

## Systemic low-grade inflammation in siblings of type 2 diabetic patients

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**ABSTRACT:** Chronic low-grade inflammation is involved in the development of diabetes mellitus type 2 (DMT2). Because of genetic inheritance, first-degree relatives of DMT2 patients are at increased risk of developing this metabolic disorder. We aimed to investigate the association of inflammation in the development of DMT2 in siblings of DMT2 patients. Twenty seven DMT2 patients and 27 healthy individuals without a family history of DMT2 were enrolled. Plasma levels of the high sensitive C-reactive protein (hs-CRP), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), and fibrinogen (FIB), as inflammatory factors were assessed in all individuals. Levels of hs-CRP, TNF- $\alpha$ , IL-6, and FIB were significantly higher in DMT2 patients than in their siblings. Furthermore, all marker levels were found to be significantly higher in the siblings group than in healthy controls. Siblings of DMT2 patients are then at higher risk for DMT2 than healthy controls. Systemic inflammatory abnormalities may play an important role in the development of diabetes mellitus in siblings via endothelial dysfunction.

**KEYWORDS:** first-degree relative, diabetes, C-reactive protein, cytokines

### INTRODUCTION

Diabetes is recognized as one of the leading cause of morbidity and mortality. The incidence of diabetes mellitus type 2 (DMT2) is increasing worldwide. It is estimated that by the year of 2025, the number of people with diabetes will increase to more than twice compared to the year 2000<sup>1</sup>. DMT2 is associated with increased blood concentrations of inflammatory markers of the acute-phase proteins, including high sensitive C-reactive protein (hs-CRP), serum sialic acid, fibrinogen (FIB), and plasminogen activator inhibitor-1<sup>2-7</sup>. The acute phase proteins are synthesized in the liver, stimulated by cytokines. Three of the most important pro-inflammatory cytokines are C-reactive protein, tumour necrosis factor-alpha (TNF- $\alpha$ ), and interleukin-6 (IL-6), all of which have been implicated in atherogenesis<sup>7,8</sup>. Chronic inflammatory processes can induce a chronic increase of IL-6 and TNF- $\alpha$  and thus turn the acute phase reaction into a chronic perpetuating state with increased levels of FIB<sup>9</sup>. Elevation of FIB levels are more common in diabetic than in non-diabetic patients<sup>10</sup>. It is, therefore, suggested that chronic inflammation may represent a triggering factor in the origin of the

metabolic syndrome, and eventually diabetes mellitus disease<sup>4</sup>.

Due to a mixture of genetic inheritance and adoption of the family lifestyle, first-degree relatives (FDR) of patients diagnosed with diabetes mellitus are at increased risk of developing the disease. In addition to the insulin resistance found in young FDR<sup>11,12</sup>, family studies have revealed that FDR of individuals with DMT2 are about 3 times more likely to develop the disease than individuals without a positive family history of the disease<sup>13</sup>. It seems important therefore to verify whether a pro-inflammatory state is present in these subjects. These findings are the basis of our study to verify whether circulating concentrations of the cytokines and acute-phase proteins including TNF- $\alpha$ , IL-6, hs-CRP, and FIB are different between siblings of DMT2 patients and individuals without a family history of DMT2.

### MATERIALS AND METHODS

#### Subjects

Of the 200 patients with DMT2 who were examined by an endocrinologist, at the Department of Endocrinology, Urmia University of Medical Sciences,

**Table 1** Demographic and clinical characteristics of controls, siblings, and DMT2 patients.

Parameters	Control ( <i>n</i> = 27)	Sibling ( <i>n</i> = 28)	DMT2 ( <i>n</i> = 27)	<i>p</i> value		
				Control versus sibling	Control versus DMT2	Sibling versus DMT2
Sex (M:F)	15:12	14:14	9:18	0.44	0.1	0.21
Age (year)	44.9 ± 8.0	44.5 ± 7.1	48.5 ± 6.8	0.996	0.131	0.153
BMI (kg/m <sup>2</sup> )	28.8 ± 4.4	30.6 ± 5.9	28.6 ± 3.6	0.172	0.906	0.131
FBS (mg/dl)	83.0 ± 6.5	90.0 ± 7.8	193 ± 48	0.384	0.0001	0.0001

Urmia, Iran; 27 patients and their sibling(s) accepted to participate in the research. Each patient was asked whether any of her/his sibling(s) had diabetes or not as per the WHO criteria<sup>14</sup>. Then the importance of study was explained to those siblings who volunteer to participate. Finally, 28 siblings of DMT2 patients were enrolled. Twenty-seven healthy individuals without a family history of DMT2 that had recently been examined were enrolled as a control group.

#### Exclusion criteria

Subjects were carefully selected to exclude conditions that could interfere with hs-CRP, TNF- $\alpha$ , IL-6, or FIB levels including coronary heart disease, smoking, hypertension, bronchial asthma, acute or chronic inflammatory diseases, autoimmune diseases, and medications like steroids and antipsychotic drugs.

#### Anthropometric and blood pressure measurements

The body weight and height of each subjects was measured in the morning. After 5 min of rest, the brachial systolic and diastolic blood pressure (SBP, DBP) was measured while the subjects remained seated.

#### Blood collection

Blood samples were taken before breakfast in the morning (between 8 AM and 11 AM), after an 8–12 h overnight fast. Ten millilitres of venous blood sample were taken and were collected in plastic tubes containing heparin and then centrifuged at 1100*g* for 10 min. Plasma was separated, aliquoted, and stored at –80 °C until analysis.

#### Biochemical and immunoassays

The hs-CRP was quantitatively determined by using the kit (Diagnostic Biochem Canada Inc.). The sensitivity and the established standard curve for hs-CRP were 10 ng/ml and 0–10 000 ng/ml, respectively. Plasma concentration of TNF- $\alpha$  and IL-6 was determined by a solid phase sandwich enzyme-linked immunosorbent assay (ELISA, eBioscience). The sensitivity and the established standard curve for

TNF- $\alpha$  were 5 pg/ml and 23–1500 pg/ml, respectively, whereas for IL-6, the sensitivity was 0.92 pg/ml, and the standard curve was linear in the range of 1.56–100 pg/ml. In addition, FIB was estimated by Clauss method<sup>15</sup> using kit (Mahsayaran Co., Iran). A glucose oxidase method was employed to measure fasting blood sugar (FBS).

#### Statistical analysis

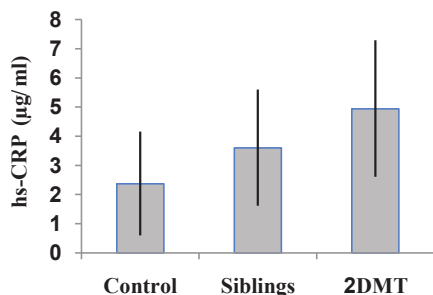
All analyses were performed using SPSS/PC statistical program (version 18.0). Results are reported as the mean ± SD. The differences between the groups were tested for significance by one-way ANOVA tests. Tukey's test was used as post hoc analysis as appropriate. Differences in proportions were tested using Pearson's chi squared test. The relation between variables was analysed by Pearson's correlation. Differences and correlations were considered significant at *p* < 0.05.

#### Research ethics

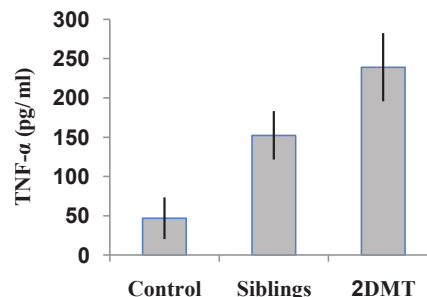
The study protocol (No. 372-2011) was approved by Research Ethics Committee of Urmia Research Administration adopted from current version of declaration of Helsinki. All participants gave written consent.

#### RESULTS

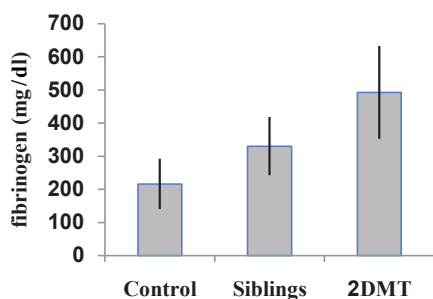
Demographic and clinical characteristics of controls, siblings, and DMT2 patients is shown in Table 1. Duration of disease in DMT2 patients was 96 ± 70 month. Plasma FBS levels in DMT2 patients (193 ± 48 mg/dl) versus siblings (90.0 ± 7.8 mg/dl) and controls (83.0 ± 6.5 mg/dl) showed significant differences (*p* = 0.0001, for all). FBS levels however were not statistically significant between siblings and healthy individuals (*p* = 0.384), Table 1. Plasma levels of hs-CRP and FIB were significantly higher in DMT2 patients to their siblings (hs-CRP: 4.9 ± 2.4 versus 3.6 ± 2.0  $\mu$ g/ml, *p* = 0.023; FIB: 493 ± 140 versus 330 ± 88 mg/dl, *p* = 0.0001, respectively) and healthy controls (hs-CRP: 2.3 ± 1.8  $\mu$ g/ml, *p* = 0.0001; FIB: 216 ± 76 mg/dl, *p* = 0.0001). Also,



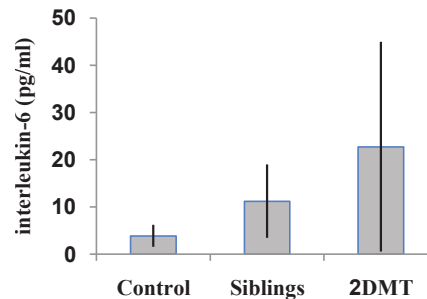
**Fig. 1** Circulating hs-CRP levels in the plasma of DMT2 patients, in their siblings, and control subjects. Levels of hs-CRP were significantly higher in DMT2 patients, in their siblings when compared with control subjects ( $p < 0.05$ ).



**Fig. 3** Levels of plasma TNF-α in DMT2 patients, in their siblings, and control subjects. Levels of TNF-α were significantly higher in DMT2 patients, in their siblings when compared with control subjects ( $p < 0.05$ ).



**Fig. 2** Plasma FIB levels in DMT2 patients, in their siblings, and control subjects. Higher levels of FIB were found in DMT2 patients and their siblings when compared to control group ( $p < 0.05$ ).



**Fig. 4** Circulating IL-6 values in the plasma of DMT2 patients, in their siblings, and control subjects. Levels of IL-6 were significantly higher in DMT2 patients, in their siblings when compared with control subjects ( $p < 0.05$ ).

the hs-CRP and FIB levels were found to be significantly higher in siblings group than in healthy controls ( $p = 0.032$  and  $0.005$ , respectively) (Figs. 1 and 2). Plasma TNF-α and IL-6 concentration in patients group were significantly higher than those in their siblings (TNF-α:  $239 \pm 43$  pg/ml versus  $152 \pm 31$  pg/ml,  $p = 0.0001$ ; IL-6:  $23 \pm 22$  pg/ml versus  $11.2 \pm 7.8$  pg/ml,  $p = 0.0001$ , respectively) and healthy controls (TNF-α:  $47 \pm 27$  pg/ml,  $p = 0.0001$ ; IL-6:  $3.9 \pm 2.3$  pg/ml,  $p = 0.0001$ , respectively). In addition, the TNF-α and IL-6 levels were found to be significantly higher in siblings group than in healthy controls ( $p = 0.0001$  and  $0.049$ , respectively) (Figs. 3 and 4).

Significant correlations were detected between hs-CRP and TNF-α with FBS in healthy individuals ( $r = 0.773$ ,  $p = 0.0001$ ;  $r = 0.508$ ,  $p = 0.01$ , respectively). Levels of hs-CRP showed significant relationship with FBS ( $r = 0.655$ ,  $p = 0.0001$ ), and BMI ( $r = 0.419$ ,  $p = 0.037$ ) in siblings. The relationship between hs-CRP, BMI and FIB levels was observed in diabetic patients ( $r = 0.464$ ,  $p = 0.02$ ;  $r = 0.570$ ,  $p = 0.026$ , respectively).

## DISCUSSION

Inflammatory markers have emerged as independent predictors of metabolic disorders such as diabetes mellitus. In past few years, many studies have been focused on hs-CRP, as a marker of low-grade inflammation<sup>16</sup>. Indeed, other inflammation markers such as TNF-α and IL-6 have been found to be increased in diabetes<sup>17,18</sup>. In addition, elevation of FIB levels and impaired fibrinolysis are more common in diabetic patients than non-diabetic subjects<sup>19</sup>. The major aim of the current study was to investigate the inflammatory status in siblings of diabetic patients who have a considerably increased risk for development of the disease later in life. These individuals showed a low-grade inflammation in our study compared to normal people without a family history of disease. Thus increased circulating levels of hs-CRP, TNF-α, IL-6, and FIB are present in non-diabetic FDR as well as their affected siblings. These data clearly indicated that augmented cytokines production is prevalent in healthy siblings of diabetic patients. It seems that in DMT2, there is an ongoing cytokine-mediated acute-phase response (part of a wide-ranging activation of innate

immunity), and this is closely involved in the pathogenesis of this metabolic disorder<sup>20,21</sup>. Our findings of a significant association between hs-CRP and BMI in siblings and patients is in agreement with previous results showing BMI was the parameter most strongly correlated with hs-CRP concentration<sup>22</sup>; however, the role of adipose tissue as a possible cause of the chronic inflammatory conditions in patients and siblings requires further study. A few studies have evaluated the association between plasma hs-CRP and FIB levels and the development of atherosclerosis in diabetic patients. The study of Hashimoto et al<sup>23</sup> reports that an elevated concentration of hs-CRP and FIB is positively correlated with atherosclerosis in subjects with a low-grade inflammation. In the current study we found that hs-CRP and FIB concentrations were statistically higher in siblings than healthy subjects, therefore it could be hypothesized that differences in these inflammatory factors may explain a low-grade inflammation in siblings which may be a risk factor for DMT2. In another study, Ozkaya et al<sup>24</sup> demonstrated the presence of a significant difference for hs-CRP levels between FDR of DMT2 and healthy subjects. Similarly another study in Denmark revealed a higher levels of hs-CRP in FDR groups than in controls<sup>25</sup>. In addition, no significant differences were found on age and BMI in mentioned studies and our results are in agreement with these findings.

There was a report that TNF- $\alpha$  was significantly higher in siblings and parents of insulin dependent diabetes mellitus compared to the appropriate control group<sup>26</sup>. Several studies have shown that hs-CRP levels is associated with blood pressure<sup>27</sup>, a result different from that observed in our study. An association between hs-CRP levels and FBS was also reported<sup>28</sup> and confirmed by Tracy et al<sup>29</sup>. We confirmed this relationship between hs-CRP levels and FBS in siblings and patients. Our data supports the opinion that chronic subclinical inflammation could be one of the components of DMT2<sup>30,31</sup>. There are however several possible explanations for these findings<sup>30</sup>. First, chronic inflammation may act as an initiating factor in the development of the eventually DMT2, as proposed by Pickup and Crook<sup>4</sup>. In their theory, stimuli such as age and over-nutrition in genetically predisposed subjects would result in increased pro-inflammatory cytokines such as IL-6 and TNF- $\alpha$ . The acute-phase response induced by pro-inflammatory cytokines could finally lead to insulin resistance and diabetes. This theory is supported with a recent report that elevated that levels of hs-CRP and IL-6 predict the development of DMT2 in healthy middle-aged women<sup>32</sup>. In a study by Choi et al<sup>33</sup>, IL-6

levels were not correlated with any component of the DMT2 patients, while TNF- $\alpha$  levels were correlated only with haemoglobin, haematocrit and some lipid profiles. The correlation between IL-6 and anthropometric measurements was not significant in our study, and there was a correlation between TNF- $\alpha$  with BMI in siblings, and TNF- $\alpha$  with FBS in controls.

Our study should be interpreted within the context of its possible limitations; we cannot exclude with certainty that there is a protective effect from other factors. Also, the number of subjects in the study was low.

## CONCLUSIONS

In summary, our study shows elevated levels of inflammatory factors hs-CRP, TNF- $\alpha$ , IL-6, and FIB in siblings than controls, and concentration of all these factors was higher in DMT2 than siblings and control groups. We have also demonstrated that healthy siblings of subjects with DMT2 exhibit over production of a range of cytokines which have been implicated in the development of this metabolic disorder. We have clearly shown that many of inflammatory abnormalities involved in diabetogenesis are shared by healthy family members.

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