Incidence of some autoimmune diseases in Basrah Province

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Abstract---All the patients diagnosed as having autoimmune disease by the clinical examiner and confirmed the diagnosis by laboratory results. Serum samples obtained from patient's blood and tested for the presence of specific autoantibodies using commercial ELISA kits. Out of 342 patients, 240(70.2%) were diagnosed with celiac disease, 94(27.5%) with Rheumatoid arthritis and 8(2.3%) with Systemic lupus erythematosus (SLE). Results of ELISA test indicated statistically significant increased level of antibodies specific to anti-gliadin (IgA and IgG) and anti-tTg (IgA and IgG) antibodies in patients with celiac disease (p-value< 0.001). Results indicated statistically significant level of (ANA) and (Anti-ccp) in individuals suffering from rheumatoid arthritis. Regarding to (anti-dsDNA), (anti-cardiolipin) and (anti-phospholipids), a statistically significant increased levels detected in individuals with SLE.

Keywords---Rheumatoid arthritis, anti-dsDNA, anti-phospholipids.

Introduction

Autoimmune diseases affect very low percentage of population and a target organs and autoimmune destruction observed. Although treatment is available, many are inadequate (1). The immune responses in these diseases generate after activation of immune cells, mostly T cells (2). Recent genetic studies described many loci considered as risk factors for autoimmunity (3). A condition in which gluten

sensitization present called celiac disease (CD), the most affected individuals are genetically susceptible children (4). This disease presents with many gastrointestinal tract problems. It showed that certain HLA alleles related to the disease (5). Regional distribution play a role in distribution of this disease and certain regions have the most incidence (6) (7). Gliadin is a dietary substrate for transglutaminase which linked to T-cell epitopes on gliadin and leads to production of anti-transglutaminase (anti-tTg) autoantibodies (8) (9). Detection of these autoantibodies by ELISA assays considered good choice for monitoring patient with this disease (10) (11). A recent study demonstrated that a new, non-invasive blood test for intestinal damage in patients with celiac disease might be possible. Although future validation will be required before it can be implemented in the clinic, the test could eliminate the requirement for biopsies in the rural or indigent areas (12). An autoimmune disease called rheumatoid arthritis (RA) affects joints leading to chronic inflammation (13). In individuals complaining from (RA) a specific type of antibodies detected, and called anti-cyclic citrullinated peptides (anti-ccp) (14)(15). Various kinds of medication are used in RA patients such as Tocilizumab and TNF-α inhibitors (16). Patients suffering from systemic lupus erythematosus (SLE) produce autoantibodies causing serious health problems. Many drugs are used to target immune cells involved in this disease (17). Individuals with this disease showed to have specific identified loci considered as risk factors (18). Anti-dsDNA antibodies are highly diagnostic of SLE by using ELISA assays (19). Studying immunological role in inducing pathology of these autoimmune diseases, agents that inhibit these targets have been focus of intense research. A number of other therapeutic targets are also actively being investigated, including therapies that target the activation of complement and cytokines (20).

Materials and Methods

This study done during (2012 – 2016). A total of 342 patients; 92(26.9%) males and 250(73.1%) females attending Al-Sadder Teaching Hospital, involved, their mean age, Std. Deviation (34.73, ±14.8). Additional documented data collected from Immunology laboratory in Al-Sadder teaching hospital and from personal communications. Confirmation of infection with disease was done clinically before taking samples from patients. Serum samples were obtained from patients blood and tested for the presence of specific auto-antibodies using AESKULISA commercial ELISA kits (Aesku. diagnostic, GmbH, Germany).

ELISA kits used for diagnosis of Celiac disease:
Anti-gliadin IgA, Anti-gliadin IgG, Anti-tissue transglutaminase (anti-tTg IgA), Anti-tissue transglutaminase (anti-tTg IgG).

ELISA kits used for diagnosis of Rheumatoid arthritis:
Anti-nuclear antibodies (anti-ANA), Anti-double stranded antibodies (anti-dsDNA) and Anti-cyclic citrullinated protein antibodies (anti-ccp).

ELISA kits used for diagnosis of (SLE):
Anti-nuclear antibodies (anti-ANA), Anti-double stranded antibodies (anti-dsDNA), Anti-cardiolipin antibodies and Anti-phospholipids antibodies.

ELISA protocol:

100µl of each patient's diluted serum was pipette into the micro wells, then 100µl cut-off calibrator, negative and positive controls were pipette into micro wells. Incubated for 30 minutes at 20-32°C then washed 3X with 300 µl washing buffer (diluted 1:50). 100 µl conjugate was pipette into each well and incubated for 30 minutes then washed 3X. 100µl TMB substrate was pipette into each well and incubated for 30 minutes. 100µl stop solution was pipette into each well and incubated 5 minutes minimum. Read absorbance at 450 nm within 30 minutes.

Statistical Analysis
Data was analyzed by using SPSS.

Results

Patients regarding to the type of autoimmune disease:

Results showed in (Figure.1) indicated that out of 342 patients with autoimmune diseases, 240(70.2%) were diagnosed clinically and according to results of ELISA test as celiac disease patients, 94(27.5%) were diagnosed as rheumatoid arthritis patients and 8(2.3%) were diagnosed as SLE patients.

Distribution of patients with autoimmune diseases according to gender:

Results showed that out of 342 patients with autoimmune diseases, 92(26.9) were males and 250(73.1) were females (Figure.2). Results showed from total 240 patients with celiac disease, 68(28.33%) were males and 172(71.67%) were females. For rheumatoid arthritis patients, results indicated that out of 94 patients, 20(21.28%) were males and 74(78.72%) were females. Out of 8 patients with SLE, males 4(50%) and females 4(50%). Difference regarding to gender was statistically significant in the three diseases.

Results of ELISA test in patients with celiac disease:

Results in (Table.2) indicated that out of 240 clinically diagnosed celiac disease patients, 210(87.5%) showed positive ELISA test for presence of IgA and IgG (anti-gliadin) and 30(12.5%) showed negative ELISA test. That means the association between clinical diagnosis and ELISA results was significant with P-value= $P < 0.001$. Out of 240 clinically diagnosed celiac disease patients, 180(75%) showed positive ELISA test for presence of anti-tTg IgA antibodies and 60(25%) showed negative ELISA test for these antibodies, the association was significant between confirmed clinical diagnosis and positive ELISA test with $P < 0.001$. Also results indicated that out of 240 clinically diagnosed celiac disease patients, 220(91.67%) showed positive ELISA test for presence of anti-tTg IgG antibodies and 20(8.33%) showed negative ELISA test for these antibodies, the association was significant between clinical diagnosis and positive ELISA results for anti- tTg with $P < 0.001$.

Results of ELISA test in patients suffering from RA:

Out of 94 clinically diagnosed Rheumatoid arthritis patients, 62(65.96%) showed positive ELISA test for presence of anti-ANA antibodies and 32(34%) showed negative ELISA test for these antibodies, the association was significant between clinically confirmed cases and positive ELISA results for ANA antibodies with P < 0.001. Also results indicated that out of 94 clinically diagnosed Rheumatoid arthritis patients, 37(39.36%) showed positive ELISA test for presence of anti-dsDNA antibodies and 57(60.6%) showed negative ELISA test for these antibodies, the association was significant between clinical diagnosis with negative ELISA results for dsDNA antibodies with P < 0.001. Results in (Table.2) indicated that out of 94 clinically diagnosed Rheumatoid arthritis patients, 94(100%) showed positive ELISA test for presence of anti-ccp antibodies and 0(0%) showed negative ELISA test for these antibodies, the association was significant between clinical diagnosis and positive ELISA results for anti-ccp antibodies with P < 0.001.

Results of ELISA test in patients with SLE:

Results showed in (Table.2) indicated that out of 8 clinically diagnosed SLE patients, 3(37.5%) showed positive ELISA test for presence of anti-ANA antibodies and 5(62.5%) showed negative ELISA test for these antibodies, the association was not significant between clinical diagnosis and positive ELISA results for ANA antibodies. Also results indicated that out of 8 clinically diagnosed SLE patients, 8(100%) showed positive ELISA test of anti-dsDNA and anti-cardiolipin, 0(0%) showed negative ELISA test for these antibodies, the association was significant between clinical diagnosis and positive ELISA results for dsDNA and anticardiolipin antibodies with P < 0.001. Results in (Table.2) indicated that out of 8 clinically diagnosed SLE patients, 4(50%) showed positive ELISA test for presence of anti-phospholipids antibodies and 0(0%) showed negative ELISA test for these antibodies, the association was significant between clinical diagnosis and positive ELISA results for anti-phospholipids antibodies with P < 0.001.

Discussion

Results showed in (Figure.1) indicated that out of 342 patients with autoimmune diseases, 240(70.2%) were diagnosed clinically and according to results of ELISA test as celiac disease patients. High frequency of patients with CD agree with other studies done on Iraqi patients and other populations (21) (22) (23) (24). Incidence of CD in Indians and in Africa showed high prevalence rate (25). Our results showed that 94(27.5%) were diagnosed as rheumatoid arthritis patients. A study carried out in Iraq during 1975 indicated that the percentage of patients with RA in Basrah was 0.87% (26). Many studies done in Iraq showed that the incidence of RA was increasing from 1.6% in 2001 to 3.02% in 2011 and the cumulative risk was in Babylon-Iraq (27). More than 21% of US adults were found to have arthritis (28). Our results showed that 8 (2.3%) were diagnosed as SLE patients. These results agree with a study done on Iraqi patients (29). Gender showed no difference in the three diseases. Regarding to positive ELISA results for anti-gliadin (IgA and IgG), anti-tTg IgA and anti- tTg IgG agree with other studies (21). Some patients with CD did not give positive ELISA results due to hemolysis which interfere with the test especially in patients with low antibodies titers (30).

Anti- tTg give indicative for CD (31). Serological tests decreased invasive diagnosis methods such as biopsy (32). Confirmation clinically was associated with ANA positive ELISA results CD and RA patients. In patients with RA, anti-ccp testing is very indicative in early diagnosis and this agree with other studies (33)(14). Association of clinical diagnosis and ANA ELISA test was not significant in SLE patients. ELISA results of dsDNA and anti-cardiolipin agree with a study done on Iraqi patients (29). Our results agree with results of a study on SLE in northern Sweden (34). Also agree with results of Villalta *et al.* in 2013 (35). Antiphospholipid presents very important serological screening test for SLE (36)(37)(38). ELISA results for anti-phospholipids agree with another study done on Iraqi patients (29).

References

1. Adriaanse MPM, Tack GJ, Lima Passos V, Damoiseaux JGM, Schreurs MWJ, van Wiick K, Riedl RG, Masclee AAM, Buurman WA, Mulder CJJ, Vreugdenhil ACE. Serum I-FABP as marker for enterocyte damage in celiac disease and its relation to villous atrophy and circulating autoantibodies. Alimentary Pharmacology & Therapeutics. 2013; 37(4): 482-490.
2. Aletaha D, Neogi T, Silman A, Funovits J, Felson D, *et al.,* 2010 Rheumatoid Arthritis Classification Criteria. ARTHRITIS & RHEUMATISM. 2010; 62(9): 2569-2581.
3. Alkazzaz A. Incidence of Rheumatoid arthritis [2001-2011]. The Iraqi Postgraduate Medical Journal. 2013; 12(4).
4. Al-Rawi AJ, Alazzawi F, Alajili FM, Alwakil R. Rheumatoid arthritis in population samples in Iraq. Annals. Of the Rheumatic diseases. 1978; 37: 73-75.
5. Arguelles-Grande C, Norman GL, Bhagat G, Green PHR. Hemolysis Interferes with the Detection of Anti-Tissue Transglutaminase Antibodies in Celiac Disease. Clinical Chemistry. 2010; 56(6): 1034-1036.
6. Arief, A. S., Pattiasina, P. J., & Remus, S. P. (2021). Relevance of human resource management on small business objective in digital era: A critical review of research evidence. International Journal of Social Sciences and Humanities, 5(2), 79–91. https://doi.org/10.29332/ijssh.v5n2.1167
7. Arif HS, Al-Hadithi R, Abdul Elah S. Some diagnostic aspects of celiac disease in Iraqi children. Iraqi J. Med. Sci. 2009; 7(3): 32-39.),(Firouzi MS, Omrani GR, Khademolhosseini F. Prevalence of Celiac Disease in Shiraz, Southern Iran. Saudi. J. Gastroenterol. 2008; 14(3): 135-138.
8. Barker C, Mitton C, Jevon G, Mock T. Can Tissue transglutaminase antibody titers replace small-bowel biopsy to diagnose celiac disease in select pediatric populations? Pediatrics. 2005; 115(5): 1341-1346.
9. Barton SH, Kelly DG, Murray JA. Nutritional deficiencies in celiac disease. Gastroenterol. Clin. North Am. 2007; 36: 93-108.
10. Baudon JJ, Johanet C, Absalon YB, Morgant G, Cabrol S, Mougenot JF. Diagnosing celiac disease: a comparison of human tissue transglutaminase antibodies with antigliadin and antiendimysium antibodies. Arch. Pediatr. Adolesc. Med. 2004; 158(6): 584-8.
11. Bizzaro N, Mazzanti G. Tonutti E, Villalta D, Tozzoli R. Diagnostic Accuracy of the Anti-Citrulline Antibody Assay for Rheumatoid Arthritis. Clinical Chemistry. 2001; 47(6): 1089-1093.

12. Bonamico M, Tiberty C, Picarelli A. *et al.* Radioimmunoassay to detect anti-transglutaminase autoantibodies is the most sensitive and specific screening method for celiac disease. Am. Gastroenterol. 2001; 96: 1536-1540.

13. Cotsapas C, Hafler DA, Immune-mediated disease genetics: the shared basis of pathogenesis. Trends. Immunol. 2013; 34(1): 22-6.

14. Cummins AG, Roberts-Thomson IC, Prevalence of celiac disease in the Asia-Pacific region. J. Gastroenterol. Hepatol. 2009; 24: 1347-51.

15. Eisenberg R, Albert D. B-cell targeted therapies in rheumatoid arthritis and systemic lupus erythematosus. Nat. Clin. Pract. Rheumatol. 2006; 2: 20-27.

16. Eriksson C, Kokkonen H, Rantapää-Dahlqvist S. Autoantibodies predate the onset of systemic lupus erythematosus in northen Sweden. Arthritis Res. Ther. 2011; 13(1): R30.

17. Eskander, A. E., Subhy, N. S., Abdelfattah, W., & Shamma, R. A. (2022). Antigastric parietal cell antibody as a screening test for Autoimmune gastritis in Egyptian children and adolescents with juvenile autoimmune thyroid disease and those with type 1 diabetes, single center experience. International Journal of Health Sciences, 6(S6), 9685–9695. https://doi.org/10.53730/ijhs.v6nS6.12529

18. Fleckenstein B, Qiao SW, Larsen MR, Jung G, Roepstorff P, Sollid LM. . Molecular characterization of covalent complexes between tissue transglutaminase and gliadin peptides. J. Biol. Chem. 2004; 279(17): 17607-16.

19. Green PH, Cellier C. Celiac disease. N. Engl. J. Med. 2007; 357: 1731-1743.

20. Harich R, Roger C, Garnier L, Bienvenu J, Fabien N. Comparison of anti-ccp autoantibodies measurement by ELISA and a bead-based assay in a large patients cohort. Clinical Biochemistry. 2014; 47(6): 485-488.

21. Helmick CG, Felson DT, Lawrence RC, Gabriel S, Hirsch R, Kwoh CK, Liang MH, Kremers HM, Mayes MD, Merkel PA, Pillemer SR, Reveille JD, Stone JH. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. Arthritis Rheum. 2008; 58(1): 15-25.

22. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, Hoffenberg EJ, Horvath K, Murray JA, Seidman EG, Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J. Pediatr. Gastroenterol. Nutr. 2005; 40(1): 1-19.

23. Hill ID. Clinical Manifestations and diagnosis of Celiac disease in children. Waltham, MA: Up to date; 2008.

24. Hom G, Graham RR, Modrek B, Tylor KE, Ortmann W, Garnier S, Lee AT, Chung SA, Ferreira RC, Pant PV, Ballinger DG, Kosoy R, Demirci FY, Kamboh MI, Kao AH, Tian C, Gunnarsson I, Bengtsson AA, Rantapää-Dahlqvist S, Petri M, Manzi S, Seldin MF, Rönnblom L, Syvänen AC, Criswell LA, Gregersen PK, Behrens TW. Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. N. Engl. J. Med. 2008; 358(9): 900-9.

25. Kang JY, Kang A, Green KA, Systemic Review: Worldwide Variation in the frequency of Coeliac Disease and Changes Over Time. Aliment. Pharmacol. Ther. 2013; 38(3): 226-245.

26. Krause I, Anaya JM, Fraser A, Barzilai O, Ram M, Abad V, Arango A, Garcia J, Shoenfeld Y, Anti-infectious antibodies and autoimmune-associated autoantibodies in patients with type I diabetes mellitus and their close family members. Annals of the New York Academy of Sciences. 2009; 1173: 633-9.

27. Masson C. Rheumatoid anemia. Joint Bone Spine. 2011; 78: 131-137.

28. McMahon MA, Keogan M,O'Connell P, Kearns G. The prevalence of antiphopholipis antibody syndrome among systemic lupus erythematosus patients. Ir. Med. J. 2006; 99: 296-8.

29. Mortensen ES, Fenton KA, Rekvig OP. Lupus nephritis: the central role of nucleosomes revealed. Am. J. Pathol. 2008; 172(2): 275-83.

30. Niewinski MM. Advances in celiac disease and gluten-free diet. Assoc. 2008; 108: 661-672.

31. Noori AS, Jawad AM, Jassim NA, Gorial FI. Prevalence of antiphospholipid antibodies in sample of Iraqi patients with systemic lupus erythematosus: A cross section study. American Journal of Clinical Medicine Research. 2013; 1(4): 61-64.

32. Petri M. Update on anti-phospholipid antibodies in SLE: the Hopkins Lupus Cohort. Lupus. 2010; 19: 419-23.

33. Rubio-Tapia A, Ludvigsson JF, Brantner TL, Murray JA, Everhart JE. The prevalence of celiac disease in United States. Am. J. Gastroenterol. 2012; 107(10): 1537-1545.

34. Sabahi R, Anlik JH. B-cell-targeted therapy for systemic lupus erythematosus. Drugs. 2006; 66(15): 1933-48.

35. Simmonds MJ, Gough SC, Genetic insight into disease mechanisms autoimmunity. Br. Med. Bull. 2004; 71: 93-113.

36. Sollid LM, Molecular basis of celiac disease. Annu. Rev. Immunol. 2000; 18: 53-81.

37. Song SJ, Iwahashi M, Tomosugi N, Uno K, Yamana J, Yamana S, Isobe T, Ito H, Kawabata H, Yoshizaki K. Comparative evaluation of effects of treatment with tocilizumab and TNF-α inhibitors on serum hepacidin anemia response and disease activity in rheumatoid arthritis patients. Arthritis Research & Therapy. 2013; 15:R141.

38. Tarr T, Lakos G, Bhattoa HP, *et al.* Clinical thrombotic manifestations in SLE patients with and without antiphospholipid antibodies: a 5-year follow-up. Clin. Rev. Allergy Immunol. 2007; 32: 131-7.

39. Thorsby E, Lie BA, HLA associated genetic predisposition to autoimmune diseases Genes involved and possible mechanisms. Transpl. Immunol. 2005; 14: 175-182.

40. Villalta D, Bizzaro N, Doria A. Anti-dsDNA Antibody isotyes in Systemic Lupus Erythematosus: IgA in addition to IgG anti-dsDNA help to identify Glomerulonephritis and active disease. 2013; 8(8): e71458.
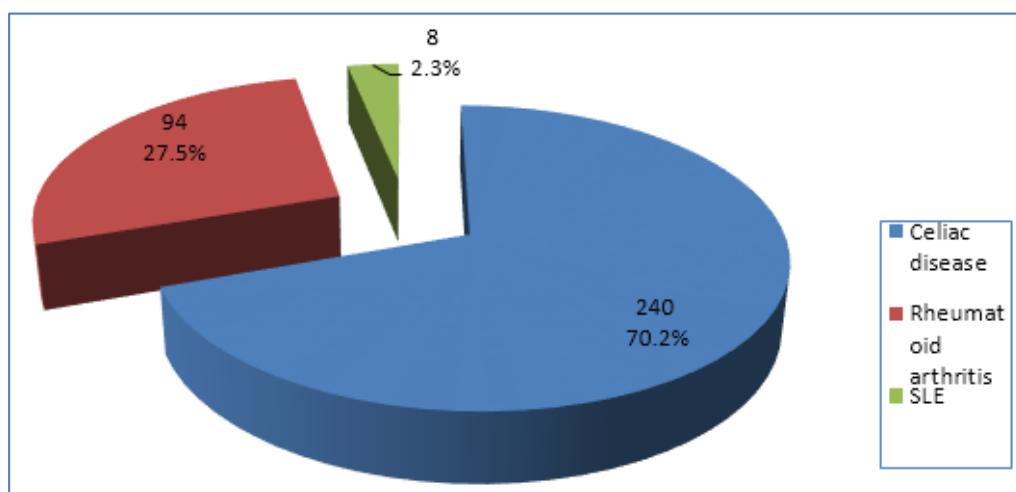
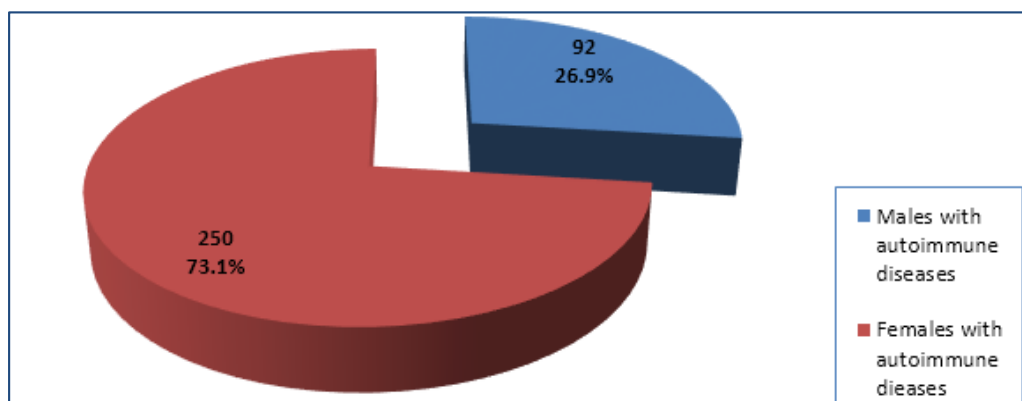Figure (1) Patients regarding to autoimmune disease

Figure (2) Patients regarding to their gender

Table (1) Patients with autoimmune diseases regarding to their gender

Autoimmune disease	Total	Males N=92	Females N=250	P-value	x^2	OD	95% CI
Celia disease	240	68(28.33%)	172(71.67%)	NS	0.84	1.29	0.75-2.19
Rheumatoid arthritis	94	20(21.28%)	74(78.72%)	NS	2.09	0.67	0.38-1.16
SLE	8	4(50%)	4(50%)	NS	2.22	2.80	0.69-11.42

Table 2 Results of ELISA tests in patients with CD, RA and SLE

Disease	No. of patients confirmed by clinical diagnosis	ELISA tests	Results of ELISA		P-value	x^2	OD	95% CI
			+ve	-ve				
Celiac disease	240	Anti- gliadin IgA	210 87.5%	30 12.5%	< 0.001	231.24	0.13	0.09-0.18
		Anti- gliadin IgG	210 87.5%	30 12.5%	< 0.001	231.24	0.13	0.09-0.18
		Anti-tTg IgA	180 75%	60 25%	< 0.001	161.50	0.25	0.20-0.31
		Anti-tTg IgG	220 91.67%	20 8.33%	< 0.001	262.11	0.08	0.06-0.13
Rheumatoid arthritis	94	ANA	62 65.96%	32 34.0%	< 0.001	176.91	94.16	35.24-251.62
		dsDNA	37 39.36%	57 60.6%	< 0.001	71.77	15.45	7.25-32.90
		Anti-ccp	94 (100%)	0 (0%)	< 0.001	342.00	N/A	N/A
SLE	8	ANA	3 (37.5%)	5 (62.5%)	NS	1.67	2.53	0.59-10.87
		dsDNA (IgG)	8 (100%)	0 (0%)	< 0.001	51.42	8.56	6.34-11.50
		Anti-cardiolipin	8 (100%)	0 (0%)	< 0.001	51.42	8.56	6.34-11.50
		anti-phospholipid	4 (50%)	4 (50%)	< 0.001	52.30	40.75	8.62-192.62