Original article

Effects of curcumin on serum cytokine concentrations in subjects with metabolic syndrome: A post-hoc analysis of a randomized controlled trial

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\textbf{A B S T R A C T}

Background: Cytokines are involved in the development of metabolic abnormalities that may result in metabolic syndrome (MetS). Since curcumin has shown anti-inflammatory properties, the aim of this study was to evaluate the effect of curcumin supplementation on serum cytokines concentrations in subjects with MetS.

Methods: This study was a post-hoc analysis of a randomized controlled trial in which males and females with diagnosis of MetS, according to the criteria defined by the National Cholesterol Education Program Adult Treatment Panel III guidelines, were studied. Subjects who met the inclusion criteria were randomly assigned to either curcumin (daily dose of 1 g/day) or a matched placebo for a period of 8 weeks.

Results: One hundred and seventeen subjects were assigned to either curcumin (n = 59) or placebo (n = 58) groups. Within-group analysis revealed significant reductions in serum concentrations of TNF-\(\alpha\), IL-6, TGF-\(\beta\) and MCP-1 following curcumin supplementation (\(p < 0.001\)). In the placebo group, serum levels of TGF-\(\beta\) were decreased (\(p = 0.003\)) but those of IL-6 (\(p = 0.735\)), TNF-\(\alpha\) (\(p = 0.138\)) and MCP-1 (\(p = 0.832\)) remained unaltered by the end of study. Between-group comparison suggested significantly greater reductions in serum concentrations of TNF-\(\alpha\), IL-6, TGF-\(\beta\) and MCP-1 in the curcumin versus placebo group (\(p < 0.001\)). Apart from IL-6, changes in other parameters remained statistically significant after adjustment for potential confounders including changes in serum lipids and glucose levels, and baseline serum concentration of the cytokines.

Conclusion: Results of the present study suggest that curcumin supplementation significantly decreases serum concentrations of pro-inflammatory cytokines in subjects with MetS.

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**Abbreviations:** MetS, metabolic syndrome; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TNF-\(\alpha\), tumor necrosis factor alpha; IL-6, interleukin 6; NCEP-ATP III, National Cholesterol Education Program Adult Treatment Panel III; TGF-\(\beta\), transforming growth factor beta; BMI, body mass index; SD, standard deviation; ANCOVA, univariate analysis of covariance; MCP-1, monocyte chemoattractant protein-1; SBP, systolic blood pressure; DBP, diastolic blood pressure; Lp(a), lipoprotein(a); hs-CRP, high-sensitivity C-reactive protein.

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1. Introduction

Metabolic syndrome (MetS) was described initially by Reaven in 1988 as Syndrome X, characterized by insulin resistance, hyperglycemia, hypertension, low high-density lipoprotein cholesterol (HDL-C), and elevated triglyceride levels [1]. Then, several definitions were proposed including obesity as a principal feature, focusing on visceral obesity since excess adipose tissue is associated with MetS components [2]. Both overweight and obesity are linked to chronic low-grade inflammation although underlying molecular mechanisms are still unclear [3]. Hypertrophied adipocytes and infiltrating macrophages and
lymphocytes contribute to the release of pro-inflammatory cytokines [4]. Tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) are considered as the most important cytokines responsible for chronic low-grade systemic inflammation, also called “metabolic inflammation”, that is commonly associated with metabolic disturbances such as type 2 diabetes [5]. Pro-inflammatory cytokines may induce the development of insulin resistance by altering the insulin signaling pathway or via triggering inflammatory pathways [6,7]. In addition, chronic systemic inflammation plays an important role in the pathogenesis of atherosclerosis and cardiovascular disease [8–10]. Although it has been suggested that the inflammatory process induced by obesity may lead to comorbidities such as atherosclerosis, dyslipidemia, hypertension, diabetes, and insulin resistance that characterize MetS; the pathophysiological mechanisms have remained unexplained yet.

Curcumin is the bioactive yellow pigment with a polyphenolic structure that is present in turmeric (Curcuma longa L.). Hitherto, several medicinal effects of curcumin have been described [10–22]. Curcumin interacts with various molecular targets including cytokines, growth factors, proteins, enzymes, and receptors [23,24]. Furthermore, this polyphenol has anti-inflammatory, antioxidant, and anti-tumor effects [25–27]. Although curcumin has been investigated in different clinical conditions, clinical trials evaluating its effect in individuals with MetS are scarce [28,29]. Therefore, in this study, we evaluate the curcumin effect on serum cytokines concentrations in subjects with MetS.

2. Material and methods

2.1. Subjects

This study is a post-hoc analysis performed on the samples obtained from our previous investigation [28]. Participants were recruited from the Cardiology and Endocrinology Clinics of the Baqiyatallah Hospital (Tehran, Iran). Inclusion criteria were males and females who were not originally receiving lipid-lowering therapy, for whom a diagnosis of MetS was made according to the criteria defined by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines as follows: ≥3 of the following conditions: waist circumference ≥102 cm (male) or ≥88 cm (female), blood pressure ≥130/85 mmHg, triglycerides ≥1.7 mmol/L, HDL-C < 1.03 mmol/L (males) or < 1.29 mmol/L (females), fasting blood glucose ≥6.1 mmol/L [30].

Exclusion criteria were pregnancy or breastfeeding, lack of compliance with the study medication (defined as not using the medication for >1 week according to the participants’ self-report), participation in a concomitant trial, hypersensitivity to the study medication, presence of inflammatory and systemic diseases, malignancies and impossibility to give informed consent. The study protocol was given approval by the institutional Ethics Committee, and written informed consent was obtained from participants.

2.2. Study design

This study was designed as a randomized double-blind placebo-controlled trial with a parallel-group design. Subjects who met the inclusion criteria were randomly assigned to either curcumin (Curcumin C3 Complex®; Sami Labs LTD, Bangalore, India; n = 59) or matched placebo (n = 58) for a period of 8 weeks. Randomization was performed via alternative allocation of participants to capsule bottles (identical in shape, size and color) labelled as “code A” or “code B”. Curcumin was administered at a daily dose of 1 g (500 mg b.i.d.), a dose that was found to be effective and safe in previous trials [17,31]. Both administering physician and the patients were blinded to the assigned intervention. In order to improve the bioavailability problem of curcumin, 5 mg piperine (Bioperine®; Sami Labs LTD, Bangalore, India) was added to each 500 mg curcumin capsule [32]. Placebo capsules contained lactose plus equal amount (5 mg) of piperine. C3 Complex® preparation that was used in the present study contained three major curcuminoids including curcumin, demethoxycurcumin and bisdemethoxycurcumin in a patented ratio. The purity of the three major curcuminoids was determined using HPLC assay.

2.3. Blood sampling

Overnight fasting blood samples were collected at baseline and at study end. The samples were allowed to clot for about 30 min and then centrifuged at 750g for 10 min to obtain serum. Sera were aliquoted and frozen at −80 °C until measurements.

2.4. Measurements

Measurement of waist circumference was performed at the level of the umbilicus, i.e. the midpoint between the lower rib margin and the iliac crest. Weight was measured with the subjects dressed in light clothing after an overnight fasting using a standard scale. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (m²) [33–35]. Serum glucose concentrations were determined using glucose oxidase method [36]. Serum concentrations of cholesterol, triacylglycerol, LDL-C and HDL-C were measured using enzymatic methods on an automated analyzer. For the measure of serum total cholesterol concentrations, a sterol esterase–cholesterol oxidase assay was used as previously described [37]. Serum LDL-cholesterol concentrations were measured directly using the sterol esterase–cholesterol oxidase method, after selective precipitation of LDL and removal of non-LDL lipoproteins. Serum HDL-C concentrations were measured with the same sterol esterase–cholesterol oxidase method used for serum cholesterol, after removal of non-HDL apolipoprotein-containing lipoproteins with magnesium–dextran sulfate precipitation [38]. Serum triglycerides concentration was measured by hydrolyzing the triacylglycerol and subsequent determination of the released glycerol [39]. Serum high-sensitivity C-reactive protein (hs-CRP) was measured using an immunoturbidimetric assay with a commercial kit [40].

Serum concentrations of IL-6, TNF-α, transforming growth factor beta (TGF-β) and chemotactant protein-1 (MCP-1) were determined the enzyme linked immunonassay technique with commercial kits. The intra-assay coefficients of variation for the measurement of IL-6, TNF-α, MCP-1 and TGF-β were 6.2%, 8.5%, 7.7% and 3.2%, respectively. The inter-assay coefficients of variation for the measurement of IL-6, TNF-α, MCP-1 and TGF-β were 7.0%, 9.8%, 6.2% and 4.9%, respectively.

2.5. Statistical analysis

Statistical analyses were performed using the SPSS software version 11.5 (SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean ± SD or number (%). Within-group comparisons were performed using paired samples t-test (for normally distributed data) or Wilcoxon signed-ranks test (for non-normally distributed data). Between-group comparisons were performed using independent samples t-test (for normally distributed data) or Mann–Whitney U test (for non-normally distributed data). Categorical variables were compared using Chi-square test. Univariate analysis of covariance (ANCOVA) using general linear model was used to adjust for the effect of potential confounders on the association between curcumin supplementation and changes in serum levels.
of TNF-α, IL-6, TGF-β and MCP-1. Statistical power was calculated using the PS software version 3.0 [41].

3. Results

One hundred and seventeen subjects met the inclusion criteria and were assigned to either curcumin (n = 59) or placebo (n = 58). One hundred subjects completed the trial. Nine subjects in the curcumin group and eight subjects in the placebo group did not complete the study due to loss to follow-up (Fig. 1). The number of drop-outs was not different between the study groups.

Curcumin and placebo groups were comparable at baseline with respect to age, gender, smoking frequency, systolic blood pressure (SBP) and diastolic blood pressure (DBP). However, BMI (p = 0.002) and serum levels of glucose concentrations (p < 0.001) were higher in the curcumin group. With respect to cytokines, there was no significant difference between the groups in terms of baseline serum TNF-α (p = 0.250) and MCP-1 (p = 0.735) concentrations. However, baseline serum IL-6 (p < 0.001) and TGF-β (p < 0.001) concentrations were significantly higher in the curcumin and placebo group, respectively (Table 1).

Within-group analysis revealed significant reductions in serum concentrations of TNF-α, IL-6, TGF-β and MCP-1 following curcumin supplementation (p < 0.001). In the placebo group, serum levels of TGF-β were decreased (p = 0.003) but those of IL-6 (p = 0.735), TNF-α (p = 0.138) and MCP-1 (p = 0.832) remained unaltered by the end of study. Between-group comparison suggested significantly greater reductions in serum concentrations of TNF-α, IL-6, TGF-β and MCP-1 in the curcumin versus placebo group (p < 0.001) (Table 2).

Univariate ANCOVA was performed to adjust the results for potential confounders including changes in serum levels of low-density lipoprotein cholesterol (LDL-C), HDL-C, total cholesterol, triglycerides, Lp(a), hs-CRP and glucose, and baseline serum concentrations of IL-6 and TGF-β (as serum levels of these two cytokines were different between curcumin and placebo groups at baseline). The impact of curcumin supplementation (versus placebo) on serum concentrations of TNF-α (p < 0.001), TGF-β (p < 0.001) and MCP-1 (p < 0.001) remained statistically significant after adjustment for potential confounders listed above. However, no significant change in serum levels of IL-6 was observed after adjustment of the analysis for the above-mentioned confounders (p = 0.581).

As reported in the original study (24), curcumin was safe and well-tolerated. In the original study, there were two reports of diarrhea, two reports of constipation, one report of headache, and two reports of skin rash in the curcumin group. Headache (n = 2) and constipation (n = 1) were reported adverse events in the placebo group. None of the drop-outs in this trial was due to the above-mentioned adverse events.

4. Discussion

Results of the present post-hoc analysis suggested that curcumin supplementation significantly decreases serum concentrations of TNF-α, IL-6, TGF-β and MCP-1 in subjects with MetS. A previous study by Ganjali et al. reported no significant effect of curcumin on serum concentrations of TNF-α, IL-6, and MCP-1 in obese individuals [42], while we found significant reduction of

![Fig. 1. Flow chart of the trial.](chart)

Table 1
Baseline characteristics of study groups.

<table>
<thead>
<tr>
<th></th>
<th>Curcumin</th>
<th>Placebo</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>44.80 ± 6.67</td>
<td>43.46 ± 9.70</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>23 (46%)</td>
<td>27 (54%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td>12 (24%)</td>
<td>8 (16%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.46 ± 2.46</td>
<td>22.80 ± 5.37</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td>91.06 ± 7.08</td>
<td>90.25 ± 14.25</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>135.56 ± 11.16</td>
<td>135.70 ± 14.74</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>88.34 ± 7.81</td>
<td>88.72 ± 8.18</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>Hs-CRP (g/L)</strong></td>
<td>6.52 ± 2.16</td>
<td>7.10 ± 1.80</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>LDL-C (mg/dl)</strong></td>
<td>190.46 ± 20.05</td>
<td>157.10 ± 17.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>HDL-C (mg/dl)</strong></td>
<td>31.50 ± 4.67</td>
<td>35.48 ± 6.54</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Total cholesterol (mg/dl)</strong></td>
<td>220.29 ± 37.72</td>
<td>184.08 ± 17.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dl)</strong></td>
<td>199.60 ± 23.44</td>
<td>185.64 ± 38.49</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Lp(a) (mg/dl)</strong></td>
<td>82.00 ± 7.35</td>
<td>84.48 ± 8.47</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>Glucose (mg/dl)</strong></td>
<td>155.46 ± 40.89</td>
<td>136.98 ± 52.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>TNF-α</strong></td>
<td>79.24 ± 8.55</td>
<td>77.48 ± 6.54</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td>3.30 ± 1.31</td>
<td>1.79 ± 0.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>TGF-β</strong></td>
<td>4.60 ± 1.37</td>
<td>5.87 ± 1.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>MCP-1</strong></td>
<td>131.52 ± 14.91</td>
<td>130.54 ± 20.41</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; hs-CRP: high-sensitivity C-reactive protein; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; Lp(a): lipoprotein(a); TNF-α: Tumor necrosis factor alpha; IL-6: interleukin 6; TGF-β: transforming growth factor beta; MCP-1: monocyte chemoattractant protein-1.
these cytokines in individuals with MetS. This discrepancy may be explained by the short duration of curcumin supplementation (30 days) in the trial of obese subjects. Moreover, a systematic review and two meta-analyses of clinical trials have shown significant reductions in C-reactive protein another key biomarker of systemic inflammation, following curcumin supplementation [10,29].

It has been suggested that curcumin has pleiotropic effects on several molecular targets involved in the inflammatory process. Specifically, curcumin regulates the inflammatory response by down-regulating the cyclooxygenase-2 activity, lipoxygenase, and inducible nitric oxide synthase enzymes; blocking the pro-inflammatory cytokines production; and down-regulating mitogen-activated and Janus kinases [43].

Several studies have reported potential beneficial effects of curcumin in the prevention and treatment of obesity, atherosclerosis, diabetes, and MetS [44]. Curcumin is involved in several mechanisms inhibiting cytokine production. Some targets curcumin can suppress key transcription factors such as nuclear factor-kappa B and activating protein-1 in sensitized macrophages and monocytes, resulting in inhibition of cytokine gene expression [45–47]. Another plausible mechanism is blocking cytokine production through down-regulation of intracellular signaling protein kinases by curcumin [48]. Moreover, it has been reported that curcumin can suppress MCP-1 release from adipocytes [47]. Also, curcumin reduces macrophage accumulation in adipose tissue by suppressing expression of inflammatory cytokines [TNF-α, MCP-1, and nitrite] and subsequent inhibition of obesity-induced inflammatory response [47]. Aside from direct inhibitory effects on cytokine production and release, mitigation of several components of MetS, such as obesity, insulin resistance, dyslipidemia, hyperglycemia, and hypertension, may be responsible for the decreased concentrations of pro-inflammatory cytokines following curcumin supplementation.

Some limitations of this study deserve mentioning. Since the interaction between pro-inflammatory and anti-inflammatory cytokines is complex, measurement of the full cytokine profile can better reflect the cytokine-modulating effects of curcumin. Another limitation is that dietary intake was not monitored during the study. Since some nutrients have inflammatory and anti-inflammatory properties, between-group differences in the daily intake of nutrients might have confounded the results. Likewise, since the original study was not designed to look at changes in anthropometric indices, the correlation between changes in inflammatory parameters with changes in body weight and BMI is unknown and deserves further investigations. In this study, we tested the effects of a single dose of curcumin, hence it is not clear if the impact of curcumin on pro-inflammatory cytokines is dose-dependent. Finally, as is the case for all post-hoc analyses, the study was limited in that it was not primarily designed to assess the impact of curcumin supplementation on serum cytokine concentrations. Although a low-grade systemic inflammation is expected to be present in subjects with metabolic syndrome, future studies are warranted to confirm the present results in populations selected according to the presence of baseline systemic inflammation.

The main strength of this post-hoc study is that the samples were driven from a randomized double-blind placebo-controlled trial which is the study design with the highest quality of evidence. Another strength of this study was the high post-hoc statistical power which excludes the possibility of insufficient of population size.

5. Conclusions

In conclusion, findings of this study suggested a significant decrease in serum cytokine concentrations following curcumin supplementation in subjects with MetS. This finding further supports the utility of curcumin as an effective supplement for MetS patients. However, still further clinical studies are required to verify if curcumin supplementation can prevent cardiovascular events in subjects with MetS, and also the dose-response association for the anti-inflammatory effect of curcumin. Also, the clinical value of adding curcumin to the therapeutic regimen of other diseases linked with systemic inflammation merits further investigation.

Conflict of interest

Muhammed Majeed is the CEO of Sabinsa Corporation and Sami Labs Ltd. Other authors have no direct competing interests to declare.

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