Serum macrophage migration inhibitory factor levels in Hashimoto's thyroiditis

Ayman Abd Elrahman M.N.[a], Nagwa S. Said[a], Azza Moustafa[b]

Departments of [a]Internal Medicine, [b]Clinical Pathology, Zagazig University, Zagazig, Egypt

Correspondence to Ayman Abd-Elrahman, MN, MD, Department of Internal Medicine, Zagazig University, Zagazig, 44519, Egypt.
Tel: +20 100 510 5535: fax: 0020552307830;
e-mail: advancedendocrine@hotmail.com

Background

Hashimoto's thyroiditis (HT) is the most common autoimmune thyroid disease. The actual mechanism by which the immunological factors lead to HT has remained obscure. Macrophage migration inhibitory factor (MIF) is recently recognized as a cytokine that has a broad range of inflammatory and immune effects. The aim of this study is to assess the role of MIF in the pathogenesis of HT.

Patients and methods

One hundred and twenty-nine patients were classified into three groups: (a) overt hypothyroidism, (b) subclinical hypothyroidism, and (c) the control group. A complete history taking, clinical examination, and laboratory investigations were done. Laboratory investigations included thyroid function tests [thyroid-stimulating hormone (TSH), triiodothyronine, and thyroxine], antithyroid antibodies (antithyroid peroxidase antibodies and thyroglobulin antibodies), and MIF level.

Results

There is highly statistically significant difference between the three groups regarding MIF, the highest level being in group 1>group 2>group 3. In both hypothyroid groups (1 and 2), there are statistically significant positive correlations between MIF with TSH, thyroid peroxidase antibodies, and thyroglobulin antibodies, but there is no statistically significant correlation between MIF with thyroid functions tests and antithyroid antibodies in the control group.

Conclusion

Serum MIF levels increased in overt and subclinical hypothyroidism and its levels were positively correlated to TSH and thyroid-specific autoantibodies. Serum MIF levels play a role in the pathogenesis of thyroid autoimmune responses in patients with HT. We recommend further larger studies to assess the MIF role in the prognosis of the HT and its treatment by a new strategy.

Introduction

Hashimoto's thyroiditis (HT) is considered as the main cause of hypothyroidism especially in iodine-sufficient areas [1]. There are specific antibodies present in excess levels in HT, mainly thyroglobulin antibodies (Tg-Abs), and thyroid peroxidase antibodies (TPO-Abs) and both are correlated with the severity of thyroid autoimmune damage [2]. Macrophage migration inhibitory factor (MIF) is one of the recently recognized cytokines that has a broad range of inflammatory and immune effects including lymphocyte proliferation, regulation of macrophage, and induction of inflammatory cytokines [3]. It also promotes the synthesis of inflammatory cytokines such as interleukin-6 (IL-6), IL-2, tumor necrosis factor alpha, and interferon-γ, IL-2, and IL-6 [4].

Moreover, MIF promotes T-cell proliferation and stimulation and has also p53-dependent apoptosis [5].

In addition, MIF gene polymorphism (rs 755622 SNP (is associated with goiter size in patients with Grave's disease, which is another common autoimmune thyroid disease [6]. Serum levels of MIF have been shown to be present in excess in several other autoimmune disorders like Wegener's granulomatosis, rheumatoid arthritis, and systemic lupus erythematosus [7].

Neutralizing MIF effects by anti-MIF antibodies have been shown to be therapeutically effective in many autoimmune diseases [8].

Although immunological factors have been considered to play a key role in the pathogenesis of autoimmune thyroid diseases, the exact mechanism by which immunological factors leads to the pathogenesis of HT have remained debatable.

Therefore, the aim of this study is to assess the role of MIF in the pathogenesis of HT by studying its levels in subclinical and overt hypothyroid patients compared with the control group.

Patients and methods

The protocol of this study was approved by the local ethics committee in March 2017 (all procedures performed were in accordance with the ethical standards of the Institutional Research Committee and with Helsinki declaration and its later amendments). The authors have no conflicts of interest, and they alone are responsible for the content and writing of the paper. This study was carried out at the Internal Medicine and Clinical Pathology Departments, Faculty of Medicine.

One hundred and twenty-nine patients were included in this study. After being informed on the purpose and procedures of the study, all subjects signed on an informed consent form. They were classified into three groups: (a) overt hypothyroidism group: It included 43 adult patients with newly diagnosed overt hypothyroidism. They were five men and 38 women. Their ages range from 26 to 60 years with a mean age of 37.7±8.4 years. (b) Subclinical hypothyroidism group: it included 43 adult patients with newly diagnosed subclinical hypothyroidism. They were six men and 37 women. Their ages ranged from 21 to 63 years with a mean age of 37.7±10.2 years. (c) Control group: it included 43 apparently healthy adult patients with normal thyroid functions and they were not receiving any medications. They were seven males and 36 women. Their ages ranged from 24 to 53 years with a mean age of 37.3±7.5 years. They matched well with patients as regards age and sex.

Patients with clinical manifestations of hypothyroid disorder and had serum thyroid-stimulating hormone (TSH) more than 5 µIU/ml with positive anti-TPO-Abs and anti-Tg-Abs. Subclincal hypothyroidism was considered if free thyroxine (T4) 0.7–1.8 ng/dl and overt hypothyroidism if free T4 less than 0.7 ng/dl.

Patients who refused to be included in the study, patients on levothyroxine, or drugs affecting thyroid functions, patients with renal, hepatic dysfunction, acute or chronic inflammatory conditions, acute infections, malignancy, and diabetic patients were all excluded from our study.

All patients were subjected to the following. Complete history taking: clinical examination and laboratory investigations which included: (a) thyroid function tests [TSH, triiodothyronine (free T3), and free T4], (b) antithyroid antibodies (anti-TPO-Abs and Tg-Abs), (c) estimation of MIF level by enzyme-linked immunosorbent assay (ELISA).

Specimen collection

A measure of 3 ml of venous blood sample was aseptically withdrawn from each patient by venipuncture. The blood sample was delivered into sterile plain vacutainer tube with a stopper, left to clot at 37°C for 10 min, and then centrifuged at 3000 rpm for 20 min Part of the serum was used for thyroid function tests and antithyroid antibodies and the other part of the serum was stored at –20°C for MIF level estimation by ELISA.

Methods

(1) Thyroid function tests and antithyroid antibodies: (a) TSH (N=0.3–5.0 µIU/ml), (b) free T3 (N=2.0–4.4 pg/ml), (c) free T4 (N=0.7–1.8 ng/dl), (d) TPO-Abs (N=0–60 IU/ml), (e) Tg-Abs (N=0–60 IU/ml). They were measured using the electrochemoluminescence method with Cobas e411 (Roche Diagnostics, Diagnostics GmbH, D-68298 Mannheim, Germany) automated analyzer.
(2) Estimation of MIF level: the concentration of MIF was measured using ELISA method. Commercially available human MIF ELISA kit (Sunred, Shanghai, China) was used.

The kit uses a double-antibody sandwich ELISA to assay the level of human macrophage MIF in serum samples. Adding human macrophage MIF to monoclonal antibody enzyme well which is precoated with human macrophage MIF monoclonal antibody, incubation; then, adding (MIF) antibodies labeled with biotin, and combined with Streptavidin–HRP to form immune complex; then incubation and washing again to remove the uncombined enzyme. Then by adding chromogen solution A, B, the color of the liquid changes to blue and with the effect of acid, the color finally becomes yellow. The chroma of the color and the concentration of human macrophage MIF of the samples are positively correlated.

Statistical analysis

The collected data were computerized and statistically analyzed using the statistical package for the social sciences, version 18. Qualitative data were represented as frequencies and relative percentages. χ^2 test was used to calculate the difference between qualitative variables. Quantitative data were expressed as mean±SD when followed the normal distribution and as median and interquartile range when they are not normally distributed. Analysis of variance test was used to calculate the difference between quantitative variables in more than two groups in normally distributed data. Kruskal–Wallis test was used to calculate the difference between quantitative variables in more than two groups in not normally distributed data. Mann–Whitney test: it is a nonparametric test that is used to compare two sample means that come from the same population, and is used to test whether two sample means are equal or not. Pearson's correlation coefficient was used to calculate the correlation between the quantitative variables. It is considered that + sign was as an indication for direct correlation, that is increased frequency of independent sign leads to an increased frequency of dependent and–sign as indication for inverse correlation, that is increase frequency of independent lead to decrease frequency of dependent. The significance level for all the above-mentioned statistical tests was done. The threshold of significance is fixed at 5% level (P value). A P value of more than 0.05 indicates nonsignificant results. A P value of less than 0.05 indicates significant results. A P value of less than 0.01 indicates highly significant results.

Results

The results of this study present in the following tables.

Table 1 shows the demographic data and the thyroid functions in all the studied groups: the mean age of the overt hypothyroid group (group 1) was 37.7±8.4 years and it ranged from 26 to 60 years. The mean age of the subclinical hypothyroid group (group 2) was 37.7±10.2 years and it ranged from 21 to 63 years. The mean age of the control group (group 3) was 37.3±7.5 years and it ranged from 24 to 53 years. As regards sex, male and female percentages were 11.6 and 88.4%, in the overt hypothyroid group; 14 and 86% in the subclinical group and 16.3 and 83.7% in the control group, respectively, with obvious female predominance among the studied groups. But no statistically significant difference between the studied groups as regards age and sex .Otherwise the groups show highly statistically significant difference regarding TSH, free T3, and free T4 ($P<0.001$) using Kruskal–Wallis test with increased TSH levels in group 1 compared with groups 2 and 3 (group 1>group 2>group 3) but decreased free T3 and free T4 levels in group 1 compared with groups 2 and 3 (group 1<group 2<group 3), respectively.

Table 2 shows there is highly statistically significant difference between the three groups regarding TPO-Abs, Tg-Abs, and MIF ($P<0.001$) using the Kruskal–Wallis test with increased anti-TPO levels in group1 (group1>group 2>group 3), Tg-Abs levels in group 2 (group 2>group 1>group 3), and increased MIF levels in group 1 (group 1>group 2>group 3).

Table 3 shows the comparison between each two groups in age, TSH, free T3, free T4, TPO-Abs, Tg-Abs, and MIF (we used the post-hoc test for age variable and used the Mann–Whitney U test for other variables) and our results have shown that there is highly statistically significant difference between the overt (group 1) and subclinical (group 2) hypothyroidism groups in TSH, free T3, free T4,

Table 1 Demographic data and basic thyroid functions of the studied groups

Variables	Group 1 (overt hypothyroidism)	Group 2 (subclinical hypothyroidism)	Group 3 (control group)	Test	P value
Age (mean±SD)	37.7±8.4	37.7±10.2	37.3±7.5	$F=0.04$	0.96
Male [n (%)]	5 (11.6)	6 (14)	7 (16.3)	$\chi^2=0.4$	0.8
Female [n (%)]	38 (88.4)	37 (86)	36(83.7)		
TSH (median) (μIU/ml)	58	53.1	2.8	$F=97.2$	<0.001
IQ range	16.7–101.2	7.3–29	2.4–3.2		
Range	10.3–150	5.2–66.3	0.9–4		
FT3 (median) (pg/ml)	1.0	2.7	2.8	$F=20.9$	<0.001
IQ range	0.7–2.7	2.6–2.8	2.6–2.9		
Range	0.2–3.0	2–2.9	2–3.3		
FT4 (median) (ng/dl)	0.3	0.9	1	F 90.4	<0.001
IQ range	0.2–0.4	0.8–1.05	0.94–1.3		
Range	0.01–0.6	0.7–1.7	0.9–1.36		

FT3, triiodothyronine; FT4, thyroxine; IQ range, interquartile range; TSH, thyroid-stimulating hormone.

Table 2 Comparison between the studied groups in thyroid peroxidase antibodies, thyroglobulin antibodies, and migration inhibitory factor

Variables	Group 1 (overt hypothyroidism)	Group 2 (subclinical hypothyroidism)	Group 3 (control group)	KW test	P value
TPO-Abs (median) (IU/ml)	1300	1067	10.1		
IQ range	381.9–1300	891.8–1216.5	8.1–11.9	87.5	<0.001
Range	9–1300	69.9–1300	3.5–18.1		
Tg-Abs (median) (IU/ml)	292	337	5.3		
IQ range	87.5–500	246.5–500	4.9–6.6	87.1	<0.001
Range	71–986	211.2–1240	2.1–10.5		
MIF (median) (pg/ml)	4904	4467	2319		
IQ range	3931–7135	2471–6641	2054–2755	26.0	<0.001
Range	2013–10 455	858.1–10 324	1733–7405		

IQ range; interquartile range; KW, Kruskal–Wallis; MIF, migration inhibitory factor; Tg-Abs, thyroglobulin antibodies; TPO-Abs, thyroid peroxidase antibodies.

Table 3 Comparison between each of the two groups in age, thyroid-stimulating hormone, triiodothyronine, thyroxine, thyroid peroxidase antibodies, thyroglobulin antibodies, and migration inhibitory factor

Variables	Groups 1, 2		Groups 1, 3		Groups 2, 3	
	Test	P value	Test	P value	Test	P value
Age	0.01	0.99	0.27	0.8	0.25	0.81
TSH	5.1	<0.001	7.9	<0.001	5.8	<0.001
Free T3	4.1	<0.001	3.6	<0.001	0.7	0.4
Free T4	14.4	<0.001	8.0	<0.001	1.4	0.3
TPO-Abs	0.04	0.9	26.5	<0.001	21.5	<0.001
Tg-Abs	1.4	0.1	45.3	<0.001	15.3	<0.001
MIF	1.1	0.27	5.3	<0.001	4.1	<0.001

Free T3, triiodothyronine; Free T4, thyroxine; MIF, migration inhibitory factor; Tg-Abs, thyroglobulin antibodies; TPO-Abs, thyroid peroxidase antibodies; TSH, thyroid-stimulating hormone.

Table 4 Correlation between migration inhibitory factor with age, thyroid function tests, and antithyroid antibodies in all groups

Variables	MIF correlation in all groups					
	Group 1		Group 2		Group 3	
	r	P	r	P	r	P
Age (years)	0.08	>0.05	0.01	0.9	−0.004	>0.05
TSH (µIU/ml)	0.6	0.01	0.5	0.04	0.01	>0.05
FT3 (pg/ml)	0.1	>0.05	0.08	0.7	0.007	>0.05
FT4 (ng/dl)	−0.4	0.04	0.05	0.5	0.04	>0.05
TPO-Abs (IU/ml)	0.6	0.02	0.3	0.03	−0.03	>0.05
Tg-Abs (IU/ml)	0.5	0.03	0.4	0.04	0.07	>0.05

FT3, triiodothyronine; FT4, thyroxine; MIF, migration inhibitory factor; Tg-Abs, thyroglobulin antibodies; TPO-Abs, thyroid peroxidase antibodies; TSH, thyroid-stimulating hormone.

and ($P<0.001$). But regarding age, TPO-Abs and Tg-Abs and MIF there were no statistically significant differences between the overt and subclinical hypothyroidism groups. Regarding overt (group 1) and control (group 3) there was highly statistically significant difference in TSH, free T3, free T4, TPO-Abs, Tg-Abs, and MIF ($P<0.001$). But regarding age, there was no statistically significant difference between the two groups. This table represents also high statistically significant difference between the subclinical hypothyroid (group 2) and control (group 3) in TSH, TPO-Abs, Tg-Abs, and MIF ($P<0.001$). But there were no statistically significant differences between the two groups regarding other variables (age, free T3, and free T4).

Table 4 shows the correlation between MIF with age, thyroid function tests, and antithyroid antibodies in all groups: as regards group 1 (overt hypothyroidism), there were statistically significant positive correlations between MIF with TSH, TPO-Abs, and Tg-Abs and negative correlation with free T4. Otherwise, there is no statistically significant correlation between MIF with age and free T3. As regards the subclinical hypothyroid group (group 2), there were statistically significant positive correlation between MIF and TSH, TPO-Abs, and Tg-Abs, but no statistically significant correlation between MIF and age, free T3, and free T4. Otherwise, there is no statistically significant correlation between MIF with age, thyroid functions tests, and antithyroid antibodies in the control group.

Discussion

Our case–control study showed no statistically significant difference between overt (group 1), subclinical (group 2) hypothyroid groups, and control group (group 3) regarding age and sex with a female predominance among the studied groups. This is in agreement with the studies proposed by Erdogan et al. [9], Ates et al. [10], and Mehmet et al. [11] which were done on newly diagnosed adult HT patients in comparison with healthy controls.

Our study showed that there was high statistically significant difference between the studied groups in TSH, free T3, and free T4 with higher TSH median levels in the overt group more than the other two

groups (subclinical and control groups), respectively, while T3 and free T4 median levels had the lowest levels in the overt group.

Our study agreed with Erdogan *et al.* [9], Halder *et al.* [12], Han *et al.* [13], Ates *et al.* [10], Xue *et al.* [14], and Mehmet *et al.* [11] which reported that there were statistically significant differences between adult newly diagnosed HT patients and control groups regarding free T3, free T4, and TSH.

The study done by Peng *et al.* [15] showed that there were no statistically significant difference between the patient group and control group regarding free T3 and free T4.

This discrimination between this study and our study can be interpreted as Peng and colleagues included euthyroid patients with positive antithyroid antibodies in their patients group and due to our small sample size.

Our results have shown that TPO-Abs median levels were higher in the overt group than in subclinical and control groups, while Tg-Abs median levels are higher in the subclinical group than in overt and control groups and the MIF levels were higher in the overt group than in the subclinical and control groups, respectively. These results were similar to the results performed by Erdogan *et al.* [9], Han *et al.* [13], Ates *et al.* [10], Xue *et al.* [14], and Mehmet *et al* [11] who found that there were statistically significant differences between overt, subclinical, and control groups.

Moreover, Xue *et al* [14] classified the groups into hypothyroid groups (patients with subclinical or overt hypothyroidism) and healthy groups which showed that the serum MIF levels increased in the hypothyroid patient group compared with healthy control with remarkable difference between the two groups which supports our results.

On the other hand, Ayaz *et al.* [16] have found higher MIF levels in patients with overt hypothyroidism compared with healthy control and subclinical patients, respectively, but not statistically different.

This discrepancy between our results and Ayaz and colleagues' results may be due to racial differences and different MIF genotype expression which affect the serum levels of MIF.

Our results have shown significant differences between overt hypothyroid group (group 1) and control group

(group 3) regarding TSH, free T3, free T4, TPO-Abs, Tg-Abs, and MIF. There was no significant difference among the two groups in age.

In comparison between overt hypothyroid group and control group, our results were the same as those of Carle? *et al.* [17], regarding TSH, TPO-Abs, and Tg-Abs which were statistically different.

Zybek-Kocik *et al.* [18], showed that the statistical difference between the overt hypothyroid and the control group was observed in TSH, free T3, and free T4 concentrations with no statistical difference regarding age.

Also Ateş *et al.* [19] reported the same result of our study regarding age, TSH, free T3, free T4, TPO-Abs, and Tg-Abs among overt hypothyroid and control groups.

Another study done by Ayaz *et al.* [16] enrolled that there were statistically significant differences regarding TSH, free T3, free T4, TPO-Abs, and Tg-Abs among overt hypothyroid and control groups.

In the subclinical hypothyroid group TSH, TPO-Abs, Tg-Abs, and MIF levels were statistically significantly higher than the control group according to our results; on the other side, there were no statistically significant differences between subclinical hypothyroid and control groups regarding age, free T3, and free T4.

Similar results were obtained from the study by Türemen *et al.* [20], who found a statistically significant difference in TSH, TPO-Abs, and Tg-Abs between subclinical hypothyroid and control groups, and showed no statistically significant difference in age and free T4 between the two groups.

Another study done by Lupoli *et al.* [21] mentioned that TSH showed statistically significant difference between subclinical hypoythyroid and control groups, while there was no statistically significant difference between the two groups regarding age, free T3, and free T4.

Also Shatynska-Mytsyk *et al.* [22], and Baskoy *et al.* [23] demonstrated that TSH, TPO-Abs, and Tg-Abs had significant difference between the two groups while age, free T3, and free T4 showed no statistically significant difference.

Regarding MIF levels, there was no significant difference between subclinical and overt hypothyroid

groups with higher levels of MIF in the overt group, while there were high statistically significant differences between overt and control and also between subclinical and control groups. This dynamic alternation in different disease stages may suggest that MIF has a role in the pathogenesis of HT.

In the overt hypothyroid group, the statistically positive correlation between MIF and TSH which is the most sensitive index reflecting thyroid functions supported the theory of the role of MIF in HT progression. Also our study showed that MIF had a statistically positive correlation with TPO-Abs and Tg-Abs which indicates the severities of autoimmune damage in the thyroid. While MIF was negatively correlated with free T4, there was no statistically significant correlation between MIF and age, free T3, and free T4.There was no statistically significant correlation between MIF and age, TSH, free T3, free T4, TPO-Abs, and Tg-Abs in the control group.

The correlation results which lastly mentioned agreed with the results of Xue *et al.* [14], regarding the correlation between MIF with TSH, TPO-Ab, and Tg-Ab.

In the context of the role of MIF in autoimmune diseases, a recent study done by Bae and Lee [24] in 2017 reported that serum MIF levels were significantly higher in the rheumatoid arthritis group than in the control group and in the same year Feng *et al.* [25] mentioned that serum levels of MIF were elevated in systemic lupus erythematosus patients and were positively associated with disease activity and accumulated damage.

From all the above, we can suggest that MIF has a role in the pathogenesis of HT and can participate in the progression of the disease.

MIF represents a potential target for anti-MIF therapy, which might attenuate the autoimmune process in type 1 diabetes mellitus [26]. Therapeutic investigations have shown that MIF and IL-17 deficiency through genetic deletion or Abs neutralization results in the protection or release from several animal models of inflammatory and autoimmune disease [27].

Conclusion
We concluded that serum MIF levels increased in overt and subclinical hypothyroidism due to HT and their levels were positively correlated with thyroid-specific autoantibodies and TSH. These findings suggest that serum MIF levels play a role in the pathogenesis and development of thyroid autoimmune responses in patients with HT not only in the initial phase but also in the later phase of the disease.

We recommend further larger studies to assess the MIF role in the prognosis of HT and its treatment by a new strategy trying to use MIF neutralizing Abs that may reduce the progression of HT.

Conflicts of interest
There are no conflicts of interest.

References

1 Brent GA, Davies TF. Hypothyroidism and thyroiditis. In: ed. Williams textbook of endocrinology. 12th 2012. Philadelphia, PA: XXXX. 423–456.

2 Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev 2014; 13:391–397.

3 Cox GM, Kithcart AP, Pitt D, Guan Z, Popovich PG, Satoskar AR, Whitacre CC, et al. Macrophage migration inhibitory factor potentiates autoimmune-mediated neuro-inflammation. J Immunol 2013; 191:1043–1054.

4 Gregory JL, Morand EF, Mckeown SJ, Ralph JA, Hall P, Yang YH, et al. Macrophage migration inhibitory factor induces macrophages recruitment via CC chemokine ligand-2. J Immunol 2006; 177:8072–8807.

5 Lue H, Kleeman R, Calandra T, Roger T, Bernhagen J. Macrophage migration inhibitory factor: mechanisms of action & role in disease. Microbes Infect 2002; 4:449–460.

6 Liu YH, Chen CC, Yang CM, Chen YJ, Tsai FJ. Dual effect of a polymorphism in the macrophage migration inhibitory factor gene is associated with new-onset Grave's disease in a Taiwanese Chinese population. PLoS ONE 2014; 9.

7 Becker H, Maaser C, Mickhols E, Doyng A. Relationship between serum level of macrophage migration inhibitory factor & the activity of antineutrophilic cytoplasmic antibody-associated vasculitides. Clin Rhaumatol 2006; 25:368.

8 Greven D, Leng L, Bucala R. Autoimmune diseases: MIF as a therapeutic target. Expert Opin Ther Targets 2010; 14:253–264.

9 Erdogan M, Kulaksizoglu M, Ganidagli S, Berdeli A. Fas/FasL gene polymorphism in patients with Hashimoto's thyroiditis in Turkish population. J Endocrinol Invest 2017; 40:82.

10 Ates I, Yilmaz FM, Altay M, Yilmaz N, Berker D, Güler S. The relationship between oxidative stress and autoimmunity in Hashimoto's thyroiditis. Eur J Endocrinol 2015; 173:791–799.

11 Erdogan M, Kösenli A, Ganidagli S, Kulaksizoglu M. Characteristics of anemia in subclinical and overt hypothyroid patients. Endocr J 2012; 59:213–220.

12 Halder T, Dastidar R, Bhattacharya S, Maji D. Prevalence of Hashimoto's thyroiditis and its association with vitamin D deficiency in West Bengal, India. Br J Med Med Res 2016; 12:1–10.

13 Han C, Xia X, Liu A, Zhang X, Zhou M, Xiong C, et al. Circulating betatrophin is increased in patients with overt and subclinical hypothyroidism. Biomed Res Int 2016; 2016:5090852.

14 Xue H, Yang Y, Zhang Y, Song S, Zhang L, MA L, et al. Macrophage migration inhibitory factor interacting with Th17 cells may be involved in the pathogenesis of autoimmune damage in Hashimoto's thyroiditis. Mediators Inflamm 2015; 2015:621072.

15 Tian X, MA J, Wang T, Tian J, Zheng Y, Peng R, et al. The long noncoding RNA IFNG-AS1 promotes T helper type 1 cells response in patients with Hashimoto's thyroiditis. Sci Rep 2015; 5:17702.

16 Ayaz T, Sahin SB, Sahin OZ, Cure MC, Sumer F, Ilkkilic K, et al. Serum macrophage migration inhibitory factor levels in Hashimoto's thyroiditis; a case control study. Thyroid Res 2014; 7:11.

17 Carlé A, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Laurberg P. Hypothyroid symptoms and the likelihood of overt thyroid failure: a population-based case-control study. Eur J Endocrinol 2014; 171:593–602.

18 Zybek-Kocik A, Sawicka-Gutaj N, Wrotkowska E, Sowiński J, Ruchała M. Time-dependent irisin concentration changes in patients affected by overt hypothyroidism. Endokrynol Pol 2016; 67:476–480.

19 Ateş I, Altay M, Topçuoğlu C, Yilmaz F. Circulating levels of irisin is elevated in hypothyroidism, a case-control study. Arch Endocrinol Metabol 2016; 60:95–100.

20 Türemen EE, Çetinarslan B, Şahin T, Cantürk Z, Tarkun İ. Endothelial dysfunction and low grade chronic inflammation in subclinical hypothyroidism due to autoimmune thyroiditis. Endocr J 2011; 58:349–354.

21 Lupoli R, Dario Di Minno MN, Tortora A, Scaravilli A, Cacciapuoti M, Barba L, *et al.* Primary and secondary haemostasis in patients with subclinical hypothyroidism: effect of Levothyroxine treatment. J Clin Endocrinol Metab 2015; 100:2659–2665.

22 Shatynska-Mytsyk L, Rodrigo R, Cioccocioppo D, Petrovic N, Lakusic N, Compostella L, *et al.* The impact of thyroid hormone replacement therapy on left ventricular diastolic function in patients with subclinical hypothyroidism. J Endocrinol Invest 2016; 39:709.

23 Baskoy K, Ay SA, Altundag A, Kurt O, Salihoglu M, Deniz F, *et al.* Is there any effect on smell and taste functions with levothyroxine treatment in subclinical hypothyroidism? PLoS ONE 2016; 11:e0149979.

24 Bae SC, Lee YH. Associations between circulating macrophage migration inhibitory factor (MIF) levels and rheumatoid arthritis, and between MIF gene polymorphisms and disease susceptibility: a meta-analysis. Postgrad Med J 2017; 93:465–471.

25 Feng X, Chen W, Xiao L, Gu F, Huang J, Tsao BP, Sun L. Artesunate inhibits type I interferon-induced production of macrophage migration inhibitory factor in patients with systemic lupus erythematosus. Lupus 2017; 26:62–72.

26 Sánchez-Zamora YI, Juarez-Avelar I, Vazquez-Mendoza A, Hiriart M, *et al.* Altered macrophage and dendritic cell response in Mif–/– mice reveals a role of Mif for inflammatory-Th1 response in type 1 diabetes. J Diabet Res 2016; 7053963.

27 Yang J, Sundrud MS, Skepner J, Yamagata T. Targeting Th17 cells in autoimmune diseases. Trends Pharmacol Sci 2015; 35:493–500.