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Determination of Antichromatin Antibodies as a Useful Marker of Disease Activity and Nephritis in Juvenile Systemic Lupus Erythematosus

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Abstract: Background: Antichromatin (aCT) antibodies were one of the first autoantibodies ever detected since they make up the majority of antibodies causing Lupus erythematosus (LE) cell formation. The prevalence of antichromatin antibodies in systemic lupus erythematosus (SLE) varies from 50% to 100%.The presence of these antibodies can be used in conjunction with clinical findings and other laboratory tests, to help in the diagnosis of SLE. Their presence has also been linked to nephritis and disease activity in such patients. 30 SLE patients and 21 age and sex matched controls,were analyzed for the presence of antichromatin antibodies using enzyme linked immunosorbent assay (ELISA).We studied it's association with nephritis and disease activity. Anti- dsDNA antibodies and ANA using indirect immunofluorescence assay and C_3 using single radial immunodiffusion were estimated. Patients were evaluated for clinical and laboratory associations. Results revealed that positive levels of (aCT) antibodies were detected in 28/30 (93.3%) of patients with SLE and in none of healthy controls. These patients have higher prevalence of nephropathy and proteinuria. In conclusion the measurement of antichromatinantibodies appears to be a useful addition to the laboratory tests that can help in the diagnosis and treatment of SLE. These antibodies are both sensitive and specific for SLE and are a useful marker for detection of an increased risk for lupus nephritis.

Key word: Autoantibodies · Autoimmune connective tissue disease · Lupus proteinuria · Cellular casts

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by multisystem inflammation and presence of circulating autoantibodies directed against self- antigens [l]. It is more commonly seen in adults, however 10-20% of SLE patients are diagnosed before 16 years old [2]. Compared with adults, children and adolescents with SLE have more severe disease and more widespread organ involvement including kidneys. It is a relapsing chronic multi -systemic disease characterized by periods of disease activity and remission. The reasons for varying course are unknown. Sun exposure, drug reactions and infection may induce inflammation which exacerbate the disease. Although the specific cause of SLE is unknown, multiple factors are associated with the development of the disease, including genetic, racial, hormonal and environmental. Many immune disturbances both innate and acquired, occur in SLE [3].

In SLE nearly every organ may be affected, however the most commonly involved are the skin, joints, kidneys, blood vessels and central nervous system. More than half of patients develop nephritis during their course of the illness. Lupus nephritis is defined as persistence of proteinuria more than 500 mg daily, or the presence of cellular casts (erythrocytes, hemoglobin, granular, tubular, or mixed casts) in the urine. SLE nephritis bears

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considerable morbidity and approximately 10% overall will progress to dialysis or transplantation which represents a major threat to long-term quality of life and survival [2].

The high frequency of renal involvement in Juvenile systemic lupus erythematosus (onset before 16 years old), contributes to the severity of the disease [4]. Renal disease in SLE is often asymptomatic; thus careful monitoring of blood pressure and urine analysis is critical [3].

The presence of certain autoantibodies is one of the factors associated with some symptoms of the disease and aids in the classification of patients with SLE into specific subsets [5]. Systemic Lupus Erythematosus is characterized by the presence of circulating auto antibodies against nuclear components (ANA). Although a positive ANA test result is not required for the diagnosis of SLE, ANA - negative lupus is extremely rare [3]. Auto - antibodies represent important players in renal damage [6]. The anti- phospholipid antibodies are clearly associated with the development of thrombotic events, while anti-ribonuclear (RNP) antibodies are associated with myositis and Raynaud's phenomenon. Moreover, anti - SSA/RO antibodies are considered markers of neonatal lupus [5].

Many efforts have been spent to find a useful and early maker of nephritis. Anti-dsDNA antibodies are often associated with lupus – nephritis [7] and their serum levels seem to correlate with kidney disease [8].

Evidence has accumulated in recent years, that anti-chromatin autoantibodies are correlated even better with lupus nephritis than anti-ds DNAantibodies [9]. Chromatin is distributed within the nucleus of eukaryotic cells and it consists of approximately 40% DNA, 40% histones and 20% non histone proteins [10]. Anti chromatin - chromatin immune complexes can bind to the glomerular basement membrane *in vivo* [5]. Antibodies to nucleosome / chromatin, revealed to be sensitive and specific for SLE. Conversely, there are growing evidences supporting their usefulness in diagnosis of lupus patients lacking anti DNA antibody [11].

Because there is evidence of the appearance of antichromatin antibody in the circulation before anti ds DNA [6], it is claimed as an early detectable biomarker of lupus disease.Moreover, studies in patients with SLE have shown a marked link between antichromatin antibodies and lupus activity including renal flareeven in the absence of anti- ds DNA antibody [12].

Therefore, the present study investigated a sample of patients having juvenile systemic lupus erythematosus (JSLE), with biopsy proven lupus nephritis. The relationship of antichromatin antibodies and renal histopathology, have been studied.

The aim of the present study was to investigate the diagnostic performance of antibodies to the chromatin antigen system in JSLE, as well as their associations with SLE clinical manifestations with emphasis on lupus nephritis.

MATERIALS AND METHODS

Patients and Study Design: Thirty children aged from 10-24 years, of whom 21 were female and 9 were male were recruited in the study. They were chosen from the Rheumatology clinic Abu El Rish Hospital Cairo University. They were diagnosed as having juvenile SLE according to the American college of Rheumatology 1997 Revised.

Classification Criteria for SLE: Patients were compared with 21 healthy age and sex matched controls. These controls were confirmed to have no medical illness, family history of SLE or any other collagen vascular disease. Our patients were submitted to: full history taking, physical examination, laboratory investigations, abdominal ultrasonography and renal biopsy to exclude nephritis. Sixteen (54%) of our patients were confirmed by renal biopsy to have renal nephritis of different grades, while the other fourteen (46%) were free of nephritis.

Samples: Seven mls of venous blood samples afterfasting for 6 – 8 hours were drawn from each child participating in the study. Two mls, was drawn into tube containing EDTA for determination of complete blood picture (CBC) on coulter counter T890 (coulter counter, Harpenden, UK). In another tube containing 0.4 ml citrate, 1.6 mls blood were put for determination of ESR using Westergren's method. The rest of blood was drawn into plain tube and left to clot and the serum was separated by centrifugation at 3000 xg for 5 minutes and CRP was determined immediately by rapid latex agglutination (wads worth and wads worth) [13] and the rest of the serum was stored at - 20°C for determination of anti-ds DNA, anti-ANA, C_3,kidney function tests (urea and creatinine) and anti chromatin antibodies.

Measurment of Antibodies: Anti ds DNA and anti-ANA were determined using indirect immuno fluorescence assay on mouth kidney and stomach slides and crithidialuciliae slides (Immco Diagnostics Inc, 640 Ellicott street, New York, USA) The slides were analyzed with Nikon epifluorescence microscope (Nikon Inc., USA).

Complement 3 (C_3) was determined by single radial immune diffusion plates (Diffuplates, Biocientifica, New Delhi, India).

Detection of antichromatin antibodies using enzyme-linked immunosorbent assay (ELISA) semi - quantitatively by QuantalitechromatinELISAsupplied from Medical Technology Promedt Consulting, GmbH (Altenhofs-trasse, 80 D-66386 street, ingbert. Germany) Gilliam *et al*. [14]. The test is considered negative if <20U, moderately positive if 20-60U and strongly positive if >60 U.

Urine Analysis: Complete urine analysis was performed for the presence of red blood cells, pus, albumin,and urinary casts.

The Statistical Paragraph in Material and Methods: Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student *t* test for independent samples in comparing 2 groups when normally distributed and Mann Whitney *U* test for independent samples when not normally distributed. Comparison of anti-chromatin levels was done using one way analysis of variance (ANOVA) test with posthoc multiple 2-group comparisons. For comparing categorical data, Chi square (x^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlation between various variables was done using Pearson moment correlation equation for normal variables and linear relation and Spearman rank correlation equation for non-normal variables/non-linear relations. *p* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

Table 1: shows demographic and clinical features of SLE patients with biopsy proven lupus nephritis in comparison to those without nephritis. The SLE patients with nephritis had similar mean age to those without nephritis. The duration of disease was slightly longer in the non nephritic group with no significant difference. There is female predominance in both groups, but the difference is not statistically significant. As regard the clinical features,there were no significant differences between SLE patients with and without nephritis except for serositis.

Table (2): shows laboratory features of SLE patients with and without nephritis, which revealed significantly higher prevalence of proteinuria in the nephritic group compared to those without nephritis. Moreover, the apparent higher creatinine, anti DNA antibodies and ESR levels observed in the nephritic group did not reach statistical significance.

Table 1: Demographic and clinical features of SLE patients with and without lupus nephritis

	Nephritis		
Variables (mean+ SD)	Yes N = 16 patients N (%)	No N= 14 patients N (%)	P value
Age (years)	16.9±4.5	15.4±4.7	>0.05
Duration (years)	5.6 ± 2.7	6.2 ± 2.7	>0.05
Sex			
Female	9 (56%)	12(86%)	
Male	7(44%)	2(14%)	>0.05
Photosensitivity	3 (19%)	2 (14%)	> 0.05
Nephrotic	2 (12.5%)	0(0.0%)	> 0.05
Epistaxis	1 (6%)	1(7%)	> 0.05
Alopecia	16 (100%)	13(93%)	> 0.05
Cerebritis	4 (25%)	2(14%)	> 0.05
Oral ulcers	3 (19%)	2 (14%)	> 0.05
Hepatomegly	1 (6%)	1 (7%)	> 0.05
Splenomegaly	1 (6%)	1 (7%)	> 0.05
Serositis	5 (31%)	0 (0.0%)	< 0.05
Lymphadenopathy	1 (6%)	0 (0.0%)	> 0.05

P*<0.05 is significant, P >0.05 is non significant

Table 2: Laboratory features of SLE patients with and without nephritis

Variables (Mean ± SD)	Nephritis: Yes N = 16 patients (53%)	Nephritis: No N= 14 patients (47%)	P value
R.B.Cs(10^6/µL)	5 ± 2.04	4.6 ±0.5	>0.05
W.B.Cs(10^3/µL)	6.5 ±2.9	6.9 ±2.5	>0.05
Platelets(10^3/µL)	313.5 ±101.7	234.6 ±11 1.5	>0.05
Urea(mg/dl)	24 ±16	25.3 ± 9.9	>0.05
Creatinine (mg/dl)	0.9 ±0.6	0.6 ±0.2	>0.05
$C_{3(mg/dl)}$ {median(range)}SD	67.4(2-167)SD(49.2).	66.9(14-128)SD(38.6).	>0.05
E.S.R{median(range)}SD mm/hr	46.5(7-125).6)SD(32.6)	25(4-90)SD(21.7)	>0.05
Anti-DNA(U/ml)(%)	11(69%)	7(50%)	>0.05
ANA(%)	14(88%)	9(64%)	>0.05
Coomb's test (%)	3(19%)	2(14%)	>0.05
CRP mg/L(%)	7(44%)	6(43%)	>0.05
Proteinuria g/24 hours(%)	11(69%)	3(21%}.	<0.05*
Cast(%)	3(19%)	0(0.0%)	>0.05

P*<0.05 is significant. C_3: complement 3; ANA:anti-nuclear antibody; CRP:C-reactive protein.

Table 3: Mean±SD and analysis of variable (ANOVA) of serum level of antichromatin antibodies in the three studied groups

	Controls	SLE without nephritis	SLE with nephritis	F	P
Anti-chromatin antibodies (U) Mean +SD	3.95±1.6	36.8±15.9	69.6±23	48.44	< 0.0001

P < 0.05 is significant

Table 4: Prevalence of SLE clinical manifestations in patients with and without antichromatin antibodies

Variables	Anti chromatin Antibodies: <20 Unit(n=2) N (%)	Anti chromatin Antibodies: >20 Unit(n=28) N (%)	P value
Photosensitivity	0(0.0%)	5(18%)	>0.05
Nephrotic	0(0.0%)	2(7%)	>0.05
Nephritis	0(0.0%)	16(57%)	<0.01*
Epistaxis	1(50%)	1(4%)	>0.05
Alopecia	1(50%)	28(100%)	>0.05
Cerebritis	0(0%)	6(21%)	>0.05
Oral Ulcers	0(0.0%)	5(18%)	>0.05
Hepatomegaly	1(50%)	1(4%)	>0.05
Splenomegaly	1(50%)	1(4%)	>0.05
Serositis	0(0.0%)	5(18%)	>0.05
Lymphadenopathy	0(0.0%)	1(4%)	>0.05

*P<0.01 is significant; > 0.05 is non-significant.

Table (3): shows serum level of antichromatin antibodies in three group: Controls, SLE without nephritis and SLE with nephritis. The serum level of antichromatin antibodies was analyzed in the control and systemic lupus patients without and with nephritis using ANOVA test. It revealed a high significant difference in serum level of antichromatin – antibodies between the three group (F = 48.44and P < 0.0001).

Table (4): shows the clinical features of SLE patients with negative anti chromatin antibodies (<20U), compared to those with positive levels (>20U), which demonstrates a statistically significant difference (<0.01) only in the frequency of nephritis, that is higher in the antichromatin positive group than in the antichromatin negative group.

Table (5): Shows laboratory features in SLE patients with positive anti chromatin antibodies which demonstrates a significant correlation between positive antichromatin antibodies levels with proteinuria and renal biopsy.

Table (6): shows comparison between levels of anti DNA antibodies and antichromatin antibodies in SLE cases, which demonstrates that patients with antichromatin antibodies level (< 20U) are anti DN A antibodies negative, while in the 16 patients with moderately positive antichromatin antibodies level (20-60 U), only 9 (56.3%) of them were positive for anti DNA antibodies. Of the 12 patients who were strongly positive for aCT(>60U), 9 (75%) were anti DNA antibodies positive.

Table 5: Correlation between levels of antichromatin antibodies with studied laboratory tests

	Anti chromatin antibodies	
Variables	R	P
CRP	0.058	0.760
Proteinuria	0.386	0.035*
Cast	0.289	0.122
ESR	0.037	0.846
Urea	0.340	0.066
Creatinine	0.250	0.182
Renal biopsy	0.730	0.001*

P*<0.05 is significant

Table 6: Comparison between levels of anti DNA antibodies and anti chromatin antibodies in SLE cases

	Anti D.N.A antibodies		
Anti chromatin antibodies	(+ve)	(-ve)	P value
<20U (n=2)	0(0.0%)	2(100%)	>0.05
20-60U(n=16)	9(56.3%)	7(43.8%)	>0.05
>60U(n=12)	9(75%)	3(25%)	>0.05

DISCUSSION

Glomerulonephritis is a major determinant for the prognosis of SLE and the underlying pathogenic mechanisms may involve the in situ formation or deposition of circulating immune complexes in the renal tissue and the direct cytotoxicity of a subset of pathogenic autoantibodies cross-reactive to intrinsic glomerular cell antigens [6].

A number of antibodies have been implicated in the development of lupus nephritis, mainly anti-ds DNA. However there are substantial evidences that neither the presence nor titers of anti-ds DNA antibody show a strict correlation with the severity or the development of lupus nephritis [15, 16].

Anti- chromatin antibodies had emerged as a potential biomarker for the diagnosis of lupus renal disease with promising results. These antibodies appear to be correlated with Juvenile SLE nephritis activity [17].

In this study, increased levels of (aCT) antibodies have been found in all SLE cases both with and without nephritis and this elevation was statistically significant when compared to normal healthy controls.Moreover, (aCT) antibodies serum level is significantly increased in SLE cases with nephritis compared to those without nephritis.This is in agreement with many authors [18-19].

Moreover, in this studya bout 53% of our patients were found to have clinical evidence of lupus nephropathy which is confirmed by renal biopsy. This result is comparable with that performed by Cervera [5], who estimated the same results, while Keusseyan *et al*., [18] and Gilliam *et al,* [14], found a lesser percentage of patient with JSLE suffering from renal involvement (40% & 37.5% respectively).

In this study, the patients with biopsy proven nephritis were compared with those without nephritis as regard all demographic features, only the duration of the disease and female gender showed a non-significant increase in those not suffering from nephritis.

When we compare the clinical and laboratory features in SLE cases with and without nephritis, only serositis and proteinuria were found to be significantly higher in the nephritic than in the non-nephritic groups (< 0.05). This ensures that proteinuria interpret the occurrence of active lupus nephritis [4].

As regard, the clinical features of JSLE with positive aCT antibodies (>20U) and those with negative aCT antibodies (<20U), no significant difference was detected in the prevalence of any of them except for nephritis, which was statistically higher (< 0.01) in the aCT antibodies positive group, compared to the negative group. These results were similar to that reported by Keusseyan *et al*., [18] and Cervera *et al*., [5]. On the other hand, Gilliam *et al*., [14], did not found any significant association between aCT antibodies and lupus nephritis.

In this study, all the laboratory features except proteinuria are not significantly different between the JSLE patients with positive anti-chromatin antibodies and those who are negative. Proteinuria was statistically higher in those with positive (aCT) antibodies than in those with negative (aCT) antibodies, as reported by Keusseyan *et al*., [18]. This is also in concordance with the results estimated by Carvalho *et al*., [6] who reported higher level of proteinuria in JSLE patients, who are (aCT) antibodies positive (level >20U), compared to those who are (aCT) antibodies negative (level < 20U), however the difference was not statistically significant.

It is noteworthy to mention that, there is a positive correlation between (aCT) antibodies and renal biopsy in the present study, which means that the more advanced, is the class of nephropathy, the higher is the (aCT) antibodies level. This is supported by the report of Carvalho *et al*., [6], who recognized the association between high titer of (aCT) antibodies with more severe class of lupus nephritis (proliferative class IV). So, (aCT) antibodies could be considered as a biomarker for lupus renal disease requiring transplantation [20].

A number of antibodies have been implicated in the development of lupus nephritis mainly anti-dsDNA [15]. In the present study anti-DNA antibodies were detected in 64% of our patients, while Gilliam *et al*., [14], found that only 56% of JSLE patients were positive for these antibodies. Moreover we detected that patients who are negative for antichromatin antibodies (< 20 U), were also negative for anti DNA antibodies, while 56.3% of patients who had moderately positive antichromatin antibodies (levels between 40-60U) were anti DNA antibodies positive and 75% of patients who were strongly positive for aCT antibodies (>60U) were anti DNA antibodies positive. This finding is similar to what reported by Souza *et al*. [21], Jesus *et al*. [19], Gilliam *et al*. [14] and kim *et al*. [22] . The previous results indicate that the detection of (aCT) antibodies can correlate with disease activity in patients with SLE who are tested negative for anti DNA antibodies. Braun *et al*. [23] identified (aCT) antibodies in SLE patients with positive ANA but negative anti -ds DNA antibodies, so persisting (aCT) antibodies indicated, SLE disease activity even if anti DNA antibodies had became negative. On the other hand, Keusseyan *et al*., [18] found that about 96% of anti-ds DNA ELISA positive samples, were (aCT) antibodies positive (>20U).

The classical complement pathways are responsible for clearing immune complexes from the body. Defects in this pathway can result in accumulation of immune complexes which can lead to autoimmune diseases like SLE [24]. In the present study, there was no significant correlation between (aCT) antibodies levels and C_3 level. This was in contrast with the study performed by Keusseyan *et al.*, [18], who found that there was a significant association between decreased C_3 levels and the frequency of aCT (>20U) positive assay. Also, Carvalho [6] estimated a significantly lower levels of C_3 in SLE patient with aCT antibodies (>40U) than in those with aCT antibodies (<40U) groups, which can be explained by complement consumption in lupus nephritis patients due to the formation of complement fixing antibodies on glomerular basement membranes as it has been proposed for anti - ds DNA antibody, however hypocomplementenemia reflects proliferative lupus nephritis but not other aspects of SLE, including membranous lupus nephritis [6].

As outlined by Kamphius and Silverman [25], pediatric onset SLE patients are more often prone to major organ involvement and more severe diseases than adult onset SLE. Early management of the disease is necessary to prevent further complications, which can be partly accomplished by monitoring autoantibody levels and understanding their significance in the disease process. So in conclusion, measurements of antichromatin antibodies at the onset of disease are essential in disease management and follow up. These antibodies are both sensitive and specific for SLE. Their association with proteinuria make them useful marker for detection of an increased risk for lupus nephritis.

However, an important limitation of the present study is the small sample size, which leads to considerable key of statistical power. Further studies are suggested to be done in a higher sample sizes,in order to confirm the influence of antichromatin–antibodies in the pathogenesis of lupus nephritis.

REFERENCES

1. Barbado, J., D. Martin, L. Vega, R. Almansa, L. Goncalves, M. Nocito, A. Jimenoi, I. Lejarazu Rod and J.F. Bermejo-Martin, 2012. M PC-I in urine as biomarker in disease activity in systemic lupus erythematosus. Cytokeine-Journal homepage: Downloaded from www.elsevier.com / locate/ issn/10434666 at Egyptian National Science. Cytokine J. cyto.2012.07.009.
2. Marks, S.D, V. Shah, Pilkingtonand K. Cand Tullus, 2010. Urinary monocyte chemoattractant protein. I correlate with disease activity in lupus nephritis. Pediatricnephrol., 25: 2283-2288.
3. Mok, C.C., 2012. Biomarker for lupus nephritis: acritical appraisal J. Biomod Bio. Technical., 10: 1155-1170.
4. Baqi, N., S. Moazami, A. Singh, H. Ahmed, S. Balachandra and A. Tejani, 1996. Lupus nephritis in children: a longitudinal study of prognostic factors and therapy. J. Am Soc. Nephrol., 7: 924-9.
5. Cervera, R., O. Vinas, M. Ramos-Casals, J. Font, M. Garcia-Carrasco, A. siso, F. Ramirez, Y. Machuca,, J. Vires, M.ingelmo and R.W. Burlingame, 2003. Anti-chromatin antibodies in systemic lupus erythematosus: a useful marker for lupus. Ann. Rheum Dis., 62: 431-434.
6. Carvalho, J.F., V.S.T. Viana, E.F. Borba, A.P. do Nasci-mento, E.P. Leon, LA. Testagrossa, R.T. Barvros and E. Bonfa, 2008. Higher titer antichromatin antibody is associated with proliferation class of lupus nephritis. Clinical Rheumatology, 27: 1417-1421.
7. Ton, E.M., P.H. Schur, R.I. Carr and H. Gkunkel, 1966. Deoxy ribonucleic acid(DNA) and antibodies to DMA in the serum of patients with systemic lupus erythematosus. J. Clin Invest, 45: 1732-40.

8. Koffler, D., R. Carr, V. Agrello, R. Thoburn and H. Gkunkel, 1971. Antibodies to polynucleotides in human sera: antigenic specifity and relation to disease. J. Exp. Med., 134: 294-312
9. Kalaaji, M., K.A. Fenton, E.S. Mortensen, R. Olsen, G. Sturfelt, Almand and O.P. Rekvig, 2007. Glomerular apoplotic nucleosomes are central target structures for nephritogenic antibodies in humans SLE nephritis. Kidney Jnt., 71: 664-672.
10. Sigal, L., 2007. Basic science for the clinician 42: handling the corpses: apoptosis, necrosesnucleosomes and (quite possibly) the immunopathogenesis of SLE. J. Clin Rheumatol., 13: 44-48.
11. Min, D.J., S.J. Kim, S.H. Park, Y.I. Seo, H.J. Kang, W.U. Kim, C.S. Choand H.Y. Kim, 2002. Autinucleosome antibody: significance in lupus patients lacking anti-double-stranded DNA antibody. Clin Exp.Rheumatol., 20: 13-18.
12. Ng, K.P., J.J. Manson, A. Rahman and D.A. Isenberg, 2006. Association of antinucleosomes antibodies with disease flare in serologically active clinically quiescentpatients with systemic lupus erythematosus. Arthritis Rheum., 55: 900-904.
13. Wadsworth, C. and E. Wadworth, 1984. Efficacy of latex agglutination and quantification methods for determination of C-reactive protein (CRP) in pediatric sera. Clin Chim Acta., 27138(3): 309-18.
14. Gilliam, B.E., A. Ombrello, R.W. Burlingame, P.H. Pepmueller and T.L. Moore, 2012. Measurement of autoantibodies in pediatric-onset systemic lupus erythematosus and their relationship with disease associated manifestations. Semin Arthritis Rheum, 1;41: 840-848.
15. Reichlin, M. and M. Wolfson – Reichlin, 2003. Correlation of antidsDNA and anti – ribosomal autoantibodies with Lupus nephritis. ClinImmunol., 168: 69-72.
16. Waldman, M. and M.P. Madaio, 2005. Pathogenic autoantibodies in lupus nephritis. Lupus, 14 : 19-24.
17. Tikly, M., T. Gould, A.A. Wadee, E. Van der Westhuizen and B.B. Mokgethwa, 2007. Clinical and serological correlates of antinucleosomes antibodies in South Africans with systemic lupus erythematosus. Clin Rheumatol., 26: 2121-2125.
18. Keusseyan, S.P., N. Pda Siwa, M.O.E. Hilario, E.M. Okuda, M. Teresa, A. Terreri and C.E.C. Andradi, 2012. Diagnostic performance and clinical association of antibodies to chromatin antigenic system in juvenile systemic lupus erythematosus.Rev Bras Rheumatol., 52(5): 695-712.
19. Jesus, A.A., L.M. Campos, B.L. Liphaus, M. Carneiro-Sampaio, C.L. Mangueira, E.A. Rosseto, C.A. Silva and M. Scheinberg, 2012. Anti-C19, anti-chromatin/ nucleosomes andanti- ds DNA antibodies in juvenile systemic lupuserythematosus patients. Rev Bras Rheumatol., 52(6): 976-981.
20. Stinton, L.M., S.G. .Baror, L.A. Tibbies, S. Yilmaz, A. Sar, H. Benedikttson and M.J. Fritzler, 2007. Autoantibodies in lupusnephritis patients requiring renal transplantation. Lupus, 15: 394-400.
21 Souza, A., L.M. da Silva, F.R. Oliveira, A.M. Roselino and P.Louzada- Junior, 2009. Antinucleosomes and anti-chromatin antibodies are present in active lupus erythematosusbut not in the cutaneous form of the disease. Lupus Mar; 18(3): 223-9.
22 Kim, H.A., J.Y. Jeon, G.S. Choi, J.M. Sung, J.M. Yun and C.H. Suh, 2008. The antichromatin antibodies can be useful as a diagnostic tool and disease activity marker of systemic lupus erythematosus in Koreans. ClinImmunol, 128(2): 277-83.
23. Braun, A., J. Sis, R. Max, K. Mueller, C. Fiehn, M. Zeier and K. Andrassy, 2007. Anti-chromatin and anti-C19 antibodies in SLE patients compared to other systemic autoimmune disease Scand J. Rheumatol., 36(4): 291-8.
24. Pickering, M.C. and M. Botto, 2010. Are antiC19 antibodies different from other SLE autoantibodies Nat Rev. Rheumatol., 6: 49-3.
25. Kamphuis, S. and E.D. Silverman, 2010. Prevelance and burden of pediatric- onset systemic lupus erythematosus. Nat Rev. Rheumatol., 6: 538-46.