JPGM
Journal of Postgraduate Medicine

Open access journal indexed with Index Medicus & EMBASE

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BRIEF REPORT

Year : 2017 | **Volume** : 63 | **Issue** : 4 | **Page** : 257-261

Association of anti-CLIC2 and anti-HMGB1 autoantibodies with higher disease activity in systemic lupus erythematosus patients

CS Syahidatulamali[1], WG Wan Syamimee[2], Y Nor Azwany[3], KK Wong[1], CH Che Maraina[1],

[1] Department of Immunology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia

[2] Department of Medical, School of Medical Sciences, Universiti Sains , Health Campus, Kelantan, Malaysia

[3] Department of Community Medicine, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kelantan, Malaysia

Correspondence Address:
C H Che Maraina
Department of Immunology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kelantan
Malaysia

Abstract

Background: Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by numerous autoantibodies. In this study, we investigated the presence of anti-chloride intracellular channel 2 (anti-CLIC2) and anti-high mobility group box 1 (anti-HMGB1) autoantibodies in SLE patients (*n* = 43) versus healthy controls ([HCs] *n* = 43), and their association with serological parameters (antinuclear antibody [ANA], anti-double-stranded DNA [anti-dsDNA], and C-reactive protein [CRP]) and disease activity using Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score (active or inactive). **Settings and Design:** Case–control study at Rheumatology Clinic of Universiti Sains Malaysia Hospital. **Subjects and Methods:** The sera of SLE patients and HCs were tested for the presence of anti-CLIC2 and anti-HMGB1 autoantibodies using human recombinant proteins and ELISA methodologies. Other serological parameters were evaluated according to routine procedures, and patients' demographic and clinical data were obtained. **Statistical Analysis:** Mann–Whitney U-test, Chi-square test, Fisher's exact test, and receiver operating characteristic analysis. **Results:** Anti-CLIC2 autoantibody levels were significantly higher in SLE patients compared to HCs (*P* = 0.0035), whereas anti-HMGB1 autoantibody levels were not significantly elevated (*P* = 0.7702). Anti-CLIC2 and anti-HMGB1 autoantibody levels were not associated with ANA pattern, anti-dsDNA, and CRP. Interestingly, SLEDAI score (≥6) was associated with anti-CLIC2 (*P* = 0.0046) and with anti-HMGB1 (*P* = 0.0091) autoantibody levels. **Conclusion:** Our findings support the potential of using anti-CLIC2 autoantibodies as a novel biomarker for SLE patients. Both anti-CLIC2 and anti-HMGB1 autoantibody levels demonstrated potential in monitoring SLE disease activity.

How to cite this article:
Syahidatulamali C S, Wan Syamimee W G, Azwany Y N, Wong K K, Che Maraina C H. Association of anti-CLIC2 and anti-HMGB1 autoantibodies with higher disease activity in systemic lupus erythematosus patients.J Postgrad Med 2017;63:257-261

How to cite this URL:
Syahidatulamali C S, Wan Syamimee W G, Azwany Y N, Wong K K, Che Maraina C H. Association of anti-CLIC2 and anti-HMGB1 autoantibodies with higher disease activity in systemic lupus erythematosus patients. J Postgrad Med [serial online] 2017 [cited 2017 Dec 11];63:257-261
Available from: http://www.jpgmonline.com/text.asp?2017/63/4/257/213899

Full Text

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease involving multiple organs.[1] Autoantibodies presence is a hallmark feature of SLE including antinuclear antibodies (ANAs) and anti-double-stranded DNA (anti-dsDNA) antibodies.[2]

Novel autoantibodies have recently been uncovered in SLE patients. Chloride intracellular channel 2 (CLIC2) protein is a member of the glutathione transferase and CLIC2 mutation is associated with atrial fibrillation and seizures,[3] suggesting that deregulation of CLIC2 is associated with autoimmune diseases. Recently, anti-CLIC2 autoantibody levels were found to be elevated in SLE patients (n = 31/110; 28.2%).[4]

High mobility group box 1 (HMGB1) is a nonhistone nuclear protein involved in the pathogenesis of SLE through the induction of anti-dsDNA antibodies.[5] Anti-HMGB1 autoantibodies are present in SLE patients and associated with lupus disease activity.[6],[7]

In this study, we set out to validate the presence of anti-CLIC2 and anti-HMGB1 autoantibodies in a local cohort of SLE patients (n = 43) versus healthy controls (HCs) (n = 43).

Subjects and Methods

Ethics

The study convention was approved by the Institutional Ethics Board, and all the participants filled the standardized consent form.

Design and site

This comparative case–control study was carried out at the Rheumatology Clinic of Universiti Sains Malaysia Hospital (HUSM).

Subjects

We recruited 43 SLE patients attending rheumatology clinic at HUSM and 43 HCs. Participant's recruitment was conducted according to the following inclusion criteria:

Age between 18 and 60 years oldAdult SLE patients who fulfilled the ACR criteria for the diagnosis of SLE [8]Healthy individual as controls without medical illness and history of autoimmune diseaseNonpregnant patients and women.

Ten milliliters of blood was taken from SLE patients or HCs. Patients' demographic and clinical presentation data were obtained from the unit records of HUSM, and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score was recorded by the attending clinician according to standardized criteria.[9]

Detection of antinuclear antibody and double-stranded DNA

Semi-quantitative measurement for ANA in human serum was conducted using the Fluoro Hanapa Test kit (MBL Aichi, Japan) according to manufacturer's protocols. Anti-dsDNA antibodies were detected Antibody Test kit (SCIMEDX Corporation, Denville, NJ, USA) according to manufacturer's i conjugated goat anti-human antibody (SCIMEDX Corporation) was used as the secondary tests, and visual inspection was conducted with a fluorescent microscope.

Detection of C-reactive protein

C-reactive protein (CRP) Direct Latex (VEDALAB, Alencon, France) was used to determine

according to manufacturer's protocols. The presence (positive detection) or absence (negative) of agglutination was observed.

Detection of anti-chloride intracellular channel 2 and anti-high mobility group box 1 autoantibodies

CLIC2 (TP304727) and HMGB1 (TP720309) recombinant proteins were purchased from OriGene (Rockville, MD, USA). ELISA methodologies were conducted according to previous studies with slight modifications.[4],[7] In brief, 1 μg/mL of CLIC2 or HMGB1 recombinant protein was diluted in phosphate-buffered saline (PBS), and 50 μL of each protein was loaded in 96-well ELISA plate in duplicate and left to coat the wells overnight at 4°C. The solutions were subsequently discarded, and wells were washed with three changes of PBS-Tween (PBST). Blocking solution (5% Marvel in PBST) was added into each well for 2 h at room temperature (RT). The wells were washed with 50 μL PBST before being loaded with 100 μL of PBS. Serum samples diluted at 1:100 for CLIC2 and 1:50 for HMGB1 in PBS were loaded and incubated for 2 h at RT before washing with PBST. Horseradish peroxidase-conjugated rabbit anti-human IgG (Dako, Glostrup, Denmark) was added and incubated for 1 h at RT. The wells were subsequently washed with PBST and loaded with 100 μL of ABTS substrate solution (Roche, Sandhofer Street, Mannheim, Denmark) for 15 min incubation at RT. Absorbance was measured at 405 nm using ELISA plate reader, and the anti-CLIC2 and anti-HMGB1 autoantibody levels were expressed in optical density (OD) values.

Statistical analysis

Differences between two groups were calculated using Mann–Whitney U-test (GraphPad Prism version 6; La Jolla, CA, USA), Chi-square test, and Fisher's exact test (SPSS Statistics version 22; Chicago, IL, USA) for continuous and categorical variables, respectively. Receiver operating characteristic (ROC) curves were constructed, according to the OD values of anti-CLIC2 and anti-HMGB1 autoantibodies detection, to calculate Youden index (J) according to the formula J = sensitivity + specificity − 1 as described previously.[10]

Results

Demographic, clinical symptoms, and laboratory investigations

In this cohort with a female predominance (n = 41/43; 95.3%), the mean and median ages were 33.5 and 32 years old, whereas in controls, 33 and 27 years old, respectively. The most common symptom observed was arthritis (n = 19; 44.2%), followed by rashes (n = 14; 32.6%), alopecia, fever, and renal disorder (n = 6 each; 14%) and other symptoms as well as laboratory investigations are summarized in [Table 1].{Table 1}

The most common ANA pattern observed was homogeneous pattern (n = 16; 37.2%), followed by speckled (n = 10; 23.3%), centromere (n = 7; 16.3%), nucleolar (n = 5; 11.6), peripheral staining (n = 2; 4.7%), and homogenous cytoplasmic staining, mitochondrial staining, and nuclear dot staining (n = 1 each; 2.3%).

Levels of anti-chloride intracellular channel 2 and anti-high mobility group box 1 autoantibodies

SLE patients showed significantly increased anti-CLIC2 autoantibody levels compared to HCs (P = 0.0035), but the anti-HMGB1 autoantibody levels did not significantly differ between SLE patients and HCs (P = 0.7702) when compared in terms of continuous variables [Figure 1].{Figure 1}

In terms of categorical variables, the optimal cutoff OD value to define positivity for anti-CLIC2 or anti-HMGB1 autoantibodies was determined through Youden index generated from ROC curves. Sampl
Youden index value was adopted to separate the group into positive or negative for both a
value of anti-CLIC2 (OD: 0.2240; sensitivity = 37.21%; specificity = 93.02%) and anti-HI
levels (OD: 0.1315; sensitivity = 81.4%; specificity = 27.91%) were most optimal to cate
into positive or negative. Based on these cutoffs, we found that our cohort of SLE patients
CLIC2 autoantibody levels (P = 0.0007) but not anti-HMGB1 autoantibody levels (P = 0.3(
3/43) and 72% (n = 31/43) showed the presence of anti-CLIC2 and anti-HMGB1 autoantil

Association of anti-chloride intracellular channel 2 and anti-high mobility group box 1 autoantibodies with antinuclear antibody pattern, anti-double-stranded DNA antibodies, C-reactive protein, and disease activity (Systemic Lupus Erythematosus Disease Activity Index score)

There was no association seen between anti-CLIC2 or anti-HMGB1 autoantibody levels with ANA staining pattern, anti-dsDNA, and CRP [Table 2]. However, higher SLEDAI score (≥6) was significantly associated with increased levels of anti-CLIC2 (P = 0.0046) and anti-HMGB1 (P = 0.0091) autoantibody levels.{Table 2}

Discussion

In this study, we examined the levels of anti-CLIC2 and anti-HMGB1 autoantibodies in a local cohort of SLE patients. Huang et al. identified CLIC2 as a novel autoantigen in SLE patients (n = 30) through screening with protein arrays containing more than 5,000 recombinant proteins and validated by ELISA (n = 110), in which 28.2% (n = 31/110) showed increased anti-CLIC2 autoantibody levels compared to controls (n = 120).[4] These observations were similar with those seen in our study where 37.2% of SLE patients (n = 16/43) versus 7% HCs (n = 3/43) were positive for anti-CLIC2 autoantibodies using modified ELISA methodologies for the detection of CLIC2 recombinant proteins.

In our study no significant difference between SLE patients and HCs was observed in terms of anti-HMGB1 autoantibody levels. This was in contrast with the previous study [6] that reported anti-HMGB1 autoantibody levels being elevated in SLE patients (n = 24/47; 51%), particularly in those with active renal involvement, and also with the study done by Wirestam et al., wherein anti-HMGB1 autoantibody levels were elevated in 23% (n = 43/188) of SLE patients.[11] Previous studies have reported that anti-HMGB1 autoantibody levels correlated with the pathogenesis of kidney diseases in patients with lupus nephritis and vasculitis with renal involvement.[12],[13],[14] Majority of the SLE patients in our cohort did not experience active renal involvement (86%), and this might partially explain the lack of significant anti-HMGB1 autoantibody levels in our study. Nevertheless, anti-HMGB1 autoantibody levels were positively associated with a higher SLEDAI score in our cohort of SLE patients, suggesting that HMGB1 plays a role in disease activity and is involved in causing organ damage. In our study anti-HMGB1 autoantibody levels were positively associated with higher SLEDAI score, a finding similar to that reported in earlier studies.[6],[7],[13],[15] In our study anti-CLIC2 autoantibody levels were likewise associated with higher SLEDAI score, which is similar to the observations reported by Huang et al.[4]

In our study anti-CLIC2 and anti-HMGB1 autoantibody levels had no association with ANA staining pattern, anti-dsDNA antibodies, or CRP, which was comparable with previous observations wherein no association was found between anti-dsDNA antibodies and anti-CLCI2 autoantibody levels.[4] However in contrast to previous observations, in our study anti-HMGB1 autoantibody levels demonstrated a positive correlation with anti-dsDNA levels.[7],[11]

Conclusion

Our findings support the potential utility of measuring anti-CLIC2 and anti-HMGB1 autoantibody levels as novel biomarkers for monitoring disease activity in SLE patients. Further studies of anti-CLIC2 and anti-HMGB1 autoantibody levels in other cohorts of SLE patients are warranted.

Acknowledgments

We would like to thank staff and trainees of Department of Immunology and Department
Medical Science, Universiti Sains Malaysia. This study was supported by Short-term Grant
Malaysia (304/PPSP/61313060).

Financial support and sponsorship

The study was supported by Short-term Grant (304/PPSP/61313060), Universiti Sains Ma

Conflicts of interest

There are no conflicts of interest.

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