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Cytokine Profile in Patients With Maturity-onset Diabetes of the Young (MODY)

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Abstract. *Background/Aim: High-sensitivity C-reactive protein (hs-CRP) is used in the differential diagnosis of maturity-onset diabetes of the young (MODY)-3, but other inflammatory markers have not been investigated in MODY patients. We aimed to compare the serum levels of anti-inflammatory and proinflammatory cytokines between MODY patients and healthy subjects and show the inflammatory features in MODY subtypes. Patients and Methods: Thirty patients with clinically suspected MODY and 34 healthy controls were included in this study. Next-generation sequencing (NGS) was used for the molecular diagnosis of MODY subtypes. Serum levels of cytokines were measured using a multiplexed cytokine assay and hs-CRP concentration was determined by the immunoturbidimetric assay. Results: The hs-CRP levels were higher in both NGS-confirmed (MODY, n=17) (p=0.009) and NGS-unconfirmed (non-MODY, n=13) patients (p<0.001) than those in controls. However, IL-1β (p=0.001), IL-6 (p=0.018), IL-31 (p=0.003), TNF-α (p<0.001), and sCD40L (p=0.007) levels of MODY patients and IL-1β (p=0.002), IL-31 (p<0.001), IL-22 (p=0.018), and sCD40L (p=0.039) levels of non-MODY patients were lower than those of controls. While hs-CRP levels were lower in MODY3 patients than non-MODY3 patients (p=0.009), IL-17A (p=0.006) and IL-23 (p=0.016) levels for the first time in this study were found to be higher in patients with MODY3 than in patients with other MODY subtypes (p<0.05). Conclusion: MODY patients had lower serum levels of the proinflammatory cytokines IL-1β, IL-6, TNF-α, IL-31, and sCD40L compared to healthy controls. High IL-17A and IL-23 levels along with low hs-CRP levels may be potential markers to distinguish MODY3 from other MODY subtypes.*

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Key Words: Pro-inflammatory cytokines, hs-CRP, HbA1c, MODY, MODY3, diabetes.

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Maturity-onset diabetes of the young (MODY) is the most common form of monogenic diabetes and comprises about 1-5% of total diabetes (1). The prevalence of MODY has been increasing like other forms of diabetes in Turkey (2). The diagnostic criteria specified in the Practice Guideline for MODY in 2018 are evidence of endogenous insulin secretion, diabetes-onset before the age of 25 in at least one family member, presence of diabetes in two consecutive generations, and absence of autoantibodies to pancreatic islet antigens (1, 3). Mutations in genes responsible for beta cells' development and function cause MODY. To date, different mutations in fourteen genes (*HNF4A*/*MODY1*, *GCK*/*MODY2*, *HNF1A*/*MODY3*, *PDX1*/*MODY4*, *HNF1B*/*MODY5*, *NEUROD1*/*MODY6*, *KLF11*/*MODY7*, *CEL*/*MODY8*, *PAX4*/*MODY9*, *INS*/*MODY10*, *BLK*/*MODY11*, *ABCC8*/*MODY12*, *KCNJ11*/*MODY13,* and *APPL1*/*MODY14*) have been described as responsible for the pathogenesis of MODY subtypes (3, 4). Recent studies have suggested that some possible biomarkers such as urine and serum amino acids, complement 5 and 8, transthyretin, urine glucose, apolipoprotein M (ApoM), lipid profile, D-glycan index, and cystatin C are useful for the differential diagnosis of

several types of MODY (5, 6). Moreover, normal serum C-peptide or urine C-peptide/creatinine ratio and mildly elevated glycated hemoglobin A1c (HbA1c) levels might be used to differentiate between MODY, type 1 (T1DM) and type 2 diabetes (T2DM) (7).

The promoter of the CRP gene has a binding site for the HNF1α transcription factor. Therefore, HNF1α mutations that disrupt the structure of the CRP binding site to the promoter of HNF1α can cause a reduction in CRP expression (8). Although low hs-CRP levels are used as a distinctive biomarker in the clinical diagnosis of MODY3 (5), other important inflammatory markers, such as serum cytokine levels, have not been as thoroughly investigated in MODY patients.

Cytokines are a cell signaling group of polypeptides/glycoproteins synthesized by different immune cells, and responsible for promoting and regulating immunity in response to inflammation, infection, and trauma (9). They are classified as pro-inflammatory and anti-inflammatory cytokines due to their role in inflammation. Tumor necrosis factor alpha (TNF-α) and interleukin (IL) 1 beta (IL-1β), which are pro-inflammatory cytokines, play a role in the disruption of insulin and lipid signaling pathways. IL-17A and IL-17F produced by effector Th17 cells are involved in the regulation of local tissue infection by inducing the expression of numerous cytokines and chemokines, including IL-1, TNF-α, IL-6, and macrophage chemotactic protein-1 (MCP-1) (10). IL-21 is a gamma chain (γc)-dependent pro-inflammatory cytokine that is expressed by several T cell subsets and natural killer (NK) cells. IL-21 increases the proliferation of CD4+ and CD8+ T lymphocytes and modulates the cytokine profile secreted by these cells. IL-22 is an IL-10 family cytokine with pro-inflammatory effects. IL-23 strongly promotes the expansion of Th17 cells, resulting in increased levels of both inflammatory cytokines (IL-17, IL-17F, IL-6, and TNF-α) and pro-inflammatory chemokines. IL-25 plays an important role in increasing Th2 cytokine production by inducing Th2-type immune responses. IL-31, mainly produced by activated Th2 cells, has been reported to play a role in various inflammatory disorders in humans. IL-33, a member of the IL-1 family, acts as a modulator in allergic, infectious and metabolic diseases. Interferon-γ (IFN-γ), the primary activator of macrophages, is an effective cytokine in the regulation of cellular and systemic metabolism as well as the effector functions of Tc cells. CD40 ligand (CD40L) and its soluble form (sCD40L), members of the TNF family, stored in platelet granules, trigger the expression of various pro-inflammatory mediators such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, IL-1, IL-6, IL-8, IL-12, TNF-α, IFN-γ, and MCP-1 (9, 10). IL-4 and IL-10, which are anti-inflammatory cytokines, suppress pro-inflammatory cytokines and down-regulate the inflammatory response. IL-6, which has both pro- and anti-inflammatory effects, is also responsible for macrophage recruitment into adipose tissue in obesity. In this way, it plays a role in the development of inflammation, insulin resistance, and T2DM (9, 11).

Abnormal levels of metabolites, such as lipids, fatty acids, and various cytokines in adipose tissue, activate monocytes and increase the secretion of inflammatory cytokines, enhancing insulin resistance (11). Although the effects of cytokines on T1DM (12) and T2DM-related (11, 13, 14) complications have been demonstrated, to the best of our knowledge, there is no detailed study in MODY patients. Therefore, to gain further insight about the potential role of inflammation in MODY diabetes, we investigated pro-inflammatory and anti-inflammatory cytokine levels in MODY patients.

Based on the current knowledge, we aimed to investigate the association of serum levels of pro-inflammatory, anti-inflammatory cytokines, and hs-CRP with clinical features of genetically confirmed MODY patients.

Patients and Methods

Subjects. This cross-sectional study comprised 30 patients with clinically suspected MODY, each attending the Diabetes Outpatient Clinic in the Division of Endocrinology and Metabolism, in Istanbul Faculty of Medicine between 2016 and 2018, and 34 healthy blood donors as the control group. Questionnaires, clinical, and laboratory records were used to identify the clinical diagnosis of MODY. A written informed consent was obtained from all participants before collecting blood samples. The study was approved by the Institutional Review Board of Istanbul University, Istanbul Faculty of Medicine (Approval no.: 2017/322, date: 24/03/2017).

The group of patients with MODY was formed according to clinical parameters such as age <40 years at onset, autosomal dominant inheritance, family history of diabetes in at least three generations (including the patient), positive serum C-peptide, negative pancreatic autoantibodies, absence of ketoacidosis. Before molecular diagnosis, the MODY probability calculator (MPC) scores were calculated in all cases (15).

Healthy blood donor subjects without any metabolic disease were included as the control group in the routine examination. All study participants examined before inclusion to the study, and those with active infection excluded from the study.

Only one patient in the MODY group had diabetic complications. A 35-year-old female patient with the HNF1B H336D mutation had neuropathy and nephropathy compatible with the MODY5 phenotype.

Mutation analysis of the MODY genes by next-generation sequencing (NGS). Genomic DNA was isolated using PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) instructions. *HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF-11, CEL, PAX-4, INS, BLK, ABCC8*, and *KCNJ11* genes were amplified with primers, which were designed for coding regions and exon-intron boundaries of the gene. DNA library was prepared *via* adding index primers and was loaded on the flow cell of the next-generation sequencing MiSeq platform (Illumina, San Diego, CA, USA) for sequencing.

Table I. *Demographic and clinical characteristics of the study groups.*

	Study groups				
	Control group (n=34)		Overall MODY group (n=30)		
Parameters	X±SEM	Median (IQR)	X±SEM	Median (IQR)	*p*-Value
Age (year)	41.5±1.6	41.0 (16.0)	43.7±2.6	42.5 (27.0)	0.494
Sex, female % (n)	88.2% (30)	–	83.3% (25)	–	0.573
BMI (kg/m^2)	27.6±0.9	27.2 (4.8)	28.5±0.9	28.2 (8.0)	0.343
Waist girth (cm)	84.7±2.3	85.5 (19.3)	95.7±2.3	98.5 (14.8)	**0.001**
SBP (mmHg)	122.4±2.3	120 (12.5)	120.3±2.1	120 (15.0)	0.511
DBP (mmHg)	76.5±1.3	75 (10.0)	74.4±2.3	70 (10.0)	0.527
Hemoglobin	15.2±0.1	15.2 (0.9)	12.8±0.3	12.3 (1.9)	**0.001**
Hematocrit	45.4±0.4	45.7 (2.6)	38.5±1.0	37.3 (5.9)	**0.001**
FBG (mg/dl)	85.6±1.3	87.0 (11.8)	147.8±10.3	132.5 (69.5)	**0.001**
HbA1c					
(%)	5.2±0.1	5.2 (0.5)	7.4±0.4	6.9 (2.4)	**0.001**
(mmol/mol)	32.8±0.7	33.1 (5.3)	56.9±3.9	52.0 (26.3)	
hs-CRP (mg/l)	1.2±0.3	0.76 (1.3)	4.5±1.0	2.29 (5.06)	**0.001**

The results are shown as mean±SEM and median IQR. Statistical analysis was performed using nonparametric Mann-Whitney *U*-test. X: Mean; SEM: standard error of mean; IQR: interquartile range; MODY: maturity-onset diabetes of the young; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood glucose; HbA1c: glycosylated hemoglobin A1c; n: number of individuals. Bold values indicate statistical significance.

Multiplex analysis. Serum samples were collected, centrifuged at 1,000×g for 5 min, and aliquoted. The aliquots were kept frozen at –80˚C until performing the assay. Before running the assay, the aliquoted samples were clot at room temperature for 30 min and centrifuged at 1,000×g for 15 min at 4˚C. After centrifugation, the samples were transferred to new clean polypropylene tubes immediately and centrifuged at 10,000×g for 10 min at 4˚C to remove platelets and precipitates completely. Then the samples were diluted 1:4 by adding Bio-Plex sample diluent HB as the manufacturer's protocol suggested (Bio-Rad, Richmond, CA, USA). The assay was performed with these freshly prepared serum samples using with MAGPIX Bio-Plex Instrument (Bio-Rad). Kit's protocol was added on the Bio-Plex Manager 6.1 Software and all information regarding standards, blanks, samples dilution, and bead regions were entered. After the run was complete, the data was acquired automatically and the concentrations of the samples were calculated.

Bio-Plex Pro Human Th17 Cytokine Panel 15-Plex (Bio-Rad, CA, USA) was chosen to investigate the most important inflammatory parameters in the present study. Magpix Bio-Plex (Bio-Rad) is a bead-based suspension array technology (xMAP Technology, Luminex Corp., Austin, TX, USA) and enables the analysis of antibodies with specificities for up to 50 different antigens in a single reaction with 96 different samples. The manufacturer's protocol was followed (Bio-Rad) and the data were acquired using the Bio-Plex MAGPIX Multiplex Reader Instrument (Bio-Rad, CA, USA). Fifteen analytes were studied: IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFN-γ, sCD40L, and TNF-α. Their concentrations were determined by Bio-Plex Manager 6.1 Software.

hs-CRP measurement. Serum concentration of hs-CRP was measured by immunoturbidimetric assay (Roche Tina-quant Cardiac High Sensitive CRP test in Roche/Hitachi 902 with Roche/Hitachi Modular P analyzers; (Mannheim, Germany)) in the Central Biochemistry Laboratory of Istanbul Faculty of Medicine. The lowest detection limit of the assay was 0.1 mg/l.

Statistical analysis. We calculated that 30 participants per group are needed for a sample size with sufficient power (85%) to detect a difference of 0.40 mg/l of hs-CRP.

The homogeneity of variance and normal distribution of variables were tested using the Shapiro-Wilk test. Clinical laboratory data are expressed as mean±standard error (X±SEM) and median (interquartile range, IQR). Mann-Whitney *U*-test was used to perform group comparisons of the mean cytokine levels, and other non-normally distributed parameters. Distribution of qualitative variables such as sex ratio was analyzed using the chi-square test. Correlation analysis was performed with Spearman's rank correlation coefficient.

Statistical data were analyzed with SPSS software, version 20.0 for Windows (SPSS, Chicago, IL, USA). *p*-Values below 0.05 were considered statistically significant.

Results

Demographic and clinical characteristics of the study population are summarized in Table I. The MODY patient and control groups have similar distributions of age, sex, body mass index (BMI), systolic (SBP) and diastolic blood pressures (DBP) ($p>0.05$). However, in MODY patients, hemoglobin and hematocrit values were lower ($p<0.001$) than the control group, but fasting plasma glucose (FPG), HbA1c, and waist girth values of MODY patients were higher as expected. Interestingly, compared to control group,

hs-CRP levels were higher in the overall MODY group ($p<0.001$).

According to the NGS results, MODY-specific missense mutations were identified in 56.6% (n=17) of MODY patients in eight genes, namely *HNF4A* (n=1), *GCK* (n=1), *HNF1A* (n=5), *PDX1* (n=1), *HNF1B* (n=1), *KLF11* (n=1), *BLK (*n=3), and *ABCC8* (n=4). The remaining 13 patients diagnosed as MODY according to clinical criteria were considered "NGS-unconfirmed (non-MODY)" due to the absence of causative gene mutations in the analyzed MODY genes (MODY 1-13). Serum levels of urea, creatinine, lipid profile, liver enzymes, C-peptide, and thyroid hormones were in the normal range in the fasting serum samples of the overall MODY group (Table II).

In the Bio-Plex cytokine inflammation panel, MODY patients showed a significantly lower susceptibility to inflammation compared to healthy subjects. As shown in Table III, the levels of hs-CRP were significantly higher in both MODY and non-MODY groups compared to control group ($p=0.009$ and $p<0.001$, respectively). The serum levels of IL-1β ($p<0.001$), IL-6 ($p=0.018$), IL-31 ($p=0.003$), TNF-α ($p=0.001$), and sCD40L ($p=0.009$) were lower in MODY group than in the controls. Also, there was a significant reduction in the levels of IL-1β ($p=0.002$), IL-31 ($p<0.001$), IL-22 ($p=0.018$), and sCD40L ($p=0.039$) in non-MODY patients compared to healthy controls. Furthermore, IL-17A, IL-21, IL-23, IFN-γ, IL-4, IL-10, IL-17F, IL-25, and IL-33 levels were similar between MODY and control groups ($p>0.05$). In the comparison between MODY and non-MODY patient subgroups, only IL-6 levels were slightly lower in the MODY group ($p=0.022$) (Table III). There was no significant difference between these two groups in terms of other cytokine levels and laboratory findings ($p>0.05$) (Table IV). In the MODY group, both BMI (r=0.755, $p<0.001$) and HbA1c levels (r=0.664, $p=0.004$) positively correlated with hs-CRP levels, however, similar correlations were not found in non-MODY group.

Mutations, related MODY subtypes, treatment choices, and inflammatory parameters found in 17 genetically confirmed cases of MODY are shown in Table V. In this group the initial treatment was sulfonylureas (SU) in 3 patients. Nine patients temporarily received insulin (INS) (in 2 patients for <1 month and in 7 patients between the first 1 and 6 months), 2 patients used metformin (MET) only, and 3 patients were on dipeptidyl peptidase 4 inhibitors (DPP-4i) plus MET. At the time of study inclusion, 2 patients were on SU, 6 patients on MET only, and 3 patients on DPP-4i plus MET. The remaining 6 patients were not using any anti-hyperglycemic drugs when included in the study. On the other hand, in the group with no genetic mutation detected but clinically had MODY criteria (n=13), 2 patients were using SU, 1 patient was using SU plus MET, 1 patient was using MET plus a DPP-4i, while 5 patients were on MET, and 4 patients were not receiving any treatment for blood glucose control.

Eleven patients in the overall MODY group were using MET. When MODY subgroups treated with and without MET were compared, no significant difference was observed between the two groups in terms of hs-CRP and other cytokines (data not shown).

There were 5 cases of MODY3 in the genetically confirmed MODY group. When we compared the MODY3 subgroup with the non-MODY3 subgroup (n=12), there was no difference between the two groups in terms of clinical features. Likewise, laboratory parameters were not different except that fasting C-peptide was lower in the MODY3 subgroup ($p=0.006$) (Table VI). As expected, the mean concentration of hs-CRP in the MODY3 subgroup was lower than that in the non-MODY3 subgroup ($p=0.009$). Moreover, the mean levels of IL-17A ($p=0.006$) and IL-23 ($p=0.016$) were significantly higher in MODY3 than in non-MODY3 cases. No significant difference was found between the two groups in terms of other inflammation parameters (Table VII). In the non-MODY3 group, correlation analyses showed that there were positive correlations between IL-17 with IL-23 levels (r=0.977, $p<0.001$), hs-CRP with waist girth (r=0.553, $p>0.05$), and BMI with C peptide (r=0.555, $p>0.05$). However, these associations were not observed in the MODY3 group.

The patient with the *GCK* L315F mutation (MODY2) (34-year-old, female) had obesity (BMI: 31.2 kg/m^2) and low serum cytokine levels. Biochemical findings of this patient were within normal limits. A 30-year-old female patient with the *PDX1* E69A mutation (MODY4) suffered from obesity (BMI: 33.20 kg/m^2). She had elevated levels of hs-CRP and inflammatory cytokines (hs-CRP: 6.25 mg/l, IL-17A: 49 pg/ml, IL-23: 48 pg/ml, TNF-α: 34 pg/ml and sCD40L: 851 pg/ml). Among the inflammatory parameters examined in the MODY5 patient carrying the *HNF1B* H336D mutation, only sCD40L levels were high (710 pg/ml) and she had neuropathy and nephropathy compatible with the MODY5 phenotype. The patient with the *KLF11* Q62R mutation (MODY7) was a 30-year-old female. She had high levels of FBG (280 mg/dl), HbA1c (13.6%), HsCRP (7.8 mg/l), sCD40L (716 pg/ml) and obesity (BMI: 36.3 kg/m^2), but she had no diabetic complications. Three patients with MODY11 did not have obesity (BMI: 27.2±4.1 kg/m^2). There was no difference in inflammatory parameters between MODY11 and non-MODY11 subgroups, however, IL-1β levels of patients with MODY11 were lower than those in the control group ($p=0.038$).

In this study, the lowest pro-inflammatory cytokine levels were detected in four MODY12 patients. In these patients, the hs-CRP levels were high (4.89±1.58 mg/l) and the mean BMI value was within the obesity limits (32.83±2.26 kg/m^2). However, serum IL-6, IL-22 and IL-33 levels were found to

Table II. *Laboratory findings of clinically suspected all MODY patients.*

Patient code	Mutation & molecular diagnosis	FBG (mg/dl)	HbA1c (%)	Triglycerides (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	Fasting C-peptide (ng/ml)	Urea (mg/dl)	Creatinine (mg/dl)	ALT (U/l)	AST (U/l)	GGT (U/l)	ACR (mg/g)	TSH (mIU/l)	FT4 (pmol/l)
1	BLK A71T(rs55758736), MODY11	118	6.1	93	37	136	18.6	3.1	9.0	0.50	18	13	15	-	2.38	13.2
2	HNF1B H336D, MODY5	135	6.6	132	48	67	26.4	2.5	33	0.80	19	16	11	35.0	1.80	16.1
3	HNF1A A98V (rs1800574), MODY3	123	7.1	57	60	94	11.4	1.8	21	0.65	9	12	8	1.5	1.96	17.4
4	No mutation*	212	10.0	333	25	106	50.0	1.2	37	1.08	33	24	38	18.3	1.68	15.4
5	BLK L503P, MODY11	121	5.4	25	82	35	5.0	1.7	29	0.78	17	15	19	-	1.33	19.5
6	KLF11 Q62R(rs35927125), MODY7	280	13.6	74	34	123	14.8	2.8	22	0.68	17	17	10	1.3	1.34	18.3
7	HNF1A A98V(rs1800574), MODY3	130	6.4	167	33	138	33.4	2.0	26	0.90	17	16	19	2.6	1.35	14.3
8	No mutation*	119	6.3	142	49	119	28.4	2.2	28	0.80	24	20	18	1.8	2.50	17.0
9	HNF1A I27L(rs1169288), MODY3	84	5.0	188	53	206	37.6	2.0	19	0.43	11	11	14	74.8	3.69	13.6
10	No mutation*	143	7.0	105	54	199	23.0	2.3	24	0.50	16	15	22	91.0	2.25	17.9
11	PDX1 E69A, MODY4	153	7.2	135	33	148	27.0	2.6	19	0.70	36	16	5	15.9	1.52	17.9
12	HNF1A A98V(rs1800574), MODY3	205	8.3	111	50	94	22.2	1.4	45	1.18	13	11	15	78.0	0.68	22.0
13	No mutation*	147	6.6	85	72	98	17	2.0	15	0.50	15	12	14	1.1	2.80	18.7
14	HNF1A I27L(rs1169288), MODY3	138	6.9	37	49	139	7.4	2.2	12	0.60	22	21	34	3.8	2.50	21.0
15	No mutation*	100	8.5	82	41	81	16.4	2.8	24	0.40	25	27	52	6.8	1.35	17.0
16	No mutation*	121	6.1	97	99	115	15.0	0.9	17	0.70	21	29	24	33.8	1.80	16.0
17	No mutation*	125	6.7	138	61	95	27.6	1.1	26	0.70	15	17	18	-	3.50	14.7
18	GCK L315F(rs1583594350), MODY2	80	5.8	41	65	134	28	3.7	23	1.10	35	26	29	3.5	1.20	17.0
19	ABCC8 K1411N, MODY12	215	9.3	152	40	130	30.4	2.0	31	0.66	17	16	14	3.8	2.50	15.0
20	ABCC8 R1103Q(rs765913448), MODY12	238	8.6	90	40	79	18.0	2.7	41	0.80	26	18	27	84.6	0.59	21.0
21	No mutation*	101	5.4	290	37	107	58.0	2.0	16	0.60	8	9	5	2.4	1.99	12.4
22	BLK R450H, MODY11	233	9.4	206	31	109	41.2	2.3	45	1.00	22	27	14	24.0	2.38	20.6
23	ABCC8 K1411N, MODY12	282	11.0	181	30	147	41.0	3.2	20	0.70	39	24	27	-	0.96	11.8
24	No mutation*	104	6.9	61	46	43	12.2	2.6	22	0.70	19	15	21	-	0.32	18.0
25	No mutation*	148	7.2	139	61	87	27.8	1.7	29	0.70	24	21	18	13.7	3.36	20.0
26	ABCC8 K1411N, MODY12	88	4.6	227	68	204	31.0	2.3	18	0.40	13	17	8	5.8	2.89	14.6
27	No mutation*	162	9.6	62	54	84	12.4	3.8	47	1.10	36	19	47	55.0	3.57	21.0
28	No mutation*	84	5.2	110	76	119	22.0	2.3	20	0.40	17	17	15	5.2	2.40	14.6
29	HNF4A T117I(rs1800961), MODY1	101	6.7	110	43	112	22.0	8.2	41	0.80	14	14	14	4.3	2.88	20.0
30	No mutation*	144	7.3	91	43	151	18.2	1.9	42	0.80	21	22	23	8.3	3.54	14.7
Total	X±SEM	149.6±10.9	7.4±0.4	125.4±13.0	50.4±3.1	116.6±7.5	24.8±2.2	2.4±0.2	26.7±1.9	0.7±0.04	20.6±1.5	17.9±0.96	19.9±2.1	23.1±5.9	2.1±0.2	17.0±0.5
	Median (IQR)	140.5 (93.3)	7.05 (2.4)	110 (81)	48.5 (23)	113.5 (45)	22.6 (14.3)	2.2 (0.8)	24 (15)	0.7 (0.4)	18 (11)	17 (8)	15 (14.5)	7.5 (31)	2.1 (1.5)	17.2 (5.2)

MODY: Maturity-onset diabetes of the young; X: mean; SEM: standard error of mean; IQR: interquartile range; HDL-C: LDL-C: and VLDL-C: high-: low-: and very low-density lipoprotein cholesterol; FPG: fasting plasma glucose; HbA1c: glycosylated hemoglobin A1c; ACR: urine albumin to creatinine ratio; ALT and AST: alanine and aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; TSH: thyroid stimulating hormone; FT4: free thyroxine. *No clinical mutations were detected in the 13 genes examined.

Table III. *Serum cytokine and hs-CRP levels of the study groups.*

	Study groups								
			Clinical MODY (n=30)						
	Control (n=34)		NGS confirmed MODY group (n=17)		NGS unconfirmed (non-MODY) group (n=13)		*p*-Values		
	X±SEM	Median (IQR)	X±SEM	Median (IQR)	X±SEM	Median (IQR)	p1	p2	p3
IL-1β (pg/ml)	17.94±1.97	14.00 (7.25)	11.1±0.9	10.0 (3.0)	11.7±0.8	10.5 (3.0)	**0.001**	**0.002**	0.341
IL-6 (pg/ml)	22.88±1.50	20.00 (5.75)	18.1±1.5	16.0 (6.8)	22.7±2.1	23.0 (5.5)	**0.018**	0.497	**0.022**
IL-17A (pg/ml)	24.79±1.06	24.50 (10.00)	25.4±3.4	19.0 (27.5)	24.8±3.4	21.0 (21.0)	0.215	0.398	0.913
IL-21 (pg/ml)	10.62±0.31	10.00 (3.00)	11.2±1.1	8.0 (7.0)	10.5±0.9	10.0 (5.0)	0.397	0.399	0.869
IL-23 (pg/ml)	30.37±1.39	30.00 (14.25)	29.9±4.3	21.0 (31.0)	29.0±3.8	26.0 (26.0)	0.308	0.460	0.934
IL-31 (pg/ml)	18.38±0.64	18.00 (5.00)	15.3±0.6	16.0 (3.5)	14.5±0.8	14.0 (4.0)	**0.003**	**0.001**	0.341
IFN-γ (pg/ml)	14.60±0.22	15.00 (1.00)	15.3±0.6	14.0 (5.0)	14.5±0.6	14.0 (4.0)	0.427	0.510	0.742
TNF-α (pg/ml)	28.03±0.93	25.75 (7.00)	22.8±2.2	19.0 (9.5)	24.3±1.8	23.0 (8.5)	**0.001**	0.174	0.157
IL-4 (pg/ml)	12.32±0.41	12.00 (3.00)	11.3±0.6	11.0 (2.0)	11.9±0.7	12.0 (5.0)	0.052	0.555	0.536
IL-10 (pg/ml)	12.85±0.34	12.50 (2.25)	13.2±0.8	12.0 (5.0)	12.5±0.6	12.0 (4.0)	0.992	0.570	0.680
IL-17F (pg/ml)	10.82±0.19	11.00 (1.25)	11.7±1.4	10.0 (2.0)	10.2±0.3	10.0 (2.0)	0.159	0.073	0.742
IL-22 (pg/ml)	13.32±0.23	13.00 (2.00)	12.9±0.8	12.0 (5.0)	12.2±0.4	12.0 (3.0)	0.118	**0.018**	0.934
IL-25 (pg/ml)	9.65±0.23	9.00 (1.00)	9.1±0.3	9.0 (2.0)	8.8±0.2	9.0 (2.0)	0.169	0.056	0.711
IL-33 (pg/ml)	21.56±0.70	20.75 (4.00)	22.5±1.8	19.0 (12.0)	23.8±2.2	21.0 (15.5)	0.688	0.943	0.385
sCD40L (pg/ml)	695.99±45.98	711.50 (383.50)	483.0±56.0	446.0 (370.5)	514.1±68.8	394.0 (433.8)	**0.007**	**0.039**	0.773
HsCRP (mg/l)	1.2±0.3	0.76 (1.3)	3.3±0.7	2.3 (5.6)	6.1±2.0	4.0 (5.8)	**0.009**	**0.001**	0.229

The results are shown as mean±SEM and median (IQR). Statistical analysis was performed using nonparametric Mann-Whitney *U*-test. P1: Control *vs*. NGS confirmed MODY; P2: Control *vs*. non-MODY patients; P3: NGS confirmed MODY *vs*. non-MODY patients. MODY: Maturity-onset diabetes of the young; X: mean; SEM: standard error of mean; IQR: interquartile range; IL: interleukin; IFN-γ: interferon-gamma; TNF-α: tumor necrosis factor-alpha; sCD40L: soluble cluster design 40 ligand. Bold values indicate statistical significance.

be lower in patients with MODY12 compared to non-MODY12 patients ($p<0.05$) (data not shown).

Discussion

In this study, 30 patients satisfying the clinical criteria of MODY were evaluated with NGS; MODY-specific mutations were detected in 13 genes examined in 17 patients (57%), and the diagnosis was confirmed. Although mutations specific to MODY in 14 known genes have been reported, many researchers suggest that there may be additional mutations in other genes (MODY X) (16-18). Therefore, considering that we examined 13 of the 14 known genes in this study, it cannot be excluded that 13 additional patients with MODY-compatible clinical features, but no MODY mutation could have MODY X. However, it should be noted that this is a low probability.

Chronic low-grade inflammation and activation of the innate immune system plays an important role in the pathogenesis of T1DM and T2DM and diabetic complications (11, 19). The levels of circulating hs-CRP and proinflammatory cytokines maybe useful markers to detect low-grade chronic inflammation with acceptable sensitivity (20).

Most of the patients in this study satisfied the clinical criteria of MODY and were referred to our clinic, which is a tertiary center. As it is known, some MODY cases may have been misdiagnosed as T2DM, especially when diabetes started in adulthood (21). The biguanide MET is the most commonly used antidiabetic drug in T2DM, and it is almost universally preferred as first-line therapy (22, 23). For this reason, MET could have been given to the patients in our study. The treatment modality was changed in patients with a confirmed diagnosis of molecular MODY, accordingly.

The development of complications in MODY patients depends on the genetic subtype (3, 24). MODY3 patients have a higher risk of cardiovascular (25) and microvascular (26) complications. MODY9 and MODY10 have been associated with ketosis-prone diabetes (27) and polycystic ovary syndrome (28), respectively, which are characterized by low-grade chronic inflammation. MODY11 is associated with a higher prevalence of obesity compared to other MODY types (3, 4). *ABCC8* gene mutations have been associated with MODY12, T2DM, gestational diabetes mellitus (GDM), and overweight/obesity (3, 4). These features justify the assessment of cytokines in MODY patients. Therefore, in the present study, we analyzed the inflammatory features of MODY patients by investigating circulating levels of the pro-

Table IV. *Comparison of laboratory findings between MODY (NGS confirmed) and non-MODY (NGS confirmed) patients.*

Parameters	Study groups				*p*-Value
	NGS confirmed MODY group (n=17)		NGS unconfirmed (non-MODY) group (n=13)		
Sex, female % (n)	82.4% (14)		84.6% (11)		0.869
	X±SEM	Median (IQR)	X±SEM	Median (IQR)	
Age (year)	42.1±3.5	37.0 (27.0)	46.2±3.7	51.0 (24.0)	0.449
BMI (kg/m^2)	29.6±1.3	31.2 (8.5)	27.4±1.2	27.9 (6.6)	0.264
SBP (mmHg)	121.3±3.1	122.5 (21.0)	118.3±1.7	120.0 (3.0)	0.750
DBP (mmHg)	72.9±2.9	75.0 (13.0)	77.5±4.0	75.0 (14.0)	0.682
FBG (mg/dl)	160.2±16.4	145.5 (121.8)	133.3±11.0	132 (51.3)	0.547
HbA1c					
(%)	7.5±0.6	7.1 (3.1)	7.3±0.5	6.8 (1.7)	0.890
(mmol/mol)	58.8±6.1	53.5 (33.8)	55.7±5.1	51.0 (18.0)	
Triglycerides (mg/dl)	119.2±15.0	133.5 (92.0)	133.4±23.2	101.0 (33.0)	0.934
HDL-C (mg/dl)	46.8±3.6	45.5 (17.0)	55.2±5.3	66.6 (31.0)	0.157
LDL-C (mg/dl)	123.2±10.6	126.5 (57.0)	108.0±10.3	106.5 (54.0)	0.229
VLDL-C (mg/dl)	24.4±2.7	26.7 (12.5)	25.2±3.9	19.5 (8.2)	0.742
Fasting C-peptide (ng/ml)	2.7±0.4	2.3 (0.7)	2.0±0.2	2.1 (0.9)	0.157
Urea (mg/dl)	26.7±2.7	23.0 (18.0)	26.7±2.8	24.0 (14.5)	0.967
Creatinine (mg/dl)	0.7±0.05	0.7 (0.3)	0.7±0.06	0.5 (0.3)	0.536
ALT (U/l)	20.3±2.2	17.0 (9.0)	21.1±2.1	19.0 (9.0)	0.621
AST (U/l)	17.1±1.2	16.0 (5.0)	19.0±1.6	19.0 (13.0)	0.281
GGT (U/l)	16.6±2.0	14.0 (4.8)	24.2±3.7	20.0 (16.3)	0.072
ACR (mg/g)	24.2±8.4	10.9 (41.2)	21.6±8.5	10.3 (43.9)	0.936
TSH (mIU/l)	1.9±0.2	1.8 (1.2)	2.4±0.3	2.4 (1.7)	0.145
FT4 (pmol/l)	17.3±0.8	18.1 (5.8)	16.7±0.7	17.5 (3.4)	0.680

The results are shown as X±SEM and median IQR. Statistical analysis was performed using nonparametric Mann-Whitney *U*-test. X: Mean; SEM: standard error of mean; IQR: interquartile range; MODY: maturity-onset diabetes of the young; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-C: LDL-C: and VLDL-C: high-: low-: and very low-density lipoprotein cholesterol; FPG: fasting plasma glucose; HbA1c: glycosylated hemoglobin A1c; ACR: urine albumin to creatinine ratio; ALT and AST: alanine and aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; TSH: thyroid stimulating hormone; FT4: free thyroxine.; n: number of individuals.

inflammatory cytokines IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFN-γ, sCD40L, and TNF-α together with hs-CRP levels. In this study, the whole patient group (MODY and non-MODY) were C-peptide positive (2.4±0.2 ng/ml), and their glycemic control was moderately impaired (HbA1c: 7.4±0.4%). The mean hemoglobin and hematocrit values of our patients in the MODY group were lower than those in the healthy control group, which may be related to normochrome-normocytic anemia of chronic disease, frequently encountered in long-term diabetes as in our MODY patients (mean duration: 13.9±2.1 years). The development of anemia may also be due to medications, a strict diet, accompanying celiac disease, or erythropoietin deficiency.

Cytokines are pleiotropic polypeptides that have autocrine, paracrine, and juxtacrine effects on the regulation of inflammatory and immune responses. These signaling molecules play important roles, directly or indirectly, in the pathophysiology of a wide range of diseases, including diabetes (27-31). Among these, IL-1β, TNF-α, and IL-6 are the major cytokines that regulate inflammation and are involved in the pathogenesis of T2DM (32). IL-1β has been reported to contribute to β-cell failure (33). TNF-α and IL-6 are implicated in the development of T2DM *via* inhibition of insulin receptor signaling, promotion of hepatic fatty acid synthesis, and induction of acute-phase proteins (32). Elevated levels of TNF-α are associated with an increased risk of diabetes and insulin resistance, this can be independent of obesity (34). Additionally, in animal studies, improving insulin resistance with TNF-α antagonist therapy supports the role of TNF-α in the development of T2DM (35). Furthermore, improved glycemia and beta-cell secretory function and reduced markers of systemic inflammation with the blockade of IL-1 with the synthetic IL-1 receptor antagonist anakinra is another strong evidence of the role of pro-inflammatory cytokines in T2DM (36). IL-22 production can be induced by IL-1β (37). Results of studies investigating the relationship between IL-22 and diabetes are limited and

Table V. *Genetic features, preferred treatment, and inflammatory status of confirmed MODY patients.*

Patient code	Sex	Age (year)	DM duration (year)	Mutation & molecular diagnosis	Treatment choice	hs-CRP (mg/l)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-17A (pg/ml)	IL-21 (pg/ml)	IL-23 (pg/ml)	IL-31 (pg/ml)	IFN-γ (pg/ml)	TNF-α (pg/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)	IL-17F (pg/ml)	IL-22 (pg/ml)	IL-25 (pg/ml)	IL-33 (pg/ml)	sCD40L (pg/ml)
1	F	26	4	BLK A71T (rs55758736), MODY11	First 1-6 months INS, currently no treatment	6.4	13	29	25.5	19	50	17	21	22	16	17	14	19	11	38	444
2	F	35	10	HNF1B H336D, MODY5	MET (1,000 mg/d) +DPP4i	1.4	10	18	21	8	21	13	14	19	10	12	9	11	9	28	710
3	F	23	15	HNF1A A98V (rs1800574), MODY3	MET (1,000 mg/d)	0.3	14	16	44	14	46	14	16	19	14	16	34	14	11	28	648
4	M	24	1	BLK L503P, MODY11	First 1-6 months INS, currently no treatment	0.5	9	12.5	15	8	17	14	13	17	10	10	10	11	8	16	522
5	F	30	11	KLF11 Q62R (rs35927125), MODY7	First 1-6 months INS, currently no treatment	7.8	11	35	23	11	35	17	15	19	18	14	10	13	10	25	716
6	M	45	8	HNF1A A98V(rs1800574), MODY3	SU	.5	10	16	48	15	45	14	16	28	11	15	10	15	9	29	454
7	F	31	1	HNF1A I27L (rs1169288), MODY3	First <1 month INS, currently no treatment	0.001	11	21	46	21	72	16	19	25	11	12	12	21	12	34	977
8	F	30	7	PDX1 E69A, MODY4	MET (1,000 mg/d) +DPP4i	6.3	12	19	49	17	48	17	17	34	11	14	11	16	10	28	851
9	F	64	35	HNF1A A98V (rs1800574), MODY3	First <1 month INS, currently MET (1,000 mg/d)	2.1	10	22	40	15	46	14	17	28	12	12	10	15	9	27.5	320
10	F	50	11	HNF1A I27L(rs1169288), MODY3	MET (850 mg/d)	1.4	8	11	15	7	17	18	12	13	9	10	9	10	8	16	180
11	M	34	1	GCK L315F (rs1583594350), MODY2	First 1-6 months SU, currently MET (1,000 mg/d)	3.1	9	12	13	8	13	12	12	17	9	10	9	10	7	15	346
12	F	59	24	ABCC8 K1411N, MODY12	SU	7.6	8	13	14	8	15	10	11	20	9	11	10	10	8	15	302
13	F	58	25	ABCC8 R1103Q (rs765913448), MODY12	MET (1,000 mg/d) +DPP4i	7.4	24	16	15	8	14	14	13	17	10	11	11	10	9	16	301

Table V. *Continued*

Table V. *Continued*

Patient code	Sex	Age (year)	DM duration (year)	Mutation & molecular diagnosis	Treatment choice	hs-CRP (mg/l)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-17A (pg/ml)	IL-21 (pg/ml)	IL-23 (pg/ml)	IL-31 (pg/ml)	IFN-γ (pg/ml)	TNF-α (pg/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)	IL-17F (pg/ml)	IL-22 (pg/ml)	IL-25 (pg/ml)	IL-33 (pg/ml)	sCD40L (pg/ml)
14	F	55	37	BLK R450H, MODY11	First 1-6 months INS, currently no treatment	4.6	10	19.5	17	8	18	18	13	52	11	21	10	12	8	17	446
15	F	37	8	ABCC8 K1411N, MODY12	First 1-6 months INS, currently MET (1,000 mg/d)	3.6	9	15.5	14	7	15	16	12	19	11	13	10	10	9	15	520
16	F	51	12	ABCC8 K1411N, MODY12	First 1-6 months INS, currently no treatment	1.0	12	14	19	10	22	18	14	23	11	18	11	12	9	19	160
17	F	64	24	HNF4A T117I (rs1800961), MODY1	First 1-6 months INS, currently MET (850 mg/d)	2.3	9	19	13	7	15	19	12	16	10	10	9	10	8	16	315

M: Male; F: female; DPP-4i; dipeptidyl peptidase-4 inhibitor; INS: insulin; SU: sulfonylurea; MET: metformin.

conflicting. While Gong *et al*. (38) reported that elevated serum IL-22 is correlated with the incidence of T2DM, Herder *et al*. (39) pointed out that higher serum IL-22 levels associate with a lower incidence of T2DM. IL-25 functions in the attenuation of IL-17-mediated inflammatory states, including diabetes (9). CD40L is a member of the TNF family and is expressed as a transmembrane protein in activated platelets. Elevated levels of sCD40L have been found in patients with obesity and diabetes mellitus (40). Furthermore, there is abundant evidence that certain inflammatory cytokines such as IL-1β, IL-6, and IL-18 are involved in the development and progression of diabetic nephropathy presenting a direct association with glomerular and tubule-interstitial damage (29-31). Elevated TNF-α levels may cause direct renal damage by changing renal hemodynamics and inducing apoptosis and neutrophil-mediated inflammatory injury. Patients with diabetic nephropathy have been reported to have higher TNF-α levels than nondiabetic persons or patients without nephropathy (41). In addition, researchers who found that IL-6 and TNF-α levels were higher in patients with proliferative diabetic retinopathy compared to those with non-proliferative diabetic retinopathy ($p<0.05$), suggested that these parameters may be useful in predicting retinopathy (42). Although the role of IL-31 in diabetes is not known, Takeuchi *et al*. (43) reported that vitreous levels of IL-31 were significantly higher in patients with retinopathy.

hs-CRP, the most widely used measure of inflammation, is an acute-phase protein (44). Elevated CRP levels have been associated with an increased risk of T2DM (7, 32). hs-CRP, TNF-α, and IL-6 together have a role in inflammatory diseases such as cardiovascular disease, hypertension, T2DM, obesity, and chronic kidney disease (45).

This study is the first to observe that serum IL-1β, IL-31, TNF-α, IL-22, IL-25 and sCD40L levels are lower in the MODY group compared to the healthy controls. In the above-mentioned studies (29-31, 41), the increase in pro-inflammatory cytokines levels has been generally associated with diabetic complications such as nephropathy and retinopathy. However, unlike cytokine levels, we showed that the serum hs-CRP level was higher in the MODY group than in the control group ($p<0.001$). High hs-CRP levels in MODY patients can be attributed to a variety of reasons. First, although recent febrile illness has been questioned, some patients in the MODY group may have a silent or subclinical infection such as periodontal disease or cystitis. Second, serum hs-CRP levels are known to be closely associated with overweight/obesity. Elevated hs-CRP levels may be related to obesity, which is associated with enlargement of adipose tissue. Hansen *et al*. (46) showed that the proposed plasma adipokines, inflammation (hs-CRP and cytokines) and T2DM is associated with more adiposity rather than insulin sensitivity and/or glycemic control. Although obesity was used as an

Table VI. *Comparison of the clinical and biochemical characteristics according to the presence of HNF1A mutation (MODY3).*

	Groups	
Parameters	MODY3 (n=5)	Non-MODY3 (n=12)
Age (year)	42.6±7.2	41.9±4.2
Diabetes duration (year)	14.0±5.7	13.7±3.3
SBP (mmHg)	123.3±8.8	120.6±3.3
DBP (mmHg)	66.7±8.8	75.0±2.5
BMI (kg/m^2)	26.3±1.9	31.0±1.6
Waist girth (cm)	92.0±5.8	99.5±4.1
Triglycerides (mg/dl)	112.0±29.5	122.2±62.5
HDL-C (mg/dl)	48.9±4.5	45.9±4.9
LDL-C (mg/dl)	134.2±20.5	118.7±12.6
VLDL-C (mg/dl)	22.4±5.9	25.3±3.0
FPG (mg/dl)	136.0±19.6	170.3±21.6
HbA1c (%) (mmol/mol)	6.74±0.54	7.85±0.75
	50.0±5.9	62.4±8.2
Fasting C-peptide (ng/ml)	**1.87±0.13***	3.09±0.49
Urea (mg/dl)	24.6±5.6	27.6±3.2
Creatinine (mg/dl)	0.75±0.13	0.74±0.05
ACR (mg/g)	32.1±18.1	19.8±8.9
ALT (U/l)	14.4±2.3	22.8±9.1
AST (U/l)	14.2±1.9	18.3±1.4
GGT (U/l)	18.0±4.4	16.1±2.3
TSH (mIU/l)	2.03±0.51	1.81±0.22
FT4 (pmol/l)	17.7±1.7	17.1±0.9

The results of the statistical analysis with nonparametric Mann-Whitney *U*-test were given as mean±SEM. MODY: Maturity-onset diabetes of the young; HbA1c: glycosylated hemoglobin A1c; BMI: body mass index; SBP and DBP: systolic and diastolic blood pressures; FPG: fasting plasma glucose; HDL-C, LDL-C, and VLDL-C: high-, low-, and very low-density lipoprotein cholesterol; ACR: urine albumin to creatinine ratio; ALT and AST: alanine and aspartate aminotransferase; GGT: gamma-glutamyl transpeptidase; TSH: thyroid stimulating hormone; FT4: free thyroxine. Bold value indicates statistical significance. *p=0.006.

Table VII. *Comparison of the inflammatory status according to the presence of HNF1A mutation (MODY3).*

	Groups	
Parameters	MODY3 (n=5)	Non-MODY3 (n=12)
hs-CRP (mg/l)	**0.9±0.38***	4.3±0.8
IL-1β (pg/ml)	10.6±1.0	11.3±1.2
IL-6 (pg/ml)	17.2±2.0	18.5±2.0
IL-17A (pg/ml)	**38.6±6.1****	19.9±2.9
IL-21 (pg/ml)	14.4±2.2	9.9±1.1
IL-23 (pg/ml)	**45.2±8.7*****	23.6±3.8
IL-31 (pg/ml)	15.2±0.8	15.4±0.8
IFN-γ (pg/ml)	16.0±1.1	13.9±0.8
TNF-α (pg/ml)	22.6±2.9	22.9±3.0
IL-4 (pg/ml)	11.4±0.8	11.3±0.8
IL-10 (pg/ml)	13.0±1.1	13.4±1.0
IL-17F (pg/ml)	15.0±4.8	10.3±0.4
IL-22 (pg/ml)	15.0±1.8	12.0±0.8
IL-25 (pg/ml)	9.8±0.7	8.8±0.3
IL-33 (pg/ml)	26.9±3.0	20.7±2 .1
sCD40L (pg/ml)	515.7±138.7	469.4±59.2

The results of the statistical analysis with nonparametric Mann–Whitney *U*-test were given as mean±SEM. hs-CRP: High sensitive C reactive protein; IL: interleukin; IFN-γ: Interferon- γ; TNF-α: tumor necrosis factor-α; sCD40L: soluble CD40 ligand. Bold value indicates statistical significance. *p=0.009; **p=0.006; ***p=0.016.

exclusion criterion for the onset of MODY in this study, similar to other parts of the world, the increase in obesity affects MODY patients as well as healthy individuals in our country. In the present study, the high hs-CRP levels in MODY patients may actually be due to the fact that most of the patients (n=12) have high BMI value above 27 kg/m^2. In other words, the high mean BMI values of our MODY patient group may explain why the hs-CRP values are high. In support, there was a strong and positive correlation between hs-CRP and both BMI (r=0.755, p<0.001) and HbA1c (r=0.664, p=0.004) in the MODY group.

Recent studies show that MET not only improves chronic inflammation by improving metabolic parameters but also through a direct anti-inflammatory effect through activation of MAPK and inhibition of mTOR pathways and autophagy (47). MET treatment has been shown to decrease IFN-γ, TNF-α, and CRP levels in some studies (48-50). Animal studies also indicate that MET treatment is associated with a decrease in plasma pro-inflammatory cytokine levels, including monocyte chemoattractant protein 1 (MCP1), IL-6, and TNF-α (51). However, some studies reported that MET treatment had no effect on hs-CRP and IL-6 levels (52-54). To assess whether MET therapy had an effect on inflammatory parameters, we compared the serum levels of hs-CRP and cytokines between the clinically suspected MODY patients with and without MET therapy (Data not shown). There was no significant difference in MET therapy between NGS-confirmed MODY patients and NGS-unconfirmed (non-MODY) patients [53.8% (n=7) *vs*. 52.9% (n=9), p>0.05]. In addition, there was no difference in terms of hs-CRP and cytokine levels between those who received and did not receive MET therapy, both in the total patient group and in MODY and non-MODY patient subgroups (p>0.05).

However, there were differences in hs-CRP and cytokine levels between MODY subtypes. The inflammatory cytokine levels were low in each of the 2 patients with MODY1 and MODY2. Serum triglycerides, ApoA1, and ApoA2 levels are low in HNF4A-MODY1 patients, but microvascular complications are frequently observed (4, 28). In our study, the patient with the MODY1 type (T117I mutation) had low serum lipid levels (no anti-hyperlipidemic medication), hs-CRP, and cytokine levels, but had obesity (BMI: 34.89 kg/m^2). The patient with *GCK* L315F mutation had a normal

lipid profile and moderate diabetes and obesity, compatible with the MODY2 phenotype (BMI: 31.16 kg/m^2). This patient also had low serum levels of cytokines.

Both *HNF1A* A98V and I27L, detected in our MODY3 group, are localized in exon 1 of the *HNF1A* gene. Both mutations, described also as an SNP, have been shown to cause decreased transcriptional activity of HNF1A (55, 56). It is known, some mutations in the *HNF1A* gene cause MODY3, whereas other mutations do not cause MODY3 but significantly increase the risk of type 2 diabetes, and some of them GDM (57). Confusingly, some *HNF1A* mutations are observed in both MODY3 and type 2 diabetes, in which case the age of onset is used for differential diagnosis. It is not fully clear why different *HNF1A* mutations cause different types of diabetes. However, it has been reported that common *HNF1A* gene mutations causing MODY3 are localized in exons 1, 2 and 4 encoding the DNA binding region of the C-terminal transactivation region, while those associated with type 2 diabetes are mostly located in exons 8 and 9 (53). As expected, hs-CRP levels in MODY3 patients were significantly lower than those in non-MODY3 patients in our study (p=0.009). MODY3 subjects had a mean BMI of 26.3±1.9 kg/m^2 and HDL-C levels (48.9±4.5 mg/dl) within the normal range, which was compatible with the MODY3 phenotype. However, MODY3 patients had higher IL-17A and IL-23 levels compared to both non-MODY3 and healthy controls and did not have any diabetic complications despite their advanced mean diabetes duration (14.0±5.7 years).

It has been shown that IL-17 activates NF-κB and thus plays a role in upregulating the expression of other inflammatory cytokine genes such as TNF-α, IL-1β, and IL-6. On the other hand, IL-23 plays a critical role in the activation of Th17 cells and in the production of IL-17 and other pro-inflammatory cytokines. It is suggested that IL-17 exerts its proatherogenic effects by inducing the production of cytokines, chemokines and matrix metalloproteinases (58). A recent study reported that serum levels of IL-17 and mRNA levels were increased in patients with newly diagnosed type 2 diabetes (59). The demonstration of increased IL-17A and IL-23 levels in patients with microvascular (60-62) and macrovascular (63) complications confirms these findings. Parhi *et al*. (64) reported that the levels of IL 17 were increased in patients with type 2 diabetes mellitus with and without diabetic complications compared to controls. However, there is no study investigating the relationship between IL-17A and IL-23 levels and HNF1A mutations in type 2 diabetes. In this study, IL-17 or IL-23 cytokines displayed a strong positive correlation in the non-MODY3 group (r=0.977, p<0.001). As expected, there were significant correlations with metabolic parameters, such as hs-CRP with waist girth (r=0,553, p<0.05), and BMI with C-peptide level (r=0.555, p<0.05) in the non-MODY3 group. However, there was no correlation between IL-17, IL-23 and BMI in non-MODY3 patients. These relationships are consistent with those observed in other forms of diabetes (65-67). However, we failed to demonstrate similar correlations in the MODY3 group in terms of both cytokine levels and metabolic parameters. The lack of these correlations in the MODY3 group may be due to the small number of patients.

The current study raises the question why IL-17 and IL-23 increase in MODY3. The main proinflammatory cytokine inducers of CRP in hepatic cells are the IL-1 and IL-6 and recently found IL-17. Patel *et al*. (68) showed that IL-17 stimulates CRP expression in hepatocytes and coronary artery smooth muscle cells and mediates CRP induction *via* p38 MAPK and ERK1/2-dependent NF-κB and C/EBPβ activation independently of IL-1β and IL-6. Moreover, a recent study showed that CRP increased IL-23 production by promoting gene transcription of IL23A (69). However, these studies cannot explain the increased expression of these cytokines under conditions of extremely low hs-CRP concentrations.

In our study, the MODY5 patient with *HNF1B* H336D mutation had neuropathy and nephropathy compatible with MODY5 phenotype. Among the inflammatory parameters examined, only sCD40L levels (710 pg/ml) were high, but the levels of other cytokines were low. So far, serum sCD40L levels of MODY5 patients have not been investigated. However, in clinical studies, circulating levels of sCD40L have been reported to be associated with kidney damage in different types of kidney disease (70). Based on this finding, it can be suggested that the level of sCD40L may also be increased in MODY5 patients in relation to nephropathy, but it needs confirmation in studies involving more patients. The female patient with the *KLF11* Q62R mutation (MODY7) had insulin requirement, uncontrolled diabetes, elevated hs-CRP (7.8 mg/l) sCD40L (716 pg/ml) and obesity (BMI: 36.3 kg/m^2), but no diabetic complications. There was no difference in inflammatory parameters between MODY11 and non-MODY11 subgroups. In MODY12 patients, hs-CRP levels were high and mean BMI was within the limits of obesity. However, the serum levels of the proinflammatory cytokines IL-6, IL-22 and IL-33 were lower than non-MODY12 patients (p<0.05). The *ABCC8* gene encodes the sulfonylurea receptor 1 (SUR-1) subunit of the ATP-sensitive potassium (K-ATP) channel in the beta-cell membrane. It is known that *ABCC8* mutations can cause structural or functional defects in the SUR1 protein, and defective K-ATP channels lead to sustained insulin release from beta cells (3). A recent study (71) reported that the SUR-1 antagonist Glibenclamide showed anti-inflammatory activity in murine microglia *in vitro* by inhibiting p38/MAPK signaling pathways and pro-inflammatory responses, and it was suggested that

Glibenclamide may be a new agent to suppress inflammatory responses in the central nervous system. Similarly, we propose that low levels of cytokines specifically associated with adipose tissue may be due to *ABCC8* gene mutations affecting SUR-1 function.

The most important limitations of this study are its cross-sectional design, the low number of patients, the high number of female participants, and the measurement of cytokine levels in serum. Serum levels may not reflect cellular cytokine secretion patterns and their contribution to local pathology. Since MODY is a rare form of diabetes, multicenter studies with a larger number of MODY patients investigating cellular cytokine secretion patterns are necessary to confirm the preliminary findings of the present study.

Conclusion

To our knowledge, this is the first study to investigate inflammatory features in terms of pro-inflammatory and anti-inflammatory cytokines in patients with MODY. First and foremost, we observed that serum levels of IL-1β, IL-6, IL-31, TNF-α, and sCD40L were significantly lower in patients with MODY compared to controls.

However, there were differences in hs-CRP and certain cytokine levels between MODY subtypes. The patient with MODY5 had increased levels of sCD40L, a marker associated with nephropathy and kidney damage. MODY3 patients had lower hs-CRP levels and higher serum levels of IL-17A and IL-23 compared to non-MODY3 patients. It has been previously reported that the levels of these cytokines are high in patients with type 2 diabetes (55, 60). However, the effect of HNF1a mutations on IL-17 and IL-23 levels in patients with type 2 diabetes was neither examined in previous studies nor in the current study. In this context, considering the high frequency of cardiovascular (25) and microvascular (26) complications similar to type 2 diabetes in MODY3 patients, we think that the relationship between *HNF1A* mutations and IL-17A and IL-23 levels is worth investigating both in type 2 diabetes patients and in the larger MODY3 group.

In conclusion, given the potential of IL-17A, IL-23, and sCD40L to affect diabetic complications, increased levels of these cytokines may affect the MODY3 and MODY5 phenotypes and perhaps also contribute to their differential diagnosis from other MODY subtypes. Therefore, we suggest detailed investigation and monitoring of these findings in multicenter and prospective studies involving larger sample groups are necessary to clarify the subject.

Conflicts of Interest

The Authors declare no conflicts of interest for this article.

Authors' Contributions

Ayca Diren: Methodology, Writing-original draft. Deniz Kanca-Demirci: Investigation. Nurdan Gul: Conceptualization, Data curation. Burcin Karacanlı: Validation, Visualization. Aykut Baykut: Validation, Visualization. Yildiz Tutuncu: Data curation, Formal analysis. Oguz Ozturk: Software, Validation. Ilhan Satman: Supervision. Hulya Yilmaz-Aydogan: Funding acquisition, Project administration, Methodology, Writing-review & editing.

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