Introduction

Obesity is linked to metabolic syndrome including insulin resistance, type-2 diabetes, and cardiovascular disease. Persistent low-grade inflammation is associated with metabolic disorders. Changes in plasma cytokines have been reported in obesity and T2D [1]. Functionally polarized CD4+ T cells are classified as Th1, Th2, Th17, and Treg subsets depending on the pattern of their cytokine production [2,3]. Th1 cytokines, such as interleukin (IL)-2 and interferon (IFN)-γ activate macrophages and are involved in inflammatory immune responses whereas Th2 cytokines, such as IL-4, IL-5, IL-10, and IL-13 have anti-inflammatory properties and are involved in antibody production, eosinophil activation, and suppression of macrophage functions [4-6]. Th1/Th2 cytokine imbalance has been reported in chronic disease progression [3,5-7] and in metabolic syndrome [8]. IL-2 is a pro-inflammatory cytokine that promotes synthesis of tumor necrosis factor (TNF)-α and IFN-γ from natural killer cells (NK) cells and is associated with atherogenesis [7,8] and T2D [9,10]. IFN-γ is related with the production of macrophage mediators, chemokines, induction of leukocyte adhesion molecules and class II major histocompatibility antigens, and potentiates the antigen presenting cell functioning [9-11]. IL-4 which is secreted by activated Th2 cells, basophils, and mast cells has pleiotropic functions including Th2 differentiation, B-cell proliferation and immunoglobulin class switching [12]. IL-10 is an anti-inflammatory cytokine which attenuates inflammation induced by IL-1, IL-6, and TNF-α while it also promotes the release of anti-inflammatory IL-1RA [13].

IL-5 is an important T cell-derived cytokine that regulates the expression of diverse genes involved in proliferation, cell survival, as well as maturation and effector functioning of B cells and eosinophils [14]. IL-5 plays a pivotal role in both the innate and adaptive immune responses and the biologic effects of IL-5 are best characterized for eosinophils in humans. It is not clear whether plasma IL-5 levels are modulated and also relate with the Th1/Th2 cytokine profile in metabolic disorders such as obesity and T2D. The aim of the study was, therefore, to determine the circulatory IL-5 levels in diabetic and non-diabetic individuals and evaluate the relationship of IL-5 levels with Th1/Th2 cytokine profile in these two populations. Here, we show that plasma IL-5 levels were significantly lower in diabetic individuals and these changes correlated with Th1/Th2 cytokines and clinical metabolic markers differentially between diabetics and non-diabetics.

Materials and Methods

Study population

This study included 43 diabetic and 22 non-diabetic adult individuals classified as obese (BMI=31-40 kg/cm²), overweight (BMI=26-30 kg/cm²), and lean (BMI=18-25 kg/cm²). The study population comprised of 22 non-diabetic and 43 diabetic participants.

Abstract

Purpose: Changes in plasma levels of T-cell cytokines may result in inflammatory disorders, including metabolic disease. The changes in circulatory IL-5 levels in obesity and type-2 diabetes (T2D) and their relationship with major Th1 and Th2 cytokines are poorly understood. The aim of this study was to determine obesity/T2D-associated perturbation in plasma IL-5 levels and investigate its association with Th1/Th2 cytokine levels in the circulation.

Experiments: Plasma samples were collected from 43 diabetic and 22 non-diabetic individuals selected over a wide range of body mass index (BMI), and further classified as obese (BMI=31-40 kg/cm²), overweight (BMI=26-30 kg/cm²), and lean (BMI=18-25 kg/cm²). Clinical metabolic parameters were determined using commercial kits. IL-5 and Th1/Th2 cytokines were measured using Luminex X-MAP® technology. The data were compared using unpaired t-test and dependence between two variables was assessed by Pearson’s correlation coefficient (r).

Results: In the diabetic cohort, circulatory IL-5 levels were significantly lower as compared to non-diabetic counterparts (Diabetic: 1.55 ± 0.23 pg/ml; Non diabetic: 3.31 ± 0.49 pg/ml; P=0.0004). Furthermore, we found that in diabetic individuals, plasma IL-5 levels were found to be significantly higher (P=0.036) in obese group (1.488 ± 0.21 pg/ml) as compared with lean+overweight group (1.03 ± 0.13 pg/ml). In diabetic individuals, IL-5 levels correlated positively with Th1/Th2 cytokines including IL-12 (r=0.68, P=0.002), IL-3 (r=0.97, P=0.001) and G-CSF (r=0.57, p=0.0001). While in non-diabetic individuals, IL-5 levels correlated positively with IL-2 (r=0.83, P=0.0005), IL-12 (r=0.66, P=0.0014), IL-3 (r=0.82, P=0.0054), IL-6 (r=0.80, P=0.009), IL-9 (r=0.88, P=0.0075), IL-10 (r=0.787, P=0.008), IL-13 (r=0.84, P=0.0002), and G-CSF (r=0.82, P=0.0001). Moreover, plasma IL-5 levels in diabetic individuals associated positively with clinical metabolic indicators including BMI (r=0.54, P=0.0001), fasting blood glucose (r=0.29, P=0.05) and glycated hemoglobin (r=0.32, P=0.034).

Conclusion: The changes in circulatory IL-5 levels show differential association with Th1/Th2 cytokines in diabetic and non-diabetic individuals which may have immunometabolic significance.

Keywords: IL-5; Th1/Th2 cytokines; Obesity; Type-2 diabetes

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Conflict of interest

The authors declare that there is no conflict of interest.

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participants were recruited through outpatient clinics of Dasman Diabetes Institute, Kuwait. Recruiting the non-diabetic individuals with clinico-demographic characteristics comparable to those of diabetics within 3 BMI subgroups was challenging and, therefore, less number of non-diabetic individuals could be recruited as compared with diabetics. The clinico-demographic data of the study participants are summarized in Table 1. All participants gave written informed consent and study was approved by Ethical Review Committee of Dasman Diabetes Institute. The diagnosis of T2D was performed by designated physician based on results of fasting blood glucose (FBG), oral glucose tolerance test (OGTT), and glycated hemoglobin (HbA1c) test. FBG levels of ≥ 126 mg/dL (≥ 7 mmol/L), 2 h-OGTT values of >200 mg/dL (11.1 mmol/L), and/or HbA1C levels of ≥ 6.5% on two separate tests were diagnosed as T2D. Anthropometric and physical measurements included body weight, height, waist circumference as well as systolic and diastolic blood pressure. Height and weight were measured with barefoot participants wearing light indoor clothing using calibrated portable electronic weighing scales and portable inflexible height measuring bars; the waist circumference at the highest point of the iliac crest and the mid-axillary line was measured using constant tension tape at the end of a normal expiration with arms relaxed at the sides. The waist-to-hip ratios were calculated, and the whole body composition including percentage of body fat, soft lean mass and total body water were measured by the use of ICI 353 Body Composition Analyzer (Jawon Medical, South Korea). Blood pressure was measured by using Omron HEM-907XL digital automatic sphygmomanometer (Omron Healthcare Inc. IL, USA). An average of the 3 blood pressure readings, with 5-10 min rest between each, was obtained. BMI was calculated using standard BMI formula i.e. body weight (kg)/height (m²). Regarding clinical laboratory measurements, peripheral blood was collected by phlebotomist through venipuncture from overnight-fasting (minimum 10 h) individuals and samples were analyzed for FBG, HbA1c, and lipid profile. Glucose and lipid profiles were measured using Siemens dimension RXL chemistry analyzer (Diamond Diagnostics, Holliston, MA, USA) and HbA1c was measured by using Variant device (BioRad, Hercules, CA, USA). All assays were carried out following instructions as recommended by the manufacturers.

### Determination of plasma cytokines

A total of 41 cytokines and chemokines were measured using panel MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel - Premixed 41 Plex - Immunology Multiplex Assay (Milliplex map kit, HCYTAMG-60K-PX41, Millipore, USA) following the manufacturer’s instructions. Data from the reactions were acquired using Luminex, Milliplex analyzer, while a digital processor managed data output and the Milliplex analyst software was used to determine mean fluorescence intensity (MFI) and analyte concentration (pg/mL).

### Statistical analysis

The data obtained were expressed as mean ± SD values; group means were compared using unpaired t-test and the linear dependence between two variables was assessed by Pearson’s correlation coefficient (r). GraphPad Prism software (version 6.05; San Diego, CA, USA) was used for statistical analysis and graphical representation of data. All P-values ≤ 0.05 were considered statistical significant.

### Results

**Decreased IL-5 levels in diabetic individuals**

IL-5 is an important Th2 cytokine and its role in metabolic disorders is not well understood. Since not much work has been done regarding changes in IL-5 expression and their relationship with Th1/Th2 cytokines in obesity and T2D, we therefore determined levels of IL-5 and Th1/Th2 cytokines in plasma samples both from diabetic and non-diabetic individuals. The data show that IL-5 levels in diabetics (1.55 ± 0.23 pg/ml) were lower (P=0.0004) than in non-diabetics (3.31 ± 0.49 pg/ml) (Figure 1A and 1B).

**Increased IL-5 levels in diabetic individuals with obesity**

Next, we asked if obesity could modulate IL-5 expression in diabetic and non-diabetic individuals. To this end, our data show that in diabetic patients, IL-5 levels were significantly higher in obese (BMI=33.96 ± 1.744 kg/m²) group (1.488 ± 0.21 pg/ml) as compared with lean+overweight (BMI=25.89 ± 2.548 kg/m²) group (1.03 ± 0.13 pg/ml) (P=0.036) (Figure 2A and 2B). In non-diabetic individuals, mean IL-5 levels in obese (BMI=34.09 ± 2.805 kg/m²) group (3.91 ± 0.71 pg/ml) were also relatively higher than in lean+overweight (BMI=23.60 ± 2.640 kg/m²) group (2.57 ± 0.59 pg/ml) but the difference between two groups did not reach statistical significance (P=0.17) (Figure 2C and 2D).

**Association between IL-5 and Th1/Th2 cytokines in diabetic and non-diabetic individuals**

As our data show that IL-5 levels were lower in diabetic patients, we further wanted to know how the IL-5 expression changes related with signature Th1/Th2 cytokines both in diabetic and non-diabetic subjects. To this effect, we found a positive/significant correlation.  

![Figure 1: Plasma samples from 43 diabetic and 22 non-diabetic individuals were analyzed for IL-5 levels using Luminex magnetic bead assay. (A) IL-5 levels (means±SD) are shown for diabetic and non-diabetic individuals. (B) Dot plot of IL-5 levels is shown for diabetic and non-diabetic subjects. Each dot represents the individual value of IL-5 and the lines represent median values in each group.](image-url)
had an association with clinico-metabolic parameters. To this end, data are summarized in Table 2.

with IFN-γ and IL-4 in both diabetic and non-diabetic individuals. The with IL-2, IL-3, IL-6, IL-9, IL-10, and IL-13 in diabetic patients and non-diabetic individuals (P=0.06). No association of IL-5 was observed with IL-5 and TNF-α did not approach the level of statistical significance in diabetic subjects only. Whereas, TG, HDL, LDL, and total cholesterol levels did not correlate with IL-5 in this population. In non-diabetics, IL-5 had no association with clinico-metabolic parameters (Table 3).

Association between IL-5 and clinical metabolic parameters in diabetic and non-diabetic subjects

We further investigated whether the modulated IL-5 expression had an association with clinico-metabolic parameters. To this end, serum levels of triglyceride, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, FBG, and HbA1c were also measured. Our data show that IL-5 levels correlated with FBG (r=0.29, P=0.05), HbA1c (r=0.32, P=0.03), as well as with BMI (r=0.54, P=0.0001) in diabetic subjects only. Whereas, TG, HDL, LDL, and total cholesterol levels did not correlate with IL-5 in this population. In non-diabetics, IL-5 had no association with clinico-metabolic parameters (Table 3).

Discussion

Obesity and T2D are marked with a state of chronic low-grade inflammation and the expanding adipose tissue plays a dual role as an organ for excessive triglyceride storage as well as an endocrine organ which secretes a variety of adipokines and inflammatory cytokines/chemokines. Whereas, the roles of adipokines and macrophage-elicited cytokines/chemokines in metabolic disease are well elucidated, little is known about the perturbation in Th2-cell cytokine IL-5 and its relationship with other Th1/Th2 cytokines in metabolic disease. Th1/Th2 cytokine balance is critical to the execution of normal immune response and perturbations in these cytokines may sway the balance from protective to deleterious consequences. The association of Th1 cytokines with metabolic disease was already reported in humans and animals [15,16]. Whereas, IL-5 is a Th2 cell-derived cytokine which is involved in diverse biological functions such as cellular proliferation, survival, maturation, as well as effector functioning of eosinophils and B cells. In this study, we show that plasma IL-5 levels were significantly lower in diabetic individuals as compared with non-diabetic counterparts. In agreement with our finding, another study also showed the suppression of Th2 serum cytokines in subjects with diabetic coronary artery disease [8]. As though the exact cause(s) for lower Th2 cytokine levels in metabolic disease is/are currently unknown, a shift in Th1 to Th2 ratio toward Th1 dominance has been earlier reported [17]. Interestingly, our data further show that in obese diabetic population, IL-5 levels were significantly higher than overweight/lean diabetic individuals. It is not clear whether obesity

Table 2: Correlation of plasma IL-5 levels with Th1/Th2 cytokines in diabetic and non-diabetic individuals.

<table>
<thead>
<tr>
<th>Plasma IL-5</th>
<th>Th1 Cytokines</th>
<th>Non-Diabetic Pearson r</th>
<th>P</th>
<th>Diabetic Pearson r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>0.51</td>
<td>0.06</td>
<td>0.185</td>
<td>0.227</td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>0.826</td>
<td>0.0005***</td>
<td>-0.316</td>
<td>0.488</td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td>0.665</td>
<td>0.0014**</td>
<td>0.684</td>
<td>0.002**</td>
<td></td>
</tr>
<tr>
<td>IL-13</td>
<td>0.839</td>
<td>0.0004***</td>
<td>-0.089</td>
<td>0.833</td>
<td></td>
</tr>
<tr>
<td>G-CSF</td>
<td>0.816</td>
<td>0.0001**</td>
<td>0.568</td>
<td>0.0001**</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Correlation between plasma IL-5 levels and metabolic parameters.

<table>
<thead>
<tr>
<th>Metabolic Parameters</th>
<th>Non-Diabetic Pearson r</th>
<th>P</th>
<th>Diabetic Pearson r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (BMI) (kg/m²)</td>
<td>0.165</td>
<td>0.445</td>
<td>0.536</td>
<td>0.0001***</td>
</tr>
<tr>
<td>Fasting Blood Glucose (mmol/l)</td>
<td>0.257</td>
<td>0.249</td>
<td>0.2908</td>
<td>0.051</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>-0.036</td>
<td>0.871</td>
<td>0.315</td>
<td>0.034*</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>-0.218</td>
<td>0.328</td>
<td>0.182</td>
<td>0.229</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>0.077</td>
<td>0.740</td>
<td>0.042</td>
<td>0.781</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>0.006</td>
<td>0.916</td>
<td>0.105</td>
<td>0.492</td>
</tr>
</tbody>
</table>

Figure 2: Plasma samples from 43 diabetic and 22 non-diabetic individuals were analyzed for the expression of IL-5 using Luminex magnetic bead assay. The diabetic and non-diabetic group, each, was classified on the basis of body mass index (BMI) as described in Materials and Methods. (A) IL-5 levels (mean±SD) are shown for obese diabetic and overweight/lean diabetic individuals. (B) Dot plot of IL-5 levels is shown for obese diabetic and overweight/lean diabetic subjects. Each dot represents the individual value of IL-5 and the lines represent median values of plasma IL-5 in each group. (C) IL-5 levels (mean±SD) are shown for obese non-diabetic and overweight/lean non-diabetic individuals. (D) Dot plot of IL-5 levels shown for obese non-diabetic and overweight/lean non-diabetic subjects. Each dot represents the individual value of IL-5 and the lines represent median values of plasma IL-5 in each group.

of IL-5 with Th1 cytokines (TNF-α, IL-2, and IL-12) as well as Th2 cytokines (IL-3, IL-6, IL-9, IL-10, IL-13, and G-CSF). Further, in this regard, IL-5 levels showed significant correlations (P<0.05) with IL-2 (r=0.83), IL-6 (r=0.80), IL-9 (r=0.89), IL-10 (r=0.79), and IL-13 (r=0.84) only in non-diabetic individuals. Whereas, both in non-diabetics and diabetics, IL-5 levels also correlated (P<0.05) with IL-12 (r non-diabetic=0.67, r diabetic=0.68), IL-3 (r non-diabetic=0.82, r diabetic=0.97), and G-CSF (r non-diabetic=0.82, r diabetic=0.57). However, the correlation between IL-5 and TNF-α did not approach the level of statistical significance in non-diabetic individuals (P=0.06). No association of IL-5 was observed with IL-2, IL-3, IL-6, IL-9, IL-10, and IL-13 in diabetic patients and with IFN-γ and IL-4 in both diabetic and non-diabetic individuals. The data are summarized in Table 2.
was a major player in upregulating IL-5 expression in diabetic patients; however, obesity associated with enhanced eosinophil numbers and IL-5 levels in a murine model of allergic asthma [18].

The further data show that IL-5 correlated positively with IL-2, IL-6, IL-9, IL-10, and IL-13 only in non-diabetic subjects whereas it was associated with IL-12, IL-3, and G-CSF in both non-diabetic and diabetic individuals. It implies that IL-5 has a broader relationship with both the Th1 and Th2 cytokines which is expressed differentially in the presence or absence of T2D. Herein, we found that IL-5 associated with IL-12, IL-3, and G-CSF whether or not diabetes was a morbid cofactor in the population studied whereas it did not associate with IL-2, IL-6, IL-9, IL-10, and IL-13 if diabetes was present as a comorbidity. It is not clear if the regulatory functions of IL-12, IL-3, and G-CSF are well preserved in obesity/T2D. IL-3 and CSF interact to promote CD11c+IL-10-producing macrophages [19]. IL-3 stimulates the proliferation of monocytes, granulocytes, and dendritic cells. G-CSF is a cytokine and a hormone that acts as a colony stimulating factor for granulocytes, stem cells, and also promotes the proliferation and differentiation of neutrophils. Elevated serum levels of IL-12 were reported in overweight and obese adult individuals that showed a strong relationship with markers of low-grade inflammation and obesity [20]. IL-12 helps in differentiation of naïve T cells into Th1 cells; it stimulates the production of IFN-γ and TNF-α and also plays a critical role in the activation of T cells and NK cells [21]. We further show that IL-5 associates with clinical metabolic markers including BMI, HbA1C and FBG in diabetic subjects which points to its clinical relevance as an immune marker in T2D. Overall, the peripheral IL-5 levels were found to be associated differentially with Th1 and Th2 cytokines in diabetic and non-diabetic individuals. Further studies involving larger cohorts will be required to verify these preliminary data as well as study the relationship of IL-5 with cytokines and chemokines produced by other immune effector cells such as monocytes/macrophages, dendritic cells, B-cells, NK cells, and adipocytes to enhance our understanding of the significant role of IL-5 in metabolic disease.

In conclusion, our data show that plasma IL-5 levels were significantly lower in diabetic as compared with non-diabetic individuals; whereas in diabetic cohort, IL-5 levels were higher in obese as compared with overweight/lean subjects. IL-5 associated differentially with Th1/Th2 cytokines in diabetic and non-diabetic individuals and also related with clinical disease markers.

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