Association of serum IgG glycosylation with disease activity of anti-transcription intermediary factor 1 gamma positive dermatomyositis

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Abstract

Objective

Dermatomyositis (DM) is an idiopathic inflammatory myopathy characterised by the presence of a variety of myositis-specific autoantibodies (MSA) in the circulation. As one of the commonly-detected MSA, anti-transcription intermediary factor 1 gamma (TIF1γ) autoantibody is strongly associated with DM-related malignancy and disease activity. We investigated the glycosylation patterns of serum IgG and to determine the clinical significance of specific glycosylation patterns in patients with anti-TIF1γ positive DM.

Methods

Lectin microarray was used to reveal the glycosylation patterns of serum IgG among 52 DM, 46 disease controls (DC) and 49 healthy controls (HC). Lectin blot was used to further validate the specific alteration of glycosylation. The correlation between glycan levels and clinical features was also evaluated.

Results

The results of lectin microarray showed that compared with the DC group, the DM group had significantly lower glycan levels of mannose and glucose. Compared with the HC group, the glycans levels of GalNAc, galactose, Galβ3GalNAc, sialic acid, and fucose were observed significantly higher in DM group. Lectin blot demonstrated that anti-TIF1γ positive DM had lower glycan level of GlcNAc (recognised by LEL) compared to patients with MSA negative DM, DC, and HC groups. Additionally, the glycan level of GlcNAc was positively associated with manual muscle test ($r=0.547$, $p=0.028$), or negatively associated with IL-6 level ($r=-0.756$, $p=0.049$) and disease activity score ($r=-0.507$, $p=0.045$).

Conclusion

Anti-TIF1γ positive DM presents a unique glycosylation pattern in serum IgG. Considering that the glycan level of GlcNAc reflects the inflammatory state and disease activity, glycosylation has a role in clinical utility by monitoring disease in patients with anti-TIF1γ positive DM.

Key words

dermatomyositis, myositis-specific autoantibodies, anti-transcription intermediary factor 1 gamma autoantibodies, glycosylation, disease activity

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Introduction

Dermatomyositis (DM) is an idiopathic inflammatory myopathy (IIM) characterised by skeletal muscle damage, skin lesions, and other tissue involvements, mainly affecting the lungs, heart, and gastrointestinal tract (1, 2). Clinically amyopathic dermatomyositis (CADM) is a subset of DM with typical skin rash and little or no clinical symptoms of myositis (3). Recent studies have shown that the mortality rate of DM was as high as 51.3 per 1000 person-years (4). Most patients died of infection, interstitial lung disease, and malignancy, causing a high economic burden on patients (5).

Myositis-specific autoantibodies (MSAs) are autoantibodies of IIM, which can be used for diagnosis, prediction of disease-related complications, and risk assessment (6-8). MSA could be detected in the serum of approximately 60% DM patients. Anti-transcription intermediary factor 1 gamma (TIF1γ) autoantibody is one of the most commonly detected MSA in adult DM, with a prevalence range from 4-13% around the world (9-11). Approximately 20% of adult DM patients develop malignancy. Presently, a large number of studies have shown that there is a robust correlation between anti-TIF1γ and malignancy in these patients.TIF1γ autoantigen abnormally expressed by tumour cells could lead to the breakdown of immune tolerance and production of anti-TIF1γ autoantibodies subsequently. For one thing, Anti-TIF1γ can trigger an anti-tumour reaction to destroy tumour cells or even eliminate tumours. For another, anti-TIF1γ could cross-react with TIF1γ antigens in muscle and skin tissues and induce inflammation process (12). Indeed, muscle and skin lesions often appear in anti-TIF1γ positive patients with DM. More recently, Shimizu *et al*. showed that the anti-TIF1γ level can be used as a biomarker of disease activity (13), but the mechanism is not very clear. The role of anti-TIF1γ in the clinical practice of DM needs to be clarified urgently.

Evolving data provide that glycosylation of serum IgG plays an important role in the pathogenesis, clinical diagnosis, and treatment of autoimmune diseases, such as rheumatoid arthritis (RA) (14, 15), systemic lupus erythematosus (SLE) (16, 17), and ANCA-related vasculitis (AAV) (18). Our previous study applied a lectin microarray to explore the glycosylation of IgG4 in IgG4 related diseases (IgG4-RD) and found that specific glycan levels can be used as predictors of multiple organ damage and assessment of disease activity (19). In addition, we also used the microarray to detect the unique glycan levels in patients with primary biliary cholangitis (PBC), and IgG glycosylation patterns were observed to be associated with the positivity of different PBC specific autoantibodies (20). Our researches demonstrated that lectin microarray can provide a powerful tool for the pathogenesis and clinical practices of autoimmune diseases.

The current study used lectin microarray to depict the serum IgG glycosylation patterns of DM, and further validated the glycan levels with lectin blot among patients with DM, SLE, RA, and heathy subjects. We also focused on the alteration in glycosylation in patients with Anti-TIF1γ positive DM and its clinical relevance.

Materials and methods

Subjects and samples

This study included 52 patients diagnosed with adult DM and clinically amyopathic dermatomyositis (CADM) who visited the Peking Union Medical College Hospital (PUMCH) between October 2017 and September 2019. The diagnosis of DM and CADM were performed according to the criteria of Bohan and Peter (21), and definitions of Sontheimer (22), respectively. Patients with juvenile dermatomyositis (JDM) or overlap syndrome were excluded from this study. A diseases control (DC) group of 46 patients included 23 patients diagnosed with SLE and 23 with RA. Besides, 49 healthy volunteers were also included as healthy controls (HC). All serum samples were collected in accordance with the requirements of glycomics researches. No sample was exposed to more than one freeze-thaw cycle before analysis. This study was approved by the Medical Ethics Committee of PUMCH (S-

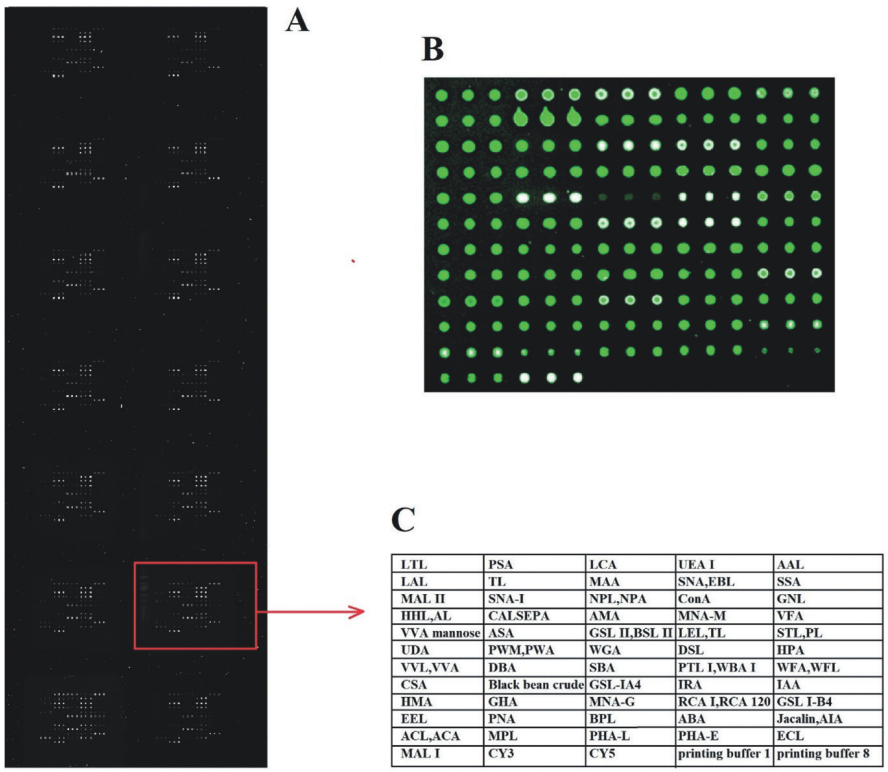
478) according to the Declaration of Helsinki. All participants provided written informed consent.

Clinical characteristics and laboratory features

We collected the clinical data from the medical records, which included the gender, age at enrolment, clinical manifestations, organ involvement (*e.g.*, ILD, malignancy), laboratory data, and disease activity. The diagnosis of ILD was performed by high-resolution chest CT based on the International Consensus Statement of Idiopathic Pulmonary Fibrosis of the American Thoracic Society (23). The assessment tool of disease activity score (DAS) in patients with DM was provided by the International Myositis Assessment and Clinical Studies (IMACS) group (http://www.niehs.nih.gov/research/resources/collab/imacs/main.cfm). The total score of DAS ranges from 0-20, a higher score indicated more active disease (24, 25). Serum MSAs in DM patients were comprehensively evaluated by line immunoassay (Euroimmune, DL 1530-4G), including anti-Mi2α, anti-Mi2β, anti-TIF1γ, anti-MDA5, anti-NXP2, anti-SAE1, anti-Ku, anti-PM-Scl100, anti-PM-Scl75, anti-Jo1, anti-SRP, anti-PL-7. Other laboratory data included levels of creatine kinase (CK), lactate dehydrogenase (LDH), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), as well as IL-6 were also collected.

Lectin microarray

A total of 56 lectins, which were immobilised on the microarray chip (BCBIO Biotech, China), were employed for detecting the glycosylation types of serum IgG in the subjects. Each lectin was spotted in triplicate (Fig. 1). The protocol of lectin microarray manipulation followed our previous study (19). Briefly, serum was diluted at 1:200 and added to a separate block at 4°C overnight. Certain glycosylation pattern of glycoproteins in serum could bind specific lectin. After two washing with PBS, anti-IgG-Cy5 conjugate diluted 1:1000 was hybridised with microarray to track the glycosylation pattern of IgG for 45 min at dark. After three



LTL	PSA	LCA	UEA I	AAL
LAL	TL	MAA	SNA,EBL	SSA
MAL II	SNA-I	NPL,NPA	ConA	GNL
HHL,AL	CALSEPA	AMA	MNA-M	VFA
VVA mannose	ASA	GSL II,BSL II	LEL,TL	STL,PL
UDA	PWM,PWA	WGA	DSL	HPA
VVL,VVA	DBA	SBA	PTL I,WBA I	WFA,WFL
CSA	Black bean crude	GSL-IA4	IRA	IAA
HMA	GHA	MNA-G	RCA I,RCA 120	GSL I-B4
EEL	PNA	BPL	ABA	Jacalin,AIA
ACL,ACA	MPL	PHA-L	PHA-E	ECL
MAL I	CY3	CY5	printing buffer 1	printing buffer 8

Fig. 1. Overview of lectin microarray.
A: the profile of lectin microarray; **B**: quality control results; **C**: spotting information. Each bright spot represents the detection signal intensity of a lectin. The brighter the color represents the higher the ability of the lectin to bind to IgG-specific glycans, and the white spot represents the lectin signal intensity at that point exceeds the upper limit of detection and has reached saturation. The ideal detection is a small amount of saturation points per microarray.

washing, the microarrays were applied to scan by GenePix 4000B BioChip Scanner System (Molecular Devices Co., USA).

Data analysis of lectin microarray

Each signal of the lectin spot is calculated by the signal-to-noise ratio (S/N) (the medium fluorescent intensity of the spot foreground relative to the background). We normalised the S/N data according to the rules of Silver *et al*. (26) to prevent inter-array bias. Fold Change (FC) [FC=log2(group1/group2)] was to characterise the differences in the binding activity of lectin between groups. According to the following rules to define the significant differences: (1) FC>1.3 or <0.769 and (2) p <0.05.

Lectin blot assay

To further verify the difference of LEL binding activity among DM, DC, and HC group, we randomly selected two small cohorts for lectin blot verification: (1) the lectin microarray cohort, which has been detected by lectin microarray, including 4 patients with anti-TIF1γ positive DM, 4 MSA negative DM, 8 DC patients, and 8 HC subjects; (2) a new cohort, 6 anti-TIF1γ positive DM, 6 MSA negative DM, 6 DC and 6 HC. We also selected serum of a healthy volunteer as reference for normalisation of the interblot. Above all, the position of IgG must be defined in the western blot. Serum was separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The gel was transferred onto the polyvinylidene fluoride (PVDF) membrane (Millipore, IPVH00010) using a Bio-Rad SemiDry apparatus (Bio-Rad, USA). The PVDF membrane was blocked with 10× Carbo-Free Blocking Solution (1:10, Vector Laboratories, USASP-5040-125) at room temperature for 2h. After washing with TBST (Tris-Hcl-tween20), the PVDF membrane was incubated with horseradish peroxidase (HRP) labeled anti-human IgG (1:1000, Solarbio, SE068) at room temperature for

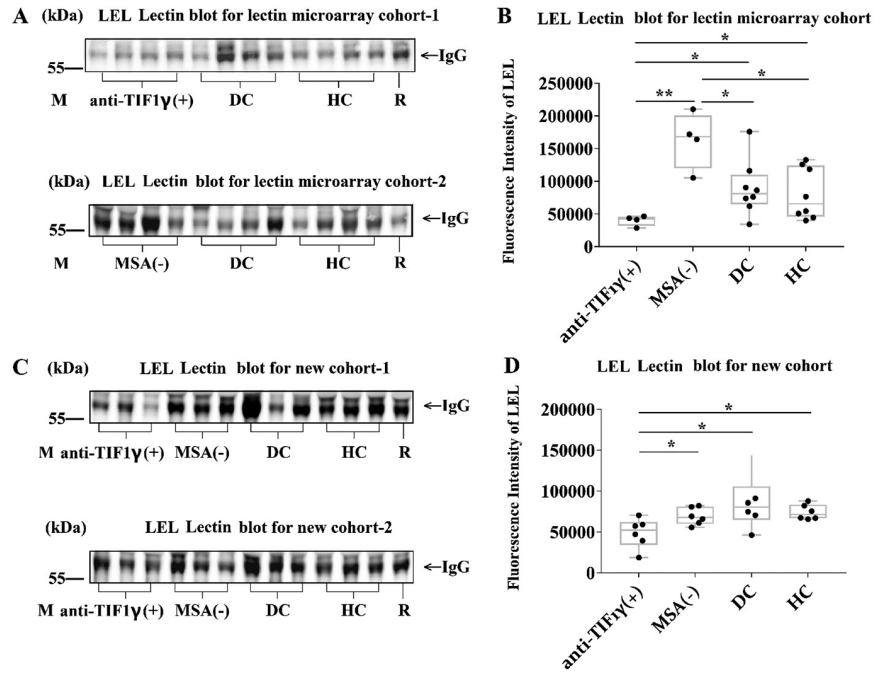


Fig. 2. The results of LEL lectin blot from lectin microarray (A and B) cohort and new cohort (C and D). **A**: Lectin blot of serum for lectin microarray cohort from DM group, DC group, and HC group, using Cy3-LEL. Each lane in the PVDF membrane near the 55kDa band shows a unique single band, indicating that IgG from each sample bind to LEL lectin in different amounts; **B**: Scatter diagram analysis of the normalized data achieved from the lectin blot for lectin microarray cohort; **C**: Lectin blot of serum for the new cohort from DM group, DC group, and HC group, using Cy3-LEL. Each lane in the PVDF membrane near 55kDa band shows a unique single band, indicating that IgG from each sample bind to LEL lectin in different amount; **D**: Scatter diagram analysis of lectin blot for the new cohort. Statistical significance of differences between groups is indicated. M: marker; DM: dermatomyositis; DC: disease controls; HC: healthy controls; R: reference; TIF1γ: transcription intermediary factor 1 gamma; MSA: myositis-specific antibody. *p<0.05, **p<0.01.

1h. The IgG presents distinct bands at specific positions when imaged by Chemiluminescence Imaging Analysis System (Tanon, China) (Fig. 2). Then, lectin blot was applied to the binding of serum to lectin. As above mentioned, after blocking non-specific binding sites, the PVDF hybridised with Cy3 labeled LEL (1:100, Vector Laboratories, L-1170-2) at 4°C overnight in the dark. After washing 3 times, the PVDF membrane was visualised by Typhoon FLA 9500 Fluorescence scanning imaging system (GE Healthcare, USA). The analysis of fluorescence signal intensity was performed by ImageJ software (National Institutes of Health, USA).

Statistical analysis

All statistical analyses were performed using SPSS 24.0 (IBM, USA) and GraphPad Prism 7 (GraphPad Software, USA) was applied to draw scatter diagrams. Continuous variables are represented by mean ± standard deviation (SD), while non-normally distributed variables are represented by median and interquartile range. Student's t-test was used for comparison of fluorescence signal intensity between groups. Pearson correlation coefficient was used to analyse the relationship between the glycan level and clinical features. A p-value <0.05 was considered statistically significant.

Results

Characteristics of serum IgG glycosylation patterns in anti-TIF1γ positive DM patients

The characteristics of all enrolled patients are shown in Table I. We used a lectin microarray which contained 56 lectins that bind to specific glycans to identify the glycosylation patterns of IgG among groups (Supplementary Table S1). Compared with the DC group, the IgG glycosylation of DM presented unique changes: the glycan levels of mannose (recognised by ConA, PSA, MNA-M, and LCA), glucose (recognised by ConA, PSA, and LCA) had significantly lower in the DM group (each<0.05). Moreover, the following glycan levels of IgG were significantly increased in DM patients compared with HC: (1) N-Acetylgalactosamine (GalNAc, recognised by GHA, IAA, and CSA); (2) galactose (recognised by GHA); (3) Galβ3GalNAc (recognised by BPL); (4) sialic acid (recognised by SNA-I); (5) fucose (recognised by UEA I) (each p<0.05). To further reveal the glycosylation patterns of specific antibodies, we divided DM patients into anti-TIF1γ positivity and MSA negativity. The results demonstrated that anti-TIF1γ positive DM patients possessed a significantly lower glycan level of N-Acetylglucosamine (GlcNAc) compared with MSA negative patients (p=0.02) and DC patients (p=0.009). However, this change was not statistically different when compared with the HC group (p=0.14) (Table II).

Decreased glycan level of GlcNAc in anti-TIF1γ positive DM patients

As above, two cohorts were applied to verify the glycan level using lectin blot assay. Initially, IgG in serum presented obvious belts in WB (Supplementary Fig. S1). The heavy and light chains of IgG were located near 55kDa and near 35kDa, respectively. Considering the relative number of heavy chains, we only analyse the bend of heavy chains. The lectin blotting of LEL showed that whether it is the lectin microarray cohort or the new cohort, the LEL binding activity in IgG bands of the anti-TIF1γ positive group was significantly weaker than that of the MSA negative group, DC and HC group (Fig. 2A, 2C). Scatter diagram analysis also showed that anti-TIF1γ positive DM patients possessed the lowest LEL binding activity among four groups (Fig. 2B, 2D) (each p<0.05). These results revealed that the glycan level of GlcNAc indeed decreased in IgG in patients with anti-TIF1γ positive DM patients.

Association between the glycan level of GlcNAc and clinical features and disease activity in anti-TIF1γ positive DM patients

Although results showed that the glycan level of GlcNAc was negatively

Table I. Clinical characteristics and laboratory features of the subjects.

	DM		Diseases controls		Healthy controls (n=49)
	Anti-TIF1γ (+) (n=26)	MSA (-) (n=26)	SLE (n=23)	RA (n=23)	
Age (y)[a]	40.9±10.7	40.4±12.3	30.8±10.5	55.9±13.3	36.9±7.9
Sex (Male/Female)	21/5	22/4	1/22	22/1	20/29
Clinical characteristics and diseases activity, *no*. (%)					
Gottron sign	6 (23.1)	1 (3.8)	0	-	-
Rash	12 (46.2)	0	11 (47.8)	-	-
Mechanics hand	1 (3.8)	0	0	-	-
Arthritis	3 (11.5)	0	3 (13)	23 (100)	-
Malignancy	5 (19.2)	0	0	-	-
ILD	7 (26.9)	2 (7.7)	-	10 (43.5)	-
MMT[b]	80 (64.5,80)	80 (80, 80)			
DAS (range 0-20)	7.1±6.2[a]	0 (0, 2)	-	-	
SLEDAI[a]	-	-	12.7±8.9	-	-
DAS28-ESR[a]	-	-	-	4.4±1.9	-
Laboratory features[a]					
CK (U/L)	80 (49, 131.5)[b]	81.5 (50, 150.3)[b]	23.9±3.3	-	-
LDH (U/L)	293.6±185.8	216.8±58.1	273.8±241.4	220.9±36.6	-
IgG (g/L)	12.2±6	12.8±2.5	18.5±7.2	-	-
IgA (g/L)	1.9±1.2	2.6±1.4	2.8±1.4	-	-
IgM (g/L)	1.2±0.6	1.1±0.6	1.2±1.1	-	-
ESR (mm)	10 (6, 17)[b]	7 (4.3, 12.5)[b]	34.1±29.4	34 (14, 86)[b]	-
CRP (mg/L)	1.21 (0.41, 2.59)[b]	0.9 (0.2, 2.7)[b]	-	47.1 (6, 142.8)[b]	-
IL-6 (U/L)	8±7.6	4±2.3	-	-	-

DM: dermatomyositis; TIF1γ: transcriptional intermediary factor 1 gamma; MSA: myositis-specific autoantibodies; SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; ILD: interstitial lung disease; MMT: manual muscle test; DAS: disease activity score; SLEDAI: systemic lupus erythematosus disease activity index; CK: creatine kinase; LDH: lactate dehydrogenase; IgG: immunoglobulin G; IgA: immunoglobulin A; IgM: immunoglobulin M; ESR: erythrocyte sedimentation rate; CRP: C reactive protein.
[a]Data are expressed mean±SD. [b]Data are expressed as median and interquartile range.

Table II. Comparison of IgG glycosylation patterns in dermatomyositis (DM), diseases controls (DC) and healthy controls (HC) using lectin microarray.

Lectin	Monosaccharide specificity	Normalised fluorescence intensity (mean ± SD)				Fold change		
		DM		DC	HC	Anti-TIF1γ (+)	Anti-TIF1γ (+)	Anti-TIF1γ (+)
		Anti-TIF1γ (+)	MSA (-)			*vs*. MSA (-)	*vs*. DC	*vs*. HC
LEL	GlcNAc	3.25 ± 1.49	5.78 ± 4.08	5.99 ± 4.86	4.62 ± 3.65	0.56*	0.54**	0.7
ConA	Man, Glc	11.94 ± 9.83	9.36 ± 4.69	19.72 ± 12.68	12.53 ± 5.43	1.28	0.61**	0.95
PSA	Man, Glc	4.97 ± 2.77	4.54 ± 1.71	7.24 ± 3.8	5.19 ± 2.98	1.09	0.69**	0.96
LCA	Man, Glc	4.95 ± 2.28	4.55 ± 1.34	7.03 ± 3.66	4.07 ± 1.92	1.09	0.7**	1.21
MNA-M	Man	4.5 ± 1.67	4.33 ± 2.86	6.41 ± 3.66	4.15 ± 1.68	1.04	0.7*	1.09
Jacalin	Galβ3GalNAc	2.33 ± 1.06	2.66 ± 1.98	3.18 ± 1.6	2.34 ± 1.13	0.88	0.73*	0.99
GHA	Gal, GalNAc	5.38 ± 3.71	5.66 ± 4.39	5.69 ± 3.73	2.74 ± 1.6	0.95	0.95	1.96***
BPL	Galβ3GalNAc	4.89 ± 2.58	6.35 ± 5.93	6.07 ± 4.52	3.12 ± 1.83	0.77	0.81	1.57**
IAA	GalNAc	3.06 ± 1.57	2.84 ± 1.23	2.79 ± 1.59	2.01 ± 0.93	1.08	1.1	1.52**
SNA-I	α-2,6 sialic acid	9.05 ± 4.81	9.73 ± 7.7	10.18 ± 5.98	5.95 ± 2.59	0.93	0.89	1.52**
CSA	GalNAc	3.33 ± 2.51	3.57 ± 3.04	3.62 ± 3.11	2.21 ± 1.47	0.93	0.92	1.51*
UEA I	Fuc	2.3 ± 1.03	2.69 ± 2.72	3 ± 2.43	1.65 ± 1.15	0.86	0.77	1.39*

TIF1γ: transcription intermediary factor 1 gamma; MSA: myositis-specific autoantibodies; LEL: lycopersicon esculentum lectin; ConA: concanavalin A Lectin; PSA: pisum sativum agglutinin; LCA: Lens Culinaris Agglutinin; MNA-M: Morniga M Lectin (black elderberry); GHA: Glechoma hederacea Lectin (ground ivy); BPL: Bauhinia Purpurea Lectin; IAA: Iberis amara Lectin; SNA-I: Sambucus nigra (Elderberry Bark); CSA: Cytisus sessilifolius Lectin (Portugal Broom); UEAI: Ulex europaeus agglutinin I; GlcNAc: N-Acetylglucosamine; Man: Mannose; Glc: Glucose; GalNAc: N-Acetylgalactosamine; Gal: galactose; Fuc: Fucose. *p<0.05; **p<0.01; ***p<0.001.

associated with serum IL-6 levels (r=-0.756, p<0.05) and DAS (r=-0.507, p<0.05) in anti-TIF1γ positive DM patients, these correlations were not found in that of other laboratory features, including CK, LDH, IgG, IgM, IgA, ESR and CRP (each p>0.05). However, neither any clinical feature nor DAS was correlated with the glycan level of GlcNAc in patients with MSA negative DM (each p>0.05) (Table III).

Table III. Correlation between the glycan level of GlcNAc and laboratory features in DM patients.

	Anti-TIF1γ (+)		MSA (-)	
	r	*p*	r	*p*
CK	-0.15	0.565	0.179	0.54
LDH	-0.306	0.233	0.05	0.871
IgG	-0.379	0.224	-0.1	0.77
IgA	-0.518	0.085	0.126	0.712
IgM	-0.296	0.35	-0.476	0.139
ESR	-0.293	0.289	-0.001	0.998
CRP	-0.404	0.152	0.098	0.761
IL-6	-0.756	0.049	0.494	0.398
MMT	0.547	0.028	0.071	0.778
DAS	-0.507	0.045	-0.095	0.717

GlcNAc: N-Acetylglucosamine; DM: dermatomyositis; TIF1γ: transcriptional intermediary factor 1 gamma; MSA: myositis-specific autoantibodies; CK: creatine kinase; LDH: lactate dehydrogenase; IgG: immunoglobulin G; IgA: immunoglobulin A; IgM: immunoglobulin M; ESR: erythrocyte sedimentation rate; CRP: C reactive protein; MMT: manual muscle test; DAS: disease activity score.

Discussion

This study demonstrated that the glycosylation patterns of serum IgG are different in DM patients compared with systematic autoimmune diseases and healthy volunteers, and the glycan level of GlcNAc was significantly lower in anti-TIF1γ positive DM. Our results also reflect an association between the level of GlcNAc in anti-TIF1γ positive DM patients and their inflammation states and disease activity. Lectin microarray could serve as a sensitive and convenient approach in measuring IgG glycosylation for clinical practice or research.

The role of IgG glycosylation in immune diseases has been extensively elucidated. Studies have shown that the presence or absence of specific glycans can alter the distinct effector functions of IgG (27, 28). In clinical settings, the traditional experimental methods for analysing glycosylation based on mass spectrometry hindered their development due to complicated and time-consuming sample purification process. Emerging high-throughput, high-speed, and high-specificity approaches such as lectin microarray can be used as an alternative option for clinical utility in glycosylation investigations (29). Our results showed distinct level of serum IgG glycosylation patterns in DM patients. The glycan levels of galactose, GalNAc, sialic acid, and fucose, were significantly higher in patients with DM than that in the HC group. High galactosylation has been proven to achieve anti-inflammatory function through increased complement-dependent cytotoxicity (CDC) activity by improving C1q binding (30, 31). Meanwhile, Gillian *et al*. recently reported that additional terminal-sialic acid increased this effect on the classical complement activation pathway (32). Besides, highly galactosylated IgG promoted the association between FcγRIIb and dectin-1 to attain anti-inflammatory properties (33). Previous researches also shown that the level of galactosylation in IIM, especially in patients with anti-synthetase syndrome (ASS) was significantly higher than that of healthy controls. An increase in IgG-galactosylation has been shown to have pro-inflammatory effects and is associated with diseases activity or progression in AIDs such as SLE, RA, and multiple sclerosis (17, 29, 34-36). However, for certain disease-specific autoantibodies such as ACPA in RA and PR3-ANCA in AAV, their levels of galactosylation were increased and associated with disease remission or relapse (15, 18). It is worth noting that our results were different from Fernandes *et al*. (37) and Perdivara *et al*. (38). The reason that causes the inconsistency with previous researches in our study may be due to the distinct inclusion criteria of patients. Patients diagnosed only with DM were included in our study, which ensure the homogeneity of subject.

To further analyse the effect of MSA on IgG glycosylation, we divided patients into anti-TIF1γ positive DM group and MSA negative DM group. The lectin microarray results showed that anti-TIF1γ positive DM had lower GlcNAc levels than that in MSA negative, DC, and HC groups. Subsequent lectin blot results for two cohorts verified the results. Studies have shown that the high-bisecting GlcNAc structure enhances the binding between antibodies and FcγRIII, thereby promoting the ADCC effect of peripheral blood target cells (39, 40). In addition, the terminal GlcNAc residues bind to the mannose receptors, thus selectively clearing the antibodies from circulation to decrease the half-life and attenuated effect function of IgG (41). Our results revealed that anti-TIF1γ positive DM patients may have lower ADCC activity.

Our results also showed that the GlcNAc level of anti-TIF1γ positive DM is negatively correlated with IL-6 and DAS, and positively correlated with MMT. As a marker for IIM diagnosis and classification, MSA is relatively stable in serum. However, MSA significant decrease over time during B cell depletion therapy, and anti-TIF1γ is correlated with indicators of disease activity, such as MMT and HAQ (42). Physiologically, TIF1γ antigen is involved in the TGF-β signalling pathway, which regulate cell proliferation, differentiation, migration, and other physiological functions in hematopoietic and non-hematopoietic systems (12, 43). For one thing, TIF1γ promotes classically activated macrophages, also known as M1 macrophages, to release inflammatory cytokines, such as IL-6, TNF-α (44, 45); for another, as a branch of TGF-β signalling, TIF1γ has a role in the proinflammatory function of Th17 cells. As evidence, Tanaka *et al*. (46, 47) confirmed that TIF1γ deficiency reduced Th17 cells differentiation in *in vitro* via decreased IL-17 expression, increased IL-10 production and downregulated CCR6, the hallmark chemokine receptor of Th17 cells. Moreover, TGF-β combined with IL-6 promotes the proliferation of IL-17 and further improved the inflammatory process (47). In view of these find-

ings, we speculate that GlcNAc could exert functional effects through reducing IgG ADCC activity, attenuating the M1 macrophages activation function and regulating the differentiation of immune effector cells in patients with anti-TIF1γ positive DM. Our results suggested that the level of serum IgG GlcNAc reflected inflammation status and disease activity in anti-TIF1γ positive DM. From a clinical point of view, the glycosylation pattern of GlcNAc can be used as an assessing index of disease activity. Thus, the findings of this study provide glycomics evidence for the pathogenesis in anti-TIF1γ positive DM and help improve the clinical management of DM in daily practice and promote the discovery of glycosylation biomarkers as well as therapeutic targets.

This study has several limitations. Methodologically, specific lectin can only bind certain glycans which only a rough characterisation of glycan profile, this limitation can by only partially corrected by the contemporary use of many lectins in a multiarray. It is necessary to correctly establish glycan profile by mass spectrometry. Second, the study has a retrospective observational design in which the patient background could not be adjusted due to the small number of patients and may not represent the real world of DM. Third, glycosylation status could alter in accordance with gender, age, or genetic background (4, 38), while our results only characterised the trend of DM patients in the Chinese population. Fourth, as mentioned previously, there might be distinct differences in patterns and function effects of glycosylation between total IgG and specific-disease antibodies in serum. Therefore, it is necessary to further clarify the role of glycosylation in disease pathogenesis using purified anti-TIF1γ antibodies.

In this study, lectin microarrays were applied to identify the unique glycosylation patterns in patients with DM. Glycan levels of GalNAc, galactose, Galβ3GalNAc, sialic acid, and fucose were observed significantly higher in DM group. Level of GlcNAc was lowest in anti-TIF1γ autoantibodies positive patients, which was also correlated with IL-6 level, MMT, and DAS. Altered IgG Glycosylation could be used as an index of disease activity in patients with anti-TIF1γ positive DM patients.

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