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ORIGINAL ARTICLE PEDIATRICS

Mannose-binding lectin (MBL) levels in children with Hashimoto's thyroiditis

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ABSTRACT

OBJECTIVE: Hashimoto's thyroiditis (HT) was described many years ago, but the etiopathogenesis remains unclear. Mannose-binding lectin (MBL) initiates complement activation in the lectin pathway. We determined MBL levels in children with HT and the associations thereof with thyroid hormone and thyroid autoantibody levels.

METHODS: Thirty-nine patients with HT and 41 controls were enrolled from the pediatric outpatient clinics. Subjects were grouped according to their thyroid functions: Euthyroid, marked hypothyroidism and clinical/subclinical hyperthyroidism. MBL levels were compared among these groups. Serum MBL levels of the subjects were determined using MBL Human ELISA kit.

RESULTS: Serum MBL levels were studied in serum samples from the 80 subjects (48 (60.0%) females). MBL levels in HT and control groups were 50.787±34.718 and 50.593±44.28 ng/ml (p=0,983), respectively. In HT group, there was no significant difference in MBL levels between thyroid function groups (p=0.869). In addition, gender was not detected as a factor for serum MBL levels. Also we found negative correlation between WBC and serum MBL levels (r=-0.532; p=0.050). Otherwise there was no correlation between TSH, anti-TPO and anti-TG with serum MBL levels.

CONCLUSION: MBL levels did not decrease in HT patients. Further research is needed to elucidate more fully any role for MBL in the development of autoimmune thyroid disease.

Keywords: Autoimmune disease and hypothyroidism; children; Hashimoto's thyroiditis; mannose binding lectin.

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Hashimoto's thyroiditis (HT) is a chronic inflammation of the thyroid gland that was described many years ago, but the etiopathogenesis remains unclear. HT is the most common cause of hypothyroidism, and the most frequent autoimmune endocrine disease [1]. The most prominent clinical manifestation is thyroid gland growth with or without hypothyroidism. An HT diagnosis should be supported by an enhanced level of circulating autoantibodies targeting thyroid antigens, and reduced echogenicity evident on a thyroid sonogram [2].

The innate immune system regulates initiation of an inflammatory response during infection and tissue regeneration. Mannose-binding lectin (MBL) is a serum protein triggering complement activation via the lectin pathway. MBL plays an important role in host defenses, particularly in infancy, when acquired immunity remains

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underdeveloped. A functional MBL serum deficiency triggers a phagocytic defect associated with an increased risk of recurrent childhood infections [3]. Complement system activation is also associated with many human diseases [4]. Functional MBL deficiency has been associated with several non-infectious disorders, including the autoimmune diseases systemic lupus erythematosus (SLE) and inflammatory bowel disease. MBL is an acute phase protein, the production of which is enhanced by inflammatory stimuli. Low MBL levels may create a risk of an excessive inflammatory response, including autoantibody production associated with poor clearance of apoptotic cellular material [5, 6].

Serum MBL levels correlate closely with those of thyroid hormones; MBL levels are markedly increased in patients with hyperthyroidism and decreased in those with hypothyroidism [7]. Previous studies suggested that MBL production was stimulated by thyroid hormones [8]. It is important to determine serum MBL levels in patients with certain diseases and prescribe plasma-purified or recombinant MBL to treat those diseases [9, 10]. Complement MBL deficiency is associated with increased susceptibility to infection and autoimmune disease. Here, we explored such an association in patients with HT, an autoimmune thyroid disease (AITD). We determined MBL levels in patients with HT and the associations thereof with thyroid hormone and thyroid autoantibody levels.

MATERIALS AND METHODS

Thirty-nine patients with Hashimoto's thyroiditis and 41 healthy controls were enrolled from the pediatric outpatient clinic of the Gaziosmanpasa University School of Medicine, Tokat, Turkiye, and from the pediatric endocrinology outpatient clinic of Erciyes University School of Medicine, Kayseri, Turkiye. Control patients had no chronic, immune, inflammatory or endocrinological disease. The study protocol adhered to all relevant tenets of the Helsinki Declaration of the World Medical Association and local ethical standards, and was approved by the Ethics Committee of Gaziosmanpasa University Faculty of Medicine (approval no. 17-KAEK-15). The parents of all patients gave written informed consent; questionnaires were completed by both parents and patients. We recorded clinical, demographic, and anthropometric data. Hashimoto thyroiditis was diagnosed by measuring the levels of anti-thyroid peroxidase antibodies (anti-TPOAb) and anti-thyroglobulin antibodies (anti-TGAb) combined with thyroid ultrasonography (USG) [11]. The USG scan was performed by radiologist with a digital sonography scanner (Aplio 500, Toshiba Medical Systems Corporation, Otawara, Japan). Patients with anthropometric measurement information, positive thyroid antibodies, and thyroid ultrasound were included in the study. Patients with missing clinical or laboratory information, who received immunosuppressive therapy such as steroid therapy or were diagnosed with another autoimmune disease, were excluded from the study. Laboratory tests, including complete blood count, thyroid-stimulating hormone (TSH), free thyroxine (fT4), free tri-iodothyronine (fT3), aspartate transaminase (AST), and alanine transaminase (ALT) were derived from blood samples drawn from all participants. All subjects were grouped by thyroid function: euthyroid (both fT4 and TSH levels within normal limits), subclinical hypothyroidism (fT4 level normal, TSH level high), marked hypothyroidism (sT4 level low, TSH level high), and clinical/subclinical hyperthyroidism (sT4 level normal/high, TSH level low). The normal upper limit of TSH was taken to be 4.50 μIU/L and that of fT4 2.00 ng/dL [12]. The normal lower limit of TSH was taken to be 0.50 μIU/L and that of fT4 0.7 ng/dL. MBL levels were compared among the groups.

Highlight key points

- MBL levels did not decrease in HT patients.
- A moderate negative correlation between the WBC and MBL level in patients with HT was found. It shows the role of lymphocytes in HT development.

TSH, fT4, anti-TG, and anti-TPO levels were measured using the chemiluminescence immunoassays (ECLIA) of the COBAS E601 system (Roche Diagnostics, Mannheim, Germany). C-reactive protein (CRP) levels were determined using a turbidimetric approach employing a UniCel DxC 880i (Beckman Coulter) analyzer. Total white blood cell counts (WBCs) were obtained using an automated counter.

Blood samples were allowed to clot for 2 h at room temperature, centrifuged at 1,000 g for 15 min at 4°C, and the sera aliquoted into tubes and stored at -80°C prior to MBL assay. Serum MBL levels were determined using an MBL Human ELISA kit (E-EL-H1305, Elabscience, Wuhan, China) according to the manufacturer's instructions. All measurements were performed in triplicate and the absorbances at 450 nm obtained using a microplate reader (HEALES Full Automatic Microplate Reader MB-530, Shenzhen, China) averaged.

TABLE 1. Demographic and clinical characteristic of subjects

	Total		Hashimoto		Control		p
	n	%	n	%	n	%	
Gender							
Female	48	60.0	30	6.9	18	3.9	0.003
Male	32	40.0	9	23.1	23	56.1	
Thyroid functions at diagnosis							
Euthyroid			11	28.2			
Subclinical hypothyroidism			11	28.2			
Hypothyroidism			14	35.9			
Hyperthyroidism			3	7.7			
USG findings at diagnosis							
Abnormal☐			37	92.6			
Normal			2	7.4			

USG: Ultrasonography; ☐: Thyroid USG findings were included at least one of the following; heterogeneity, increased blood supply, granular and/or hypoechoic appearance.

Statistical Analyses

Quantitative data are expressed as arithmetic means with standard deviations. Categorical variables are given as numbers with percentages. The independent-samples t-test or one-way analysis of variance was used to compare continuous, normally distributed data among groups. The Turkiye HSD test was used for multiple comparisons. The chi-squared test was employed to compare categorical data between/among groups. We derived Pearson correlation coefficients when examining relationships among variables. To determine the effect of group on MBL level, we controlled for age when analyzing covariance. A p-value <0.05 was considered to reflect statistical significance. All statistical analyses were performed with the aid of SPSS ver. 19 software (IBM SPSS Statistics 19, SPSS Inc., Somers, NY, USA).

RESULTS

We measured MBL levels in serum samples from 39 patients with Hashimoto's thyroiditis and 41 controls. Of the 80 subjects, 48 (60.0%) were female. Table 1 lists the demographic and clinical characteristics. The means ages of HT patients and controls were 14.2±3.0 and 12.4±3.2 years (p=0.021), respectively. The serum MBL levels, and the laboratory and demographic data of the groups, are listed in Table 2. The MBL levels in the HT and control groups were 50.787±34.718 and 50.593±44.28 ng/mL (p=0.983), respectively. The MBL levels by the categorical variables are shown in Table 3 for the HT group. The MBL level did not differ significantly by thyroid function status at diagnosis (p=0.896) or gender. In the HT group, negative correlations were evident between the MBL level, and the body mass index and the WBC (r=-0.434; p=0.034; r=-0.532; p=0.050 respectively). On the other hand, we found no correlation between the MBL level and the level of any of TSH (r=-0.026; p=0.897), anti-TPO (r=-0.132; p=0.244), or anti-TG (r=-0.091; p=0.667).

DISCUSSION

To the best of our knowledge, this is the first analysis of plasma MBL levels in children and adolescents with HT; we sought associations between that level and those of functional thyroid markers and autoantibodies. We found no such associations. The lectin pathway is an effective antimicrobial defense mechanism and an important scavenger system [13]. MBL is associated with autoimmunity in that apoptotic cells are cleared when their altered membrane carbohydrates bind to MBL [14]. Klecha et al. [15] studied thyroid specimens of HT patients and found nuclear DNA fragmentation of thyroid follicular cells surrounded by infiltrates of mononuclear cells, indicative of apoptosis. It has been suggested that anti-TGAb reflect an im-

Table 2. Distribution of serum levels of MBL and laboratory/demographic data according to groups

	Hashimoto thyroiditis, Mean±SD	Control, Mean±SD	p
MBL (ng/ml)	50.787±34.718	50.593±44.28	0.983
Age (year)	14.20±3.0	12.4±3.2	0.021
Age of diagnosis (year)	11.3±3.7		
Length of diagnosis time (year)	2.7±2.3		
BMI (kg/m^2)	21.21±4.59	20.42±6.14	0.591
TSH at diagnosis (µIU/L)	13.52±22.42		
fT4 at diagnosis (ng/dL)	1.19±0.32		
TSH recently (µIU/L)	1.39±0.85	2.37±1.19	
fT4 recently (ng/dL)	1.23±0.2	1.32±0.17	
Anti-TPO Ab (IU/mL)	267.78±187.26		
Anti-TG Ab (IU/mL)	739.84±1038.37		
Hemoglobin (g/dL)	13.61±1.14	13.01±1.37	0.145
WBC (x1000/mm^3)	7.93±1.47	6.84±1.87	0.052
Platelets (x1000/mm^3)	298.07±63.43	288.2±57.7	0.592
CRP (mg/dL)	2.16±2.93	3.1±5.34	0.585
AST (IU/L)	20.62±10.45	21.91±6.8	0.605
ALT (IU/L)	18.04±18.05	15.91±7.75	0.546

SD: Standard deviation; MBL: Mannose-binding lectin; BMI: Body mass index; TSH: Thyroid stimulating hormone; fT4: Free T4; Anti-TPO: Anti-thyroid peroxidase antibodies; Anti-TG: Anti-thyroglobulin antibodies; WBC: White blood cell; CRP: C-reactive protein; AST: Aspartate transaminase; ALT: Alanine transaminase. Gender correction is applied for quantitative variables.

mune reaction to antigenically modified thyroglobulin rather than thyroid autoimmunity [16]. The progressive reduction of thyroid function as autoimmunity develops is associated with a gradual increase in serum TSH levels in patients with autoimmune thyroiditis. Potlukova et al. [7] reported that serum MBL levels were lower in HT patients with hypothyroidism than in patients who were euthyroid; at follow-up, serum MBL levels in HT patients increased in parallel with thyroid status. To our surprise, we found no significant difference in MBL levels between the HT and control groups. This was a cross-sectional work; we studied patients who had been diagnosed with HT and were being followed-up. We suggest that euthyroidism may have masked the low levels of MBL in such patients. It has been reported that when MBL levels were normalized in terms of thyroid function, the differences in serum MBL levels between HT and control groups disappeared [7].

Filho et al. [17] found that the MBL 2 O allele (associated with defective MBL synthesis in homozygous O/O patients) increased the HT risk 2.5-fold. It was suggested that patients homozygous for MBL2, thus lacking MBL, only ineffectively cleared apoptotic cells, associated with a risk of self-antigen spread and development of autoimmune diseases. Potlukova et al. [18] found that women exhibiting thyroid dysfunction and/or anti-TPOAb had lower MBL levels during pregnancy than controls. The MBL levels decreased markedly

Table 3. Distribution of MBL levels according to groups and thyroid functions

	MBL (ng/ml)	p
Thyroid function group at diagnosis		
Euthyroid	56.85±39.57	0.896
Subclinical hypothyroidism	54.65±36.49	
Hypothyroidism	26.5±9.17	
Hyperthyroidism	45.76±30.75	
Control	50.59±44.28	
Gender		
Female	51.72±40.5	0.730
Male	48.3±39.73	

MBL: Mannose-binding lectin. Gender correction is applied for quantitative variables in thyroid function groups.

after delivery, unassociated with any significant change in the TSH or FT4 serum levels. However, we found no relationship between MBL and thyroid autoantibody levels. As the physiology of pregnancy is unique, we cannot compare the data of (18) to those of the present study. Karbownik et al. [19] found that MBL levels did not correlate with either anti-TPOAb or anti-TGAb status in women of childbearing age. Also, MBL levels did not differ significantly between groups that did and did not express such antibodies [19]. MBL levels seem to be unaffected by autoantibody and autoimmunity status.

We found a moderate negative correlation between the WBC and MBL level in patients with HT. As MBL levels decrease, the WBC would be expected to increase. Although we did not specifically examine lymphocyte status, MBL suppresses T lymphocyte proliferation and inhibits T cell activity [20]. T lymphocyte proliferation in patients with autoimmune diseases would thus be expected. We suggest that this negative correlation reflects the role played by lymphocytes in HT development. MBL deficiency may increase the risk of several T cell-mediated autoimmune diseases including HT. Also, tissue accumulation of MBL may reduce serum MBL levels in patients with autoimmune diseases.

Our patient numbers were small, our data limited, and the study was cross-sectional in nature. Although we performed statistical corrections, the groups differed in terms of age and gender. Since Hashimoto's disease is more common in late adolescence period and female adolescents. These are limitations. The strengths of our study are that it is the first to measure MBL levels in children with HT and that we emphasize that MBL measurement at the time of diagnosis may shed light on the pathophysiological mechanism of HT.

Conclusion

In summary, MBL levels did not decrease in HT patients. The low levels of serum MBL in such patients may reflect hypothyroidism rather than any role for MBL in the pathophysiology of HT. As mentioned above, the increases in MBL levels after correction of the hypothyroidism of HT patients supports this interpretation. If MBL in fact plays a role in the pathophysiology of HT, the levels must be measured soon after diagnosis. Further research is needed to elucidate more fully any role for MBL in the development of autoimmune thyroid disease.

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REFERENCES

1. McLeod DS, Cooper DS. The incidence and prevalence of thyroid autoimmunity. Endocrine 2012;42:252–65. [CrossRef]
2. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmunity Rev 2014;13:391–7. [CrossRef]
3. Super M, Lu J, Thiel S, Levinsky R, Turner M. Association of low levels of mannan-binding protein with a common defect of opsonisation. Lancet 1989;334:1236–9. [CrossRef]
4. Singh SS, Cheung RC, Wong JH, Ng TB. Mannose Binding lectin: a potential biomarker for many human diseases. Curr Med Chem 2016;23:3847–60. [CrossRef]
5. van de Geijn FE, Dolhain RJ, van Rijs W, Hazes JM, de Groot CJ. Mannose-binding lectin genotypes and pre-eclampsia: a case-control study. Hum Immunol 2007;68:888–93. [CrossRef]
6. Christiansen OB, Nielsen HS, Lund M, Steffensen R, Varming K. Mannose-binding lectin-2 genotypes and recurrent late pregnancy losses. Hum Reprod 2009;24:291–9. [CrossRef]
7. Potlukova E, Jiskra J, Freiberger T, Limanova Z, Zivorova D, Malickova K, et al. The production of mannan-binding lectin is dependent upon thyroid hormones regardless of the genotype: a cohort study of 95 patients with autoimmune thyroid disorders. Clin Immunol 2010;136:123–9. [CrossRef]
8. Riis ALD, Hansen TK, Thiel S, Gravholt CH, Gjedde S, Gormsen LC, et al. Thyroid hormone increases mannan-binding lectin levels. Eur J Endocrinol 2005;153:643–9. [CrossRef]
9. Petersen SV, Thiel S, Jensenius JC. The mannan-binding lectin pathway of complement activation: biology and disease association. Mol Immunol 2001;38:133–49. [CrossRef]
10. Valdimarsson H, Stefansson M, Vikingsdottir T, Arason GJ, Koch C, Thiel S, et al. Reconstitution of opsonizing activity by infusion of mannan-binding lectin (MBL) to MBL-deficient humans. Scand J Immunol 1998;48:116–23. [CrossRef]
11. Çorakçı A. Tiroid hastalıklarına yaklaşım. [Article in Turkish]. Turkiye Klinikleri J Endocrin 2004;2:1–3.
12. Dugaw KA JR, Rutledge J. Pediatric reference ranges for TSH, free T4, total T4, total T3 and T3 uptake on the Vitros ECi analyzer. Clin Chem 2001;47:A108.

13. Garred P, Genster N, Pilely K, Bayarri-Olmos R, Rosbjerg A, Ma YJ, et al. A journey through the lectin pathway of complement—MBL and beyond. Immunol Rev 2016;274:74–97. [CrossRef]
14. Worthley DL, Bardy PG, Mullighan CG. Mannose-binding lectin: biology and clinical implications. Intern Med J 2005;35:548–55.
15. Klecha AJ, Barreiro Arcos ML, Frick L, Genaro AM, Cremaschi G. Immune-endocrine Interactions in autoimmune thyroid diseases. Neuroimmunomodulation 2008;15:68–75. [CrossRef]
16. Fiore E, Rago T, Latrofa F, Provenzale MA, Piaggi P, Delitala A, et al. Hashimoto's thyroiditis is associated with papillary thyroid carcinoma: role of TSH and of treatment with l-thyroxine. Endocr Relat Cancer 2011;18:429–37. [CrossRef]
17. Filho C, Rodrigues F, Segat L, Fonseca A, Araujo J, Arahata C, et al. Association of MBL2 gene exon 1 variants with autoimmune thyroid disease in Brazilian patients. Int J Immunogenet 2012;39:357–61. [CrossRef]
18. Potlukova E, Freiberger T, Limanova Z, Jiskra J, Telicka Z, Bartakova J, et al. Association between low levels of Mannan-binding lectin and markers of autoimmune thyroid disease in pregnancy. PloS One 2013;8:e81755. [CrossRef]
19. Karbownik-Lewinska M, Stepniak J, Marcinkowska M, Krygier A, Lewinski A. High normal TSH is associated with lower mannan-binding lectin in women of childbearing age. BMC Endocr Disord 2020;20:1. [CrossRef]
20. Zhao N, Wu J, Xiong S, Zhang L, Lu X, Chen S, et al. Mannan-binding lectin, a serum collectin, suppresses T-cell proliferation via direct interaction with cell surface calreticulin and inhibition of proximal T-cell receptor signaling. FASEB J 2017;31(6):2405–17.