Expression of interleukins 7 & 8 in peripheral blood mononuclear cells from patients with metabolic syndrome: A preliminary study


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Background & objectives: Metabolic syndrome (MS) is a common but serious public health problem in developed countries. Chronic inflammation plays a key role in MS. Interleukins (IL)-7 and 8 are considered to have proinflammatory effects and may be involved in the pathogenesis of MS. Therefore, the aim of this study was to determine gene expression level of IL-7 and IL-8 in peripheral blood mononuclear cells (PBMCs) of patients with MS compared to healthy control subjects.

Methods: Using real-time RT-PCR, the relative amounts of IL-7 and IL-8 mRNA were determined in PBMCs from 20 female patients with MS and compared with those of 20 healthy control subjects. Biochemical and anthropometric parameters of MS were also assessed.

Results: Total cholesterol, triglyceride, and fasting blood sugar were significantly higher in MS patients compared to healthy subjects. There were no significant differences in HDLc and LDLc between the two groups. IL-8 expression in PBMC was significantly decreased in MS versus control subjects (fold of change was 0.395 ± 0.1824), while no difference in the IL-7 expression was detected between them. IL-8 expression had negative correlation with MS components especially with triglyceride and total cholesterol (r=0.5, P<0.001).

Interpretation & conclusions: In this preliminary study, no detectable differences were found in IL-7 expression and decreased expression of IL-8 in PBMCs of MS patients as compared to those of control subjects. Study on a larger population and investigating the mechanisms involved can reveal more details.

Key word Gene expression - IL-7 - IL-8 - metabolic syndrome - PBMC - real-time PCR

Metabolic syndrome (MS) is one of the major public health challenges worldwide that is characterized by clustering of waist circumference, blood triglycerides (TG), high density lipoprotein (HDL) cholesterol, fasting glucose and blood pressure with different cut-off values. MS affects approximately 25 percent of the adult population in western countries and also is quickly increasing in young populations. The aetiology is complex, genetic and environmental factors both have important roles. Metabolic syndrome and its components are risk factors of cardiovascular disease and type 2 diabetes mellitus. MS can cause
dyslipidaemia, pro-thrombotic and pro-inflammatory states, fatty liver disease and finally increased cardiovascular disease (CVD) related to and all-cause mortality. It is believed that visceral obesity can lead to development of insulin resistance, impaired glucose tolerance, hyperglycaemia, and type 2 diabetes mellitus.

Inflammation is now thought to play a key role in the pathophysiology of MS. Moreover, inflammation is closely related to obesity. Adipose tissue releases a variety of molecules referred to adipocytokines. Adipose tissue enlargement leads to an increase in the immune cells in this tissue. The process plays a role in inflammation, frequently through cytokines derived from macrophages infiltrated in the tissue. Monocytes are shown to play an important role in the pathogenesis of MS, like inflammation of adipose tissue.

Interleukin (IL)-7, known as B cell precursor growth factor, has a key role in lymphocyte homeostasis; especially in basal metabolism of glucose. It maintains high glucose uptake and expression of GLUT1 which results in adequate glycolytic flux. There are evidences to show that IL-7 contributes to inflammation in several chronic inflammatory processes. Another key role of IL-7 is its effect on immune mediators which modulate metabolic functions. Therefore, it may contribute to local pro-inflammatory process observed in MS.

IL-8 is known as a cytokine that involves in inflammation via neutrophil chemoattraction. Basal production of IL-8 is normally low but it can be quickly induced by different stimuli and is secreted from a variety of cells. It is responsible for macrophage infiltration into adipose tissue in obesity and also associated with some disorders which are related to obesity. IL-8 is secreted from adipose tissue in vitro and is shown to be involved in the pathogenesis of type 2 diabetes. Plasma levels of IL-8 may be higher in patients with heart failure suffering from MS compared with non-MS group. This study was undertaken with the aim to assess the IL-7 and IL-8 expression in PBMC of patients with MS. Detection of possible correlation between their expression and the components of disease was also studied.

Material & Methods

This study was conducted in the Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, Iran during January-March, 2011.

Twenty female patients with recent diagnosis of metabolic syndrome (MS), referred to the endocrinology department of Shahid Beheshti Hospital of Hamadan University of Medical Sciences (Iran), and 20 age matched healthy women who were referred for check up to the hospital laboratory in the same period were included in this study. All patients having the inclusion criteria were selected consecutively. MS diagnosis was carried out according to the modified criteria for MS from the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III). Based on these criteria, the patients had three or more of the following conditions: (i) Central obesity (waist circumference > 95 cm); (ii) High blood pressure ≥ 130/86 mmHg or documented use of antihypertensive therapy; (iii) High fasting glucose level (≥ 100 mg/dl); (iv) Hypertriglyceridaemia (≥ 150 mg/dl) and (v) Decreased high density lipoprotein cholesterol (HDLc) (< 50 mg/dl).

The diabetic and hypertensive patients received routine medicine of diabetes and hypertension. Participants having the following conditions were excluded from the study: estro-progesterone or testosterone treatment including oral contraceptives; smoking; pregnancy; amenorrhea; polycystic ovarian syndrome. The subjects were not affected by chronic illness, and not taking drugs or supplements known to modify the immune system. Past medical and habitual history of participants were negative for chronic diseases (hepatic, renal, thyroid, cardiac), smoking, alcohol consumption, or taking drugs. There was no family history of early onset cardiovascular disease.

Informed written consent was obtained from all participants. Study protocol was approved by the Hamadan University of Medical Sciences ethics committee. Each participant underwent a baseline visit after an overnight fasting. Height, weight, waist circumference and blood pressure were measured prior to sample collection.

Following an overnight fasting (≥ 10 h) venous blood sample (6 ml) was collected. Samples for CBC (cell blood count) and gene expression analysis were collected in tubes containing 1 g EDTA/l. Serum was separated within an hour after obtaining the blood sample.

Analytical methods: Height and weight of all of participants were measured by a stadiometer (Seca, USA) and digital scale (Escali, USA), respectively. Blood pressure was measured in the sitting position.
after five minutes rest using a sphygmomanometer (Welch Allyn, USA). FBS (fasting blood glucose), TC (total cholesterol), TG, LDLc (low density lipoprotein cholesterol) and HDLc were measured using an autoanalyzer (Hitachi 911 sunrise Corporate company Kobe, Japan) and a colorimetric method kit (ParsAzmun - Iran). The intra- and inter-assay precisions for these biochemical parameters were 2.3-3.1 and 5.8-6.4 per cent, respectively. CBC analysis was carried out by haematology analyzer Sysmex Kx-21N (Sysmex Corporation, Japan).

Peripheral blood mononuclear cell (PBMC) isolation: Peripheral blood (4 ml) was diluted 1:1 (v/v) in BSS (Balanced salt solution); and layered on 3 ml of Ficoll-Hypaque solution (Amersham Biosciences, USA). Density gradient centrifugation was carried out at 400 × g for 35 min. PBMCs were harvested from the interface layer and washed twice with BSS. Harvested PBMCs were used for RNA extraction.

RNA extraction and cDNA synthesis: Total RNA was extracted from PBMCs using RNeasy Mini Kit (Qiagen, Hilden, Germany). Quantity and purity of the extract were measured by Nanodrop spectrophotometer (Epoch, BioTek) and the ratio of A260/A280 nm of all the samples was about 2. RNA integrity was assessed using 1 per cent agarose gel. RNA was considered suitable for the next step if intact bands corresponding to 18S and 28S ribosomal RNAs were detected on electrophoresis.

Equal amounts of total RNA, approximately 0.5 micrograms, was reverse transcribed into single-stranded cDNA using QuantiTect Reverse Transcription kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Briefly, after one step gDNA wipeout at 42°C, cDNA synthesis was performed at 42°C for 20 min, followed by RT inactivation at 95°C for 3 min.

Quantitative real-time PCR: PCR analyses were performed using C1000 Thermocycler and CFX96 real time system (BioRad) and QuantiFast SYBR Green PCR Kit (Qiagen, Hilden, Germany) in a final volume of 25 μl with 10 picomol of each primer. Each reaction was performed on 1 μl of 1:9 (v/v) dilution of the first cDNA strand. Positive and negative controls were used for quality control of process.

The reaction mixture was incubated at 95°C for 5 min, followed by 40 cycles of 15 sec at 95°C, 30 sec at annealing temperature, 30 sec at 72°C and then fluorescence was measured. Primers, designed by software AlleleID7.6, were: IL7-forward (5'-GGCAAACAATATGAGAGT -3'); IL7-reverse (5'-CCTTTAGCATCAGATA -3'); IL8-forward (5'-AGCATACTCCACCCCTTT -3'); IL8-reverse (5'-GCTCTTCTCCATCAGAA -3'); β-actin-forward(5'-AAGATCAAGATCATTGCT -3'); and β-actin-reverse (5'- TACGCACACTAAGTC -3'). Gene numbers of different variants of IL-7 were NM_001199886, NM_001199887, NM_001199888, NM_000880 and the number for IL-8 was NM_000584.

Annealing temperature and size of PCR products were 48.5°C and 130, 49.5°C and 114 and 47.5°C and 177 for IL7, IL8 and β-actin, respectively. Specificity of PCR amplifications was verified by melting curve programme (70-95°C with a heating rate of 0.5°C/sec and a continuous fluorescence measurement) and analyzed by electrophoresis on a 1 per cent agarose gel, 1× TBE. Expression values were obtained as relative expression of the target gene versus the constitutively expressed β-actin gene as reference gene (ΔCT = Target gene CT - Reference gene CT). Fold change for each of studied genes was calculated by means of ΔΔCT formula (ΔΔCT of each gene = ΔCT in MS group - ΔCT in non-MS group; Fold change = 2 – ΔΔCT).

Statistical analysis: The values were expressed as means ± SD and gene expression level was reported as means ± SEM of three independent experiments. P<0.05 was considered significant. Results were analyzed using Mann-Whitney U test for comparison between normal and MS subjects. Spearman correlation coefficient was used for the determination of relations among variables. All statistical analyses were conducted using SPSS software (Version10) (SPSS Inc., USA).

Results

The biochemical and anthropometric parameters of patients and controls are shown in Table I. All patients in MS group and 40 per cent of the subjects of non-MS had waist circumferences more than 95 cm. Both groups had approximately similar rates of positive family history of disease and no remarkable difference was reported (55 and 60% in non-MS, and MS, respectively). All subjects with MS had waist circumferences more than 95 cm as a diagnostic factor of MS. Frequency of other components in MS group were high FBS: 75 per cent, hypertension: 65 per cent, hypertriglyceridaemia: 60 per cent, low HDLc: 45 per cent. Correlation between MS and its components is shown in Table II. Results showed significant correlations between MS and WC, FBS, TG, total cholesterol and hypertension.
For each person and gene, ΔCT was computed and compared between the two groups. There was a significant difference in the IL-8 expression between the two groups (ΔCT 3.48 ± 1.45 in MS group versus 2.14 ± 1.12 in Non-MS subjects, \( P = 0.002 \)), but IL-7 expression was not significantly different between the two groups (12.79 ± 1.1 versus 12.77 ± 0.7 in MS and non-MS groups \( P = 0.94 \), respectively). Fold changes of gene expression in MS group compared to non-MS subjects was calculated by \( 2^{-\Delta\Delta CT} \) formula. MS patients expressed 1.01 ± 0.4 (mean ± SE) fold IL-7 expression compared to non-MS subjects. IL-8 expression in PBMCs was significantly decreased in MS versus control subjects (fold change was 0.395±0.182).

Correlation study of both genes in the two groups showed that IL-7 was only associated with WBC count. A strong relationship was observed between IL-8 expression (ACT) and MS components (waist circumferences, systolic and diastolic blood pressure, TG, total cholesterol and LDLc). This association for TG and tChol was stronger than other factors (\( P=0.001 \), \( r = 0.511 \) and \( r = 0.500 \), respectively), while there was no association with HDLc and FBS. In MS group, IL-8 ΔCT showed a direct association with TG (\( r = 0.470, \) \( P=0.036 \)) but this relation was not observed in non-MS subjects.

### Discussion

In the present study, we quantified the relative gene expression of IL-7 and IL-8 in PBMCs in MS subject and investigated correlation between these gene expression and other biochemical parameters. A study on mice showed that IL-7 had a regulatory effect on adipose tissue mass through a lymphocyte-independent mechanism but protective role of this cytokine on glucose homeostasis would be mediated by immune cells. Relationship between this cytokine and insulin sensitivity and also adipose tissue mass indicated that the gene expression of IL-7 might be altered in process of metabolic syndrome. Regulatory function of IL-7 in glucose utilization by lymphocytes supports this theory. However, we did not find any change in gene expression of the cytokine.

IL-8 is hypothesized to involve in macrophage infiltration into adipose tissue in obesity and is reported to be associated with the development of obesity related disorders. It is also reported that circulating IL-8 is increased in MS. Thus, an increase in IL-8 expression in PBMC of MS subjects was expected compared to normal subjects, but our results showed vice versa. Therefore, it can be deduced that most of the IL-8 secreted in blood is from other sources including adipose tissue. It has been recently reported that IL-8 is released from adipose tissue in vitro; our result also displayed that all MS subject had central obesity. It is possible that the increase in production from other sites induce negative feedback control in PBMC IL-8 expression.

### Table I. Demographic data of the studied population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with MS</th>
<th>Non-MS controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>53 ± 10</td>
<td>47 ± 6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.7 ± 4.3</td>
<td>27.3 ± 3.5</td>
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<tr>
<td>WC (cm)</td>
<td>101 ± 7</td>
<td>91 ± 6</td>
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<tr>
<td>SBP (mmHg)</td>
<td>139 ± 26</td>
<td>114 ± 13</td>
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<tr>
<td>DBP (mmHg)</td>
<td>90 ± 13</td>
<td>78 ± 16</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>123 ± 38</td>
<td>96 ± 6</td>
</tr>
<tr>
<td>tChol (mg/dl)</td>
<td>218 ± 50</td>
<td>177 ± 45</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>187 ± 120</td>
<td>87 ± 32</td>
</tr>
<tr>
<td>HDLc (mg/dl)</td>
<td>57 ± 21</td>
<td>57 ± 16</td>
</tr>
<tr>
<td>LDLc (mg/dl)</td>
<td>127 ± 39</td>
<td>111 ± 38</td>
</tr>
</tbody>
</table>

MS, metabolic syndrome; BMI, body mass index; WC, waist circumference; SBP, systolic pressure; DBP, diastolic pressure; FBS, fasting blood sugar; tChol, total cholesterol; TG, triglyceride; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; NS, non significant

Values are mean ± SD (n=20)

\( P<0.05, \) \( "<0.01, \) \( ""<0.001 \) compared to healthy controls

### Table II. Correlation between metabolic syndrome (MS) and some biochemical parameters

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Parameters</th>
<th>WC</th>
<th>FBS</th>
<th>TG</th>
<th>tChol</th>
<th>HDLc</th>
<th>SysP</th>
<th>DiasP</th>
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<tbody>
<tr>
<td>r</td>
<td>0.692</td>
<td>0.691</td>
<td>0.576</td>
<td>0.394</td>
<td>0.022</td>
<td>0.593</td>
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<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.012</td>
<td>0.894</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

FBS, fasting blood sugar; TG, triglyceride; HDLc, high density lipoprotein cholesterol; WC, waist circumferences; tChol, total cholesterol; SysP, systolic pressure; DiasP, diastolic pressure
It has been shown that calcium is a second messenger in many cell types and plays a key role in different cellular processes, additionally it has been previously reported that Ca\(^{2+}\) is required for IL-8 production in neutrophils and mast cells. Increase in intracellular Ca\(^{2+}\) may induce IL-8 gene expression and protein secretion through transcriptional and posttranscriptional regulation\(^\text{31,32}\). Further, results of another study from our laboratory with a large sample size (n=400) showed that Ca\(^{2+}\) was diminished in MS subjects (Our unpublished data). Therefore, reduction in IL-8 expression may be considered as one of the Ca\(^{2+}\) downfall consequences.

The major limitation of our study was small sample size that can could lead to type II errors. Another limitation was the fact that we intended the control group not to have any parameter of MS but finding age-matched women without any MS component was very difficult.

In summary, our preliminary findings showed an absence of detectable differences in IL-7 expression and decreased expression of IL-8 in PBMCs of patients with MS compared to control subjects. Study on a larger population and detecting synthesis of these proteins can reveal more details.

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References


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