in MS [5].

Earlier studies have reported increased levels of inflammatory cytokines like tumor necrosis factor–$\alpha$ [6], interleukin (IL)-6 [7], high-sensitivity C-reactive protein [7], and IL-18 [7] and low levels of anti-inflammatory cytokine IL-10 in MS [8]. There are no data on the recently characterized T-cell–derived cytokine IL-17 in MS, and studies on the anti-inflammatory cytokine transforming growth factor (TGF)–$\beta$

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(also secreted from T-cells) are also limited. Apart from infectious and autoimmune diseases, IL-17 has recently been shown to be associated with obesity in women [9]. Transforming growth factor–$\beta$ is a multifactorial cytokine that regulates immune system by immunosuppression and also has specific effects on differentiation, migration, development, tissue remodeling, and repair [10]. The aim of the present study was to estimate the levels of IL-17 and TGF-$\beta$ in subjects with and without MS.

## 2. Research design and methods

Study subjects were recruited from the Chennai Urban Rural Epidemiology Study (CURES), an ongoing epidemiologic study conducted on a representative population of Chennai. The methodology of the CURES has been published elsewhere [11]. *Metabolic syndrome* was defined according to the National Cholesterol Education Program Adult Treatment Panel III criteria modified for waist according to World Health Organization Asia Pacific guidelines for obesity [12]. *Metabolic syndrome* was defined as the presence of any 3 of the following abnormalities: abdominal obesity defined as waist circumference of at least 90 cm for men and at least 80 cm for women, high blood pressure (systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg), elevated fasting glucose (fasting plasma glucose [FPG] ≥100 mg/dL), hypertriglyceridemia (≥150 mg/dL), or low HDL-C (<40 mg/dL for men and <50 mg/dL for women).

For the present study, 156 subjects with and 98 subjects without MS were randomly selected using computer-generated numbers from the CURES samples as shown in Fig. 1. Subjects with known infectious or inflammatory diseases were excluded. Approval of the institutional ethical committee of the Madras Diabetes Research Foundation was obtained for the study, and informed consent was obtained from all the study subjects.

### 2.1. Anthropometry and biochemical measurements

Anthropometric measurements including height, weight, and waist circumference were obtained using standardized techniques as detailed elsewhere [11]. Fasting plasma glucose (glucose oxidase-peroxidase method), serum cholesterol (cholesterol oxidase-peroxidase-amidopyrine method), serum triglycerides (glycerol phosphate oxidase-peroxidase-amidopyrine method) and HDL-C (direct method-polyethylene glycol–pretreated enzymes) were measured using Hitachi-912 Autoanalyzer (Boehringer Mannheim, Mannheim, Germany). The intra- and interassay coefficient of variation for the biochemical assays ranged between 3.1% and 7.6%. Low-density lipoprotein cholesterol was calculated using the Friedewald formula. Glycosylated

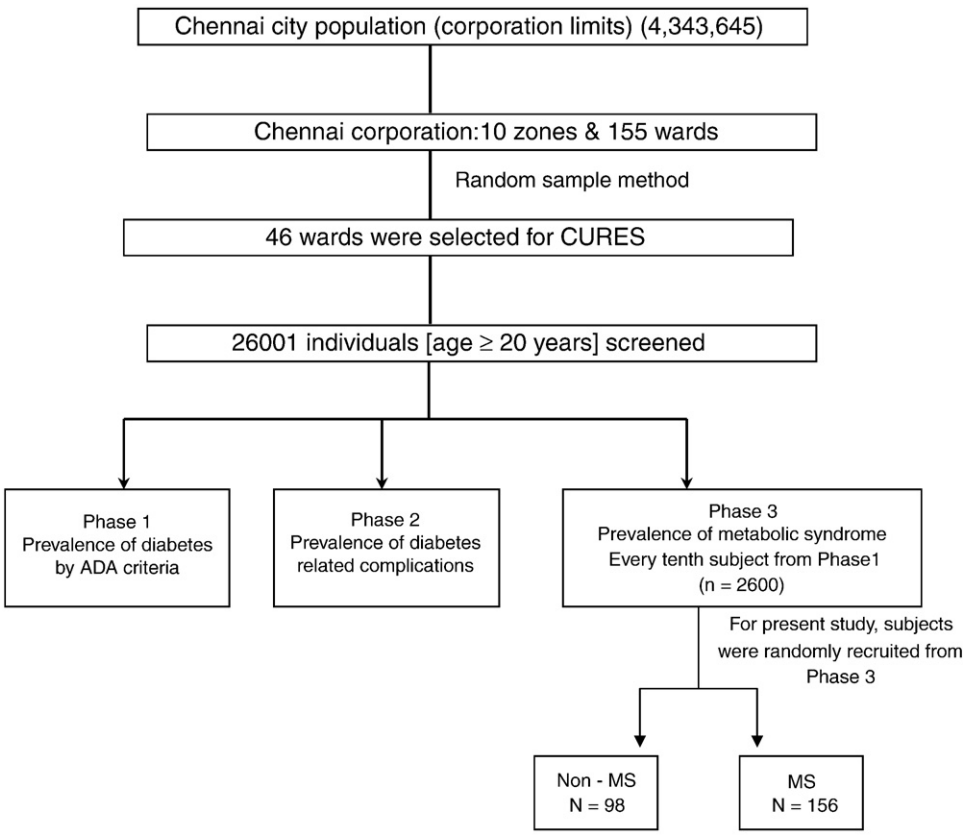


Fig. 1. Flowchart describing the methodology of CURES.

hemoglobin ($HbA_{1c}$) was estimated by high-pressure liquid chromatography using the Variant machine (Bio-Rad, Hercules, CA). The intra- and interassay coefficient of variation of $HbA_{1c}$ was less than 10%.

### 2.2. Serum IL-17 and TGF-β

Serum IL-17 and TGF-β were measured by enzyme-linked immunosorbent assay (R&D Systems, Abingdon, United Kingdom). In brief, monoclonal antibody specific for IL-17 and TGF-β were coated onto microplates followed by the addition of standards and samples. After washing unbound cytokines, enzyme-linked monoclonal antibody specific for IL-17 and TGF-β were added to the wells. After wash, substrate solution was added; and the plates were incubated in the dark until color develops. Absorbance was read at 450 nm. The values were expressed in picograms per milliliter. The intra- and interassay coefficients of variation were less than 5% and 10%, respectively, for both IL-17 and TGF-b.

### 2.3. Statistical analysis

Student *t* test was used to compare groups for continuous variables, whereas $\chi^2$ test or Fisher exact test (as appropriate) was used to compare proportions. Spearman correlation analysis was carried out to determine the association of TGF-β and IL-17 with other risk variables. Logistic regression analysis was used to determine the association of IL-17 and TGF-β with MS components. Kruskal-Wallis test was used for parameters that did not show normal distribution. All the analyses were done using SPSS statistical package (Version 15.0; SPSS, Chicago, IL) and $P$ value < .05 was considered significant.

## 3. Results

Table 1 shows the clinical and biochemical features of the study subjects. Subjects with MS were significantly older than non-MS subjects ($P$ < .001). As expected, body mass index, waist circumference, blood pressure (both systolic and diastolic), FPG, $HbA_{1c}$ and triglycerides were higher in subjects with MS ($P$ < .001), whereas HDL-C levels were lower ($P$ < .001). In our study subjects, 67% of non-MS subjects and 96% of MS subjects had elevated $HbA_{1c}$ (>5.6) levels.

Table 1
Clinical and biochemical characteristics of study subjects

| Parameters | Non-MS (n = 98) | MS (n = 156) | *P* |
|---|---|---|---|
| Age (y) | 44 ± 17 | 54 ± 13 | **<.001** |
| Sex (M/F) (%) | 49/51 | 43/57 | **NS** |
| Waist (cm) | 82.4 ± 12.3 | 92.0 ± 9.2 | **<.001** |
| Body mass index (kg/m$^2$) | 23.4 ± 4.2 | 27.0 ± 5.0 | **<.001** |
| Systolic blood pressure (mm Hg) | 116 ± 19 | 134 ± 15 | **<.001** |
| Diastolic blood pressure (mm Hg) | 73 ± 11 | 81 ± 9 | **<.001** |
| FPG (mg/dL) | 99 ± 37 | 146 ± 56 | **<.001** |
| $HbA_{1c}$ (%) | 6.1 ± 1.2 | 8.3 ± 5.7 | **<.001** |
| Total cholesterol (mg/dL) | 164 ± 34 | 166 ± 42 | .690 |
| Serum triglycerides (mg/dL) | 96 ± 37 | 162 ± 70 | **<.001** |
| HDL-C (mg/dL) | 46 ± 9 | 39 ± 9 | **<.001** |
| Low-density lipoprotein cholesterol (mg/dL) | 86 ± 23 | 91 ± 31 | .156 |

NS indicates not significant.

Fig. 2A shows that IL-17 levels were lowest in subjects with MS (geometric mean, 60.3 pg/mL) followed by those with 2 (63 pg/mL) and 1 (81.3 pg/mL) metabolic abnormalities ($P$ for trend < .001). Fig. 2B shows that the TGF-β values were highest in subjects with MS (geometric mean, 477.4 pg/mL) followed by those with 2 metabolic risk factors (182.0 pg/mL) and 1 risk factor (141.3 pg/mL) ($P$ for trend < .001). Even after adjusting for age and sex, both IL-17 ($P$ < .01) and TGF-β ($P$ < .01) retained the statistical significance.

Table 2 depicts the results of Spearman correlation analysis between IL-17 and TGF-β with various components of MS. Whereas IL-17 showed a negative correlation,

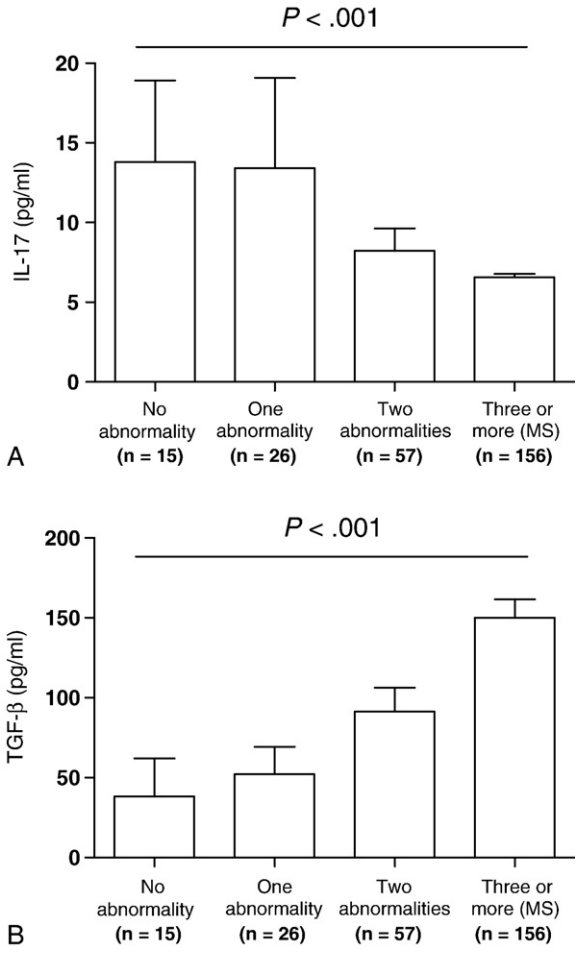


Fig. 2. Decreased levels of IL-17 and increased levels of TGF-β in MS subjects. A, Decreasing levels of serum IL-17 with increasing risk factors of metabolic syndrome; $P$ < .001 denotes trend. B, Increasing levels of serum TGF-β with increasing risk factors of metabolic syndrome; $P$ < .001 denotes trend.

Table 2
Spearman correlation analysis of IL-17 and TGF-$\beta$ with the components of MS in the whole study population

| Parameters | IL-17 | | TGF-$\beta$ | |
|---|---|---|---|---|
| | $r$ value | $P$ value | $r$ value | $P$ value |
| Waist | −0.049 | .435 | 0.084 | .181 |
| Systolic blood pressure | −0.155 | **<.05** | 0.307 | **<.001** |
| Diastolic blood pressure | −0.100 | .111 | 0.151 | **<.05** |
| FPG | −0.260 | **<.001** | 0.362 | **<.001** |
| Serum triglycerides | −0.075 | .236 | 0.136 | **<.05** |
| HDL-C | 0.058 | .361 | −0.023 | .717 |

TGF-$\beta$ showed a positive association with most of the risk factors. In the total study population, IL-17 and TGF-$\beta$ showed a significant correlation with systolic blood pressure (IL-17; $r = -0.155$, $P < .05$) (TGF-$\beta$; $r = 0.307$, $P < .001$) and FPG (IL-17; $r = -0.260$, $P < .001$) (TGF-$\beta$; $r = 0.362$, $P < .001$). Diastolic blood pressure ($r = 0.151$, $P < .05$) and serum triglycerides ($r = 0.136$, $P < .05$) showed an association only with TGF-$\beta$.

Table 3 presents the results of the logistic regression analysis. After adjusting for age and sex, both IL-17 and TGF-$\beta$ showed an association only with blood pressure and FPG.

## 4. Discussion

This is the first report, to the best of our knowledge, on IL-17 levels in subjects with and without MS and shows that, whereas IL-17 levels are low, TGF-$\beta$ levels are elevated in subjects with MS. Previously, Santos et al [13] observed significant increase in the serum TGF-$\beta$ levels in the Mas knockout mice model of MS that developed a severe form of disease. Suthanthiran et al [10] showed a significant correlation between serum TGF-$\beta$ levels with MS among blacks, but not in the white population, indicating ethnic differences in the utility of biomarkers for MS and end-stage renal disease. Increased levels of TGF-$\beta$ in MS were expected because earlier reports have shown increased levels of this cytokine in obesity [14], diabetes [15], hypertension [16] and cardiovascular disease [17]. Therefore, all the above-mentioned conditions, which are essential components of MS, might have acted synergistically in bringing about the marked increase in TGF-$\beta$ levels in our study subjects with MS. It is possible that TGF-$\beta$, being a major anti-inflammatory cytokine, is secreted in excess to counterbalance inflammation, which is an integral component of MS [18].

Table 3
Logistic regression analysis using various components of MS as dependent variable and IL-17 and TGF-$\beta$ as independent variables

| | Parameters | Unadjusted | | | Age, sex adjusted | | |
|---|---|---|---|---|---|---|---|
| | | OR | 95% CI | $P$ | OR | 95% CI | $P$ |
| IL-17 | Waist | 0.86 | 0.31-2.30 | .756 | 0.96 | 0.34-2.71 | .945 |
| | BP | 0.28 | 0.09-0.81 | **<.05** | 0.24 | 0.07-0.73 | **<.05** |
| | FPG | 0.36 | 0.13-0.99 | .050 | 0.23 | 0.07-0.74 | **<.05** |
| | TG | 1.07 | 0.39-2.94 | .890 | 1.05 | 0.38-2.88 | .928 |
| | HDL | 0.42 | 0.14-1.18 | .099 | 0.43 | 0.15-1.25 | .123 |
| TGF-$\beta$ | Waist | 1.05 | 0.79-1.38 | .754 | 0.86 | 0.62-1.18 | .362 |
| | BP | 1.89 | 1.41-2.51 | **<.001** | 1.55 | 1.14-2.11 | **<.01** |
| | FPG | 2.28 | 1.69-3.07 | **<.001** | 1.88 | 1.32-2.66 | **<.001** |
| | TG | 1.18 | 0.88-1.57 | .269 | 1.16 | 0.85-1.58 | .335 |
| | HDL | 1.09 | 0.81-1.45 | .581 | 1.14 | 0.83-1.55 | .423 |

OR indicates odds ratio; CI, confidence interval; BP, blood pressure; TG, triglyceride.

However, the decreased levels of IL-17 noted in subjects with MS were unexpected. Interleukin-17 is a recently discovered novel T-cell cytokine that has been widely associated with autoinflammatory (irritable bowel syndrome) and autoimmune diseases (rheumatoid arthritis and multiple sclerosis) [19]. However, its role in type 1 diabetes mellitus (T1DM) is unclear. In streptozotocin-induced T1DM, IL-23 treatment substantially increased IL-17 and interferon-$\gamma$–mediated pancreatic $\beta$-cell destruction and diminished insulin production [20]. In NOD mice, both anti–IL-17 antibody therapy and recombinant IL-25 (a potent inhibitor of IL-17) showed significant improvement in disease outcome (reduced pancreatic loss and reduced GAD65 specific autoantibody production and increased Treg production) [21]. However, recently, in mycobacterium-based adjuvant therapy against T1DM, IL-17–producing cells were shown to protect against disease manifestation [22]. The, role of IL-17 in metabolic diseases like dyslipidemia, obesity, insulin resistance, hypertension, and cardiovascular diseases is largely unexplored. Sumarac-Dumanovic et al [9] have reported increased IL-23/IL-17 proinflammatory axis in obese women. Interleukin-17 is a unique cytokine, which requires both proinflammatory IL-6 and anti-inflammatory TGF-$\beta$ for its synthesis and secretion [23]. Interleukin-17 has also been shown to be associated with hypertension [24] and type 2 diabetes mellitus [25]. Earlier studies carried out by us [26] and others [7] have shown increased levels of IL-6 in metabolic diseases. Hence, we expected to see high levels of IL-17, but found that IL-17 levels were lower in MS subjects. It should be noted that TGF-$\beta$ has a dual effect on IL-17 synthesis: at lower concentrations, it induces its secretion, whereas at higher concentrations, it inhibits its secretion [19]. Alternatively, the other T-cell cytokines like interferon-$\gamma$ and IL-4 can also inhibit IL-17 secretion [23]. It should be noted that, recently, Arababadi et al [25] have reported a significant increase in serum IL-17 levels in type 2 diabetes mellitus subjects, which start declining as the subjects develop progressive end-stage nephropathy. More work is obviously needed on the mechanistic processes involved to explain this phenomenon.

One of the limitations of this study is that we have looked only at the IL-17 levels. Studying the levels of upstream regulators of IL-17 synthesis like IL-23 and IL-21 and other members of IL-17 family (like IL-17F and IL-25) could provide a better insight into the biochemical processes

involved. Secondly, as this is a cross-sectional study, no conclusions regarding a causal relationship between IL-17/TGF-$\beta$ and MS can be made. Longitudinal follow-up studies are needed to explore the exact role of these cytokines in MS.

In summary, this study suggests a linear negative association between IL-17 and MS and a positive association between TGF-$\beta$ and MS. In particular, both these cytokines showed an independent association with hypertension and hyperglycemia. Future studies should attempt to elucidate the role of IL-17 (and other members of its family) and TGF-$\beta$ in MS in general and insulin resistance in particular.

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