

Dysregulation of complement factor H in juvenile-onset systemic lupus erythematosus patients

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ABSTRACT

Objectives: This study aims to evaluate the expression pattern of factor H in peripheral blood and the frequency of factor H autoantibodies in plasma of juvenile-onset systemic lupus erythematosus (jSLE) patients compared to healthy controls.

Patients and methods: Between March 2019 and October 2019, a total of 30 healthy individuals (3 males, 27 females; mean age: 26±7.4 years; range, 18 to 40 years) and 65 jSLE patients (age of onset ≤16 years) (2 males, 63 females; mean age: 23.4±7 years; range, 15 to 38 years) were included. Factor H expression pattern was examined in blood of all subjects using quantitative real-time polymerase chain reaction and the frequency of factor H autoantibodies was estimated in plasma using enzyme-linked immunosorbent assay.

Results: Factor H expression was significantly downregulated in jSLE patients compared to healthy controls ($p<0.01$). A significant underexpression of factor H was observed in jSLE patients with nephritis compared to those without nephritis ($p<0.03$), while there was no association of factor H expression levels with any of the other clinical and serological features, disease activity or disease damage index of patients. Only 5% of jSLE patients were positive for factor H autoantibodies without any correlations with the clinical data or disease activity of patients.

Conclusion: Our study results suggest that factor H expression can be dysregulated in jSLE patients.

Keywords: Complement factor H, factor H autoantibodies, juvenile-onset systemic lupus erythematosus, nephritis.

Juvenile-onset systemic lupus erythematosus (jSLE) affects up to 20% of all systemic lupus erythematosus (SLE) patients before the age of 16 years. It is characterized by more severe clinical courses and more common complications than adult-onset SLE. Its pathophysiology is different from that of adult-onset SLE, as indicated by the variability in sex and age distribution and also the presence of monogenic etiology of disease in early childhood.¹⁻³ The processes involved in the pathogenesis of SLE include generation of autoantibodies, deposition of immune complex,

defective clearance of apoptotic cells, and chronic activation of complement.⁴

Complement plays a central role in both innate and adaptive defense mechanisms, as it destroys microbes, and controls the inflammatory and adaptive immune response. It is also involved in the disposal of dead cells and misfolded proteins.⁵⁻⁷ The complement system contains soluble and membrane-bound proteins that act through three (classical, alternative and lectin) pathways.⁸ Deficiency of complement components or regulatory complement proteins due to genetic

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deficiencies or a gene copy number variation or the presence of autoantibodies is strongly linked to lupus pathogenesis.⁹⁻¹¹

Chronic activation of complement causes self-tissue damage due to a self-amplifying and aggressive inflammation that should be controlled by some circulating and membrane-associated regulators.^{12,13} Failure to control complement that involves continuous activation or aberrant inhibition results in pathological processes that ends in some autoimmune and inflammatory diseases.^{7,14-18} Several studies have shown that there is a strong correlation between alternative complement pathway activation and disease activity, or flares in SLE patients.¹⁹⁻²¹ Factor H and factor H-like protein 1 are the most important soluble inhibitors of the alternative pathway.²²

Factor H, a soluble 150-kDa glycoprotein, regulates activation of the alternative pathway by inactivating complement component 3b (C3b) mediated by factor I, inhibiting the formation of complement 3 (C3)-convertase (C3bBb) and enhancing its degradation.²³⁻²⁵ It has been demonstrated that factor H contributes to clearance of the apoptotic or necrotic cells by promoting their phagocytosis and inhibits cytokine release of macrophages, such as tumor necrosis factor alpha and interleukin-8.²⁶

Mutations and genetic variations of factor H and genes related to factor H and autoantibodies against factor H could result in its deficiency and dysfunction, dysregulation of the alternative pathway,^{13,27} exhaustion of C3 in plasma and its deposition in the glomeruli,²⁸ and these defects are associated with several diseases such as atypical hemolytic uremic syndrome (aHUS) and SLE.^{13,27-29} In this study, we aimed to evaluate the expression pattern of factor H in peripheral blood and the frequency of factor H autoantibodies in plasma of jSLE patients compared to healthy controls.

PATIENTS AND METHODS

This case-control study was conducted at Kasr Al Ainy Hospital, Cairo University clinics of Rheumatology and Rehabilitation outpatient between March 2019 and October 2019. A total of 30 healthy individuals (3 males, 27 females;

mean age: 26 ± 7.4 years; range, 18 to 40 years) and 65 jSLE patients (age of onset ≤ 16 years) (2 males, 63 females; mean age: 23.4 ± 7 years; range, 15 to 38 years) were included. All patients fulfilled the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE.³⁰ Demographic and cumulative clinical manifestations were recorded, and laboratory investigations included complete blood count (CBC), erythrocyte sedimentation rate (ESR), urine analysis, serum creatinine, liver function tests, serum complement C3 and C4, antinuclear antibody (ANA) and anti-double stranded deoxyribonucleic acid (anti-dsDNA), as well as 24-h urinary protein. An ANA titer of $>1/80$ measured by enzyme-linked immunosorbent assay (ELISA) was considered positive. Disease activity at the last visit was assessed through the Systemic Lupus Erythematosus Disease Activity Index-2K (SLEDAI-2K).³¹ Disease damage index was determined through SLICC Damage Index (SDI)/ American College of Rheumatology (ACR) Damage Index (DI).³² Lupus nephritis (LN) was defined as clinical and laboratory manifestations meeting the American College of Rheumatology (ACR) renal criteria (persistent proteinuria >0.5 g per day (24-h urinary protein) or greater than 3+ dipstick, cellular casts including red cell, hemoglobin, granular, tubular or mixed, and renal biopsy for histopathological examination demonstrating immune complex-mediated glomerulonephritis compatible with LN).³³

Healthy controls were selected from age- and sex-matched individuals. Patients having chronic autoimmune, allergic or neoplastic disease and those having any acute infection within the past 15 days prior to study entry were excluded from the study.

A written informed consent was obtained from each patient. The study protocol was approved by the National Research Centre, Cairo, Egypt Ethics Committee (16/109). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Ribonucleic acid (RNA) extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted and isolated from fresh blood of all individuals of the study populations using the QIAamp RNA Blood

Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. For reverse transcription, RNA was reverse-transcribed to complementary DNA (cDNA) using HiSenScript™ RH[-] cDNA Synthesis Kit (iNtRON Biotechnology, Korea) according to the manufacturer's instructions. Reverse transcription was performed under the following conditions: 5 min at 25°C, 45 min at 45°C, followed by 10 min at 85°C and the resulting cDNA was kept at -80°C until use.

The quantitative real-time PCR was carried out to quantify the expression levels in triplicate of factor H using TaqMan® factor H Assay kits and TaqMan® Universal Master Mix (Applied Biosystems) using 7500 fast real-time PCR system according to the manufacturer's instructions.

The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as endogenous control to normalize the expression levels of factor H. Relative quantification (Rq) was calculated using the $2^{-\Delta\Delta CT}$ threshold cycle method. The ΔCT was determined by subtracting the cycle threshold (CT) values for GAPDH from the CT values for the gene of interest. The qRT-PCR was performed under the following conditions: 2 min at 50°C, 10 min at 95°C, followed by 45 cycles at 95°C for 15 sec and at 60°C for 1 min.^{34,35}

ELISA testing

Plasma complement factor H autoantibodies of all study individuals were determined using the human anti-complement factor H antibody IgG ELISA kit (Bioneovan Co., Ltd., Beijing, China) according to the manufacturer's instructions.

Table 1. Clinical, laboratory, and baseline characteristics of jSLE patients

Characteristic	n	%	Median	IQR
Family history of SLE or other autoimmune diseases	15	23		
Nephritis	52	80		
Constitutional	50	77		
Arthritis	44	68		
Neuropsychiatric	17	26		
Antiphospholipid syndrome	30	46		
Mucocutaneous	19	29		
Serositis	17	26		
Vasculitis			13	20
SLEDAI-2K			10	9
SDI			1.5	3
ANA	57	87.7		
Anti-ds DNA	52	80		
Hypocomplementemia	30	46		
Leukopenia	28	43		
Proteinuria (g/day)			0.8	2.1
ESR (mm/h)			30	30
Histopathological classes of nephritis patients				
Class I	2	3		
Class II	8	12.3		
Class III	14	21.5		
Class IV	18	27.7		
Class V	10	15.4		

jSLE: Juvenile-onset systemic lupus erythematosus; IQR: Interquartile range; SLE: Systemic lupus erythematosus; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index-2K; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; ANA: Antinuclear antibodies; Anti-ds DNA: Anti-double stranded DNA antibodies; Hypocomplementemia: low levels of C3 and/or C4 in blood; Leukopenia: Low white blood cell count (<4000/mm³); Proteinuria: 24 hour urinary protein (levels of protein in urine per day); ESR: Erythrocyte sedimentation rate.

Statistical analysis

Study power analysis and sample size calculation were performed using the G*Power version 3.1 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany).³⁶ Accordingly, a priori power analysis indicated a sample size of 31 subjects in each group for a power of 0.80 and a significance level of 0.05. Statistical analysis was performed using the IBM SPSS version 19.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean \pm standard deviation (SD), median (interquartile range [IQR]) or number and frequency, where applicable. A *p* value of <0.05 was considered statistically significant.

RESULTS

Clinical, laboratory and baseline characteristics of jSLE patients

Of a total of 65 jSLE patients, the mean age at onset and at time of sampling 13 ± 2.7 and 23.4 ± 7 years, respectively. The median disease duration was 120 (IQR: 110) months. Cumulative clinical and laboratory characteristics of the patients

at the time of sampling are summarized in Table 1. Medications taken by lupus patients at time of sampling were as follows: glucocorticoids in 55 (100%), hydroxychloroquine in 45 (69.2%), azathioprine in 22 (33.8%), mycophenolate mofetil in 14 (21.5%), and cyclophosphamide in 10 (15.4%) of patients.

Expression pattern of factor H in peripheral blood of jSLE patients

Our study results showed that factor H expression was significantly downregulated in jSLE patients compared to healthy controls. A 2.9-fold downregulation of factor H was found in patients compared to healthy individuals. Relative quantification (median) of factor H expression was 0.35 at $p<0.01$ in jSLE patients (Figure 1a).

Frequency of factor H autoantibodies in jSLE patients

Our findings revealed that only 5% of our jSLE patients were positive for factor H autoantibodies (Figure 1b) while no autoantibodies were detected in healthy controls. In addition, there were no significant correlations between the factor H autoantibodies and the clinical data or disease activity of patients.

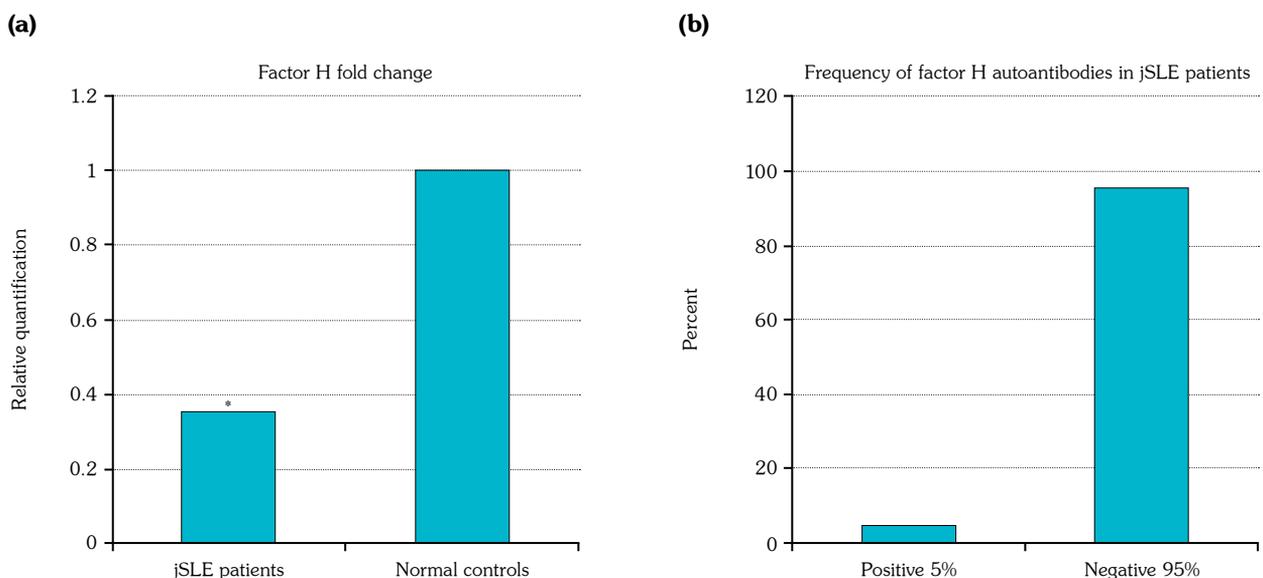


Figure 1. (a) Fold change of factor H in jSLE patients relative to normal controls. Bar graph represents median of fold change. (b) Frequency of factor H autoantibodies in jSLE patients.

* Statistically significant at $p<0.01$ versus controls (by Mann Whitney U test); jSLE: Juvenile-onset systemic lupus erythematosus.

Table 2. Comparison of factor H expression levels between patients based on the presence or absence of different clinical and serological data

	Factor H expression levels	
	Rq median	<i>p</i> *
Nephritis		
Presence	0.16	0.03
Absence	0.5	
Arthritis		
Presence	0.38	0.773
Absence	0.3	
Antiphospholipid		
Presence	0.68	0.739
Absence	0.35	
Mucocutaneous		
Presence	0.32	0.984
Absence	0.37	
Serositis		
Presence	0.30	0.78
Absence	0.38	
Neuropsychiatric		
Presence	0.45	0.41
Absence	0.31	
Anti-dsDNA		
Presence	0.37	0.98
Absence	0.51	
Hypocomplementemia		
Presence	0.27	0.15
Absence	0.53	

* Significant at $p < 0.05$ (by Mann-Whitney U test). Rq: Relative quantification.

Association of factor H with different clinical characteristics and serological data

Our study showed a significant underexpression of factor H in jSLE patients with nephritis compared to those without nephritis (Table 2). On the other hand, there was no association of factor H expression levels with any of the other clinical and serological features (Table 2). Interestingly, there was a significant negative correlation between factor H expression levels and ESR of patients ($r = -0.775$, $p = 0.041$). However, no associations were found between factor H expression levels with SLEDAI-2K, SDI, and proteinuria of patients.

DISCUSSION

In our study, dysregulation of factor H was associated with lupus patients. We found that factor H expression was significantly downregulated in

jSLE patients compared to healthy controls. In addition, a significant underexpression of factor H was observed in jSLE patients with nephritis compared to those without nephritis. Our findings also revealed that only 5% of our jSLE patients were positive for factor H autoantibodies. Downregulation of factor H observed in patients may be due to its overconsumption during regulation of complement activation, generation of autoantibodies against it or a mutation affecting its expression or function.

The significant downregulation of factor H expression in our study agrees with the significant decrease in mean serum factor H level in SLE with LN patients compared to both SLE without renal involvement and control groups, in a study by Wang et al.¹² They also observed an inverse correlation between serum factor H levels and disease activity scores in LN patients and in patients being positive compared to those being negative for ANA and anti-ds-DNA in LN. On the other hand, serum factor H levels increased significantly in the remission phase.

In addition, dysfunctions of factor H through the defective regulation of complement alternative pathway and the aberrant disposal of apoptotic cells were observed in some active LN patients and correlated with their clinical manifestations.²⁹ Furthermore, Bao et al.³⁷ showed that absence of factor H in Murphy Roths Large (MRL)/lymphoproliferation (lpr) mice promoted the development of LN in a markedly accelerated manner.

Furthermore, autoantibodies have been demonstrated to affect the role of complement factors and predispose to inflammatory diseases. It has been documented that autoantibodies produced toward complement factor H is associated with aHUS.¹³ In a study by Foltyn Zadura et al.,¹³ plasma analysis of SLE patients showed positive results in 6.7% for the presence of autoantibodies against factor H. They found that factor H autoantibodies could only be found in active lupus patients without significant association between their titers and disease activity. They also showed a significant high percentage of factor H autoantibodies in rheumatoid arthritis patients from two independent cohorts compared to controls. In contrast, Wang et al.¹² could not

observe any factor H autoantibodies in serum of patients with LN and healthy controls.

The main limitations of our study include the limited number of individuals in the groups. Also, the drugs used might have affected the expression pattern of factor H or its autoantibody positivity rate. However, studying the association between factor H and lupus pathogenesis is of great importance in exploring the etiology of lupus.

In conclusion, factor H expression was significantly downregulated in jSLE patients compared to healthy controls, particularly in patients with nephritis compared to those without nephritis, while there was no association of factor H expression levels with any of the other clinical and serological features, disease activity or disease damage index of patients. Only 5% of jSLE patients were positive for factor H autoantibodies without any correlations with the clinical data or disease activity of patients. Taken together, we conclude that factor H expression can be dysregulated in jSLE patients. However, further well-designed, large-scale studies are needed to confirm these findings.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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