

## COOMBS TEST POSITIVITY AND ITS ASSOCIATION WITH ANTI-CARDIOLIPIN ANTIBODIES AND DIFFERENT CLINICAL MANIFESTATIONS IN PAEDIATRIC SLE PATIENTS

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### ABSTRACT

**Background:** Anemia is a well-known hematological abnormality in paediatric SLE patients (pSLE). The etiology of anemia in pSLE is multifactorial, including iron deficiency anemia, anemia of chronic disease and autoimmune hemolytic anemia (AHA). Coombs positive hemolytic anemia has specific clinical and serological characteristics compared to the others. **Aims:** To assess the coombs test frequency in pSLE patients and its association with anti-cardiolipin (aCL) antibodies and different clinical manifestations. **Method:** This was a cross-sectional study. Sixty five newly diagnosed pSLE patients who fulfilled the American College of Rheumatology 1997 revised classification criteria and attended in the department of paediatrics, Bangabandhu Sheikh Mujib Medical University (BSMMU) during the study period were included. Detailed history, examination, laboratory investigations including direct and indirect coombs test and aCL antibodies were done at diagnosis. **Results:** The mean age of the pSLE patients was 11.77 years and the female to male ratio was 6:1. Constitutional symptoms (92.3%) followed by mucocutaneous (87.7%), hematological (87.7%), renal (52.3%), musculoskeletal (50.8%) and neurological (23%) manifestations were the predominant manifestations in this cohort. Coombs test was positive in 52.3% of pSLE patients. Coombs test had significant positive association with aCL antibodies but no significant association was found between coombs test positivity and different clinical manifestations. **Conclusion:** Hematological manifestations were very common in pSLE patients. A Significant association of positive coombs test with aCL antibodies was observed in this study.

**KEYWORDS:** Anticardiolipin (aCL) antibodies, Coombs test, paediatric SLE (pSLE).

### INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem, chronic autoimmune disorder that involves many organ systems. It has a wide variety of clinical features, numerous auto-antibodies and widespread immunologically determined tissue damage.<sup>[1]</sup> Children with SLE have more severe and aggressive disease with more widespread organ involvement compared to adults and disease damage also develops more quickly in children.<sup>[2,3]</sup>

Among different systemic features musculoskeletal, renal and neuropsychiatric manifestations are most prevalent in paediatric SLE patients.<sup>[5]</sup> Some other studies have observed a higher frequency of renal disease, central nervous system involvement and hematological abnormalities in these patients.<sup>[6]</sup> Coombs positive hemolytic anemia is an important hematological finding in pSLE, caused by autoantibody mediated destruction of red blood cells.<sup>[7]</sup> The coombs test is positive in approximately 30 to 40% of patients, but only

10 to 15 % have overt hemolysis.<sup>[8]</sup> Gorce et al. has shown, 86% hematological abnormalities in their series and 81% of them were anemic with 76.6% had positive coombs test.<sup>[9]</sup> In a Bangladeshi study, 100% pSLE patients were anemic and 16% had positive coombs test.<sup>[10]</sup>

Renal and neuropsychiatric manifestations had a significant association with positive coombs test in the different studies.<sup>[11,12]</sup> Patients with coombs positive hemolytic anemia were found to have a higher frequency of positive ANA, anti-dsDNA and anti-cardiolipin antibodies (aCL).<sup>[11]</sup> Assaliev et al. reported a significant positive correlation between aCL antibodies and coombs positive hemolytic anemia.<sup>[12]</sup> Evidence suggests that the presence of aCL antibodies is a secondary phenomenon caused by hemolysis and contributes to the pathogenesis of coombs positive hemolytic anemia by acting as an anti-erythrocyte antibody.<sup>[13]</sup> Elevated aCL antibody titer was found in

64.7% of SLE patients with coombs positive anemia in another study.<sup>[14]</sup>

The current study was aimed to assess the frequency of positive coombs test, its association with aCL antibodies and different clinical manifestations in pSLE patients.

## METHODOLOGY

A cross-sectional study was conducted in the Paediatric Rheumatology follow-up clinic and in-patient department of Paediatrics, Bangabandhu Sheikh Mujib Medical University (BSMMU) from December 2017 to June 2019. Newly diagnosed 65 pSLE who fulfilled ACR 1997 revised criteria were included in this study. WHO guideline 2011 was used to define anemia (mild, moderate and severe) in this study.<sup>[15]</sup> Informed written consent was taken from the parents after explaining the objectives and method of the study. Ethical clearance was taken from the Institutional Review Board (IRB) of BSMMU prior to the study. Baseline investigations to diagnose the disease including Coombs test and aCL antibody were done in all the patients. Direct coombs test was done by mixing washed cells with anti-human globulin and then centrifuged and the agglutination was seen under light microscope. Indirect coombs test was done by mixing patient's serum with known red cells and then incubated. After that anti human globulin was added with mixture of serum and cells and the agglutination was seen under light microscope. Anti-cardiolipin (aCL) antibodies were detected by commercially available ELISA kit and the value >15 U/mL was considered positive. History, clinical examination and relevant investigations were recorded in the predesigned questionnaire.

Statistical analysis was performed by using SPSS for Windows version 22. Numeric data were expressed as mean  $\pm$  standard deviation and qualitative data were expressed as frequency and percentage. Chi-square test, Mann-Whitney U test and Fisher exact test were done to identify the association and p-value <0.05 was considered as significant.

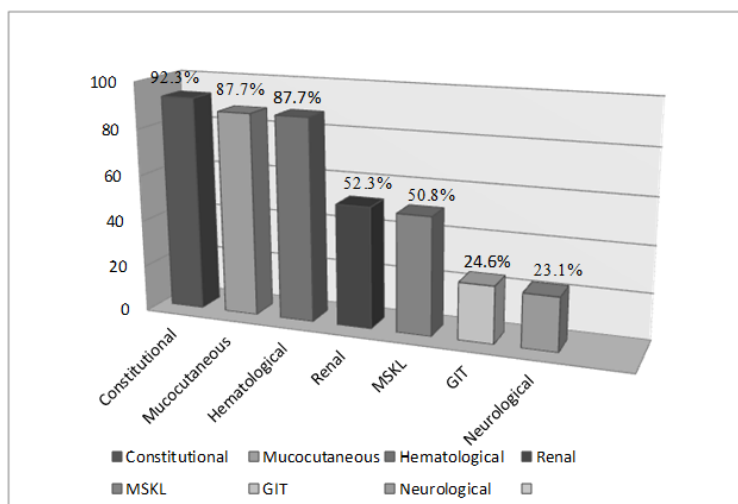
## RESULT

A total number of 65 patients were enrolled in the study. The patients' mean age was 11.76 years, and most of them (66.2%) were more than 10 years old. The mean duration of illness of this study group was 5.4 months. 86% were female and the female-male ratio was 6:1. (Table -I) Common systemic manifestations were constitutional symptoms (92.3%) followed by mucocutaneous (87.7%), renal (52.3%), musculoskeletal (50.8%), gastrointestinal (24.6%) and neurological (23%) manifestations. Hematological abnormalities were found in 87.7% of pSLE patients (Figure 1). The majority (52.3%) of the patients were found to have positive coombs test (Figure 2). Sixty one percent coombs positive patients had moderate and 26.4% had severe anemia in this study. The association of positive coombs test to the degree of anemia was not statistically significant (Table- II ). Anti-cardiolipin antibodies (aCL) were found positive in 52.9% coombs positive patients and the association of aCL antibodies and positive coombs test was found significant (Table-III). No significant association was found between the positive coombs test and different clinical manifestations in pSLE patients (Table- IV).

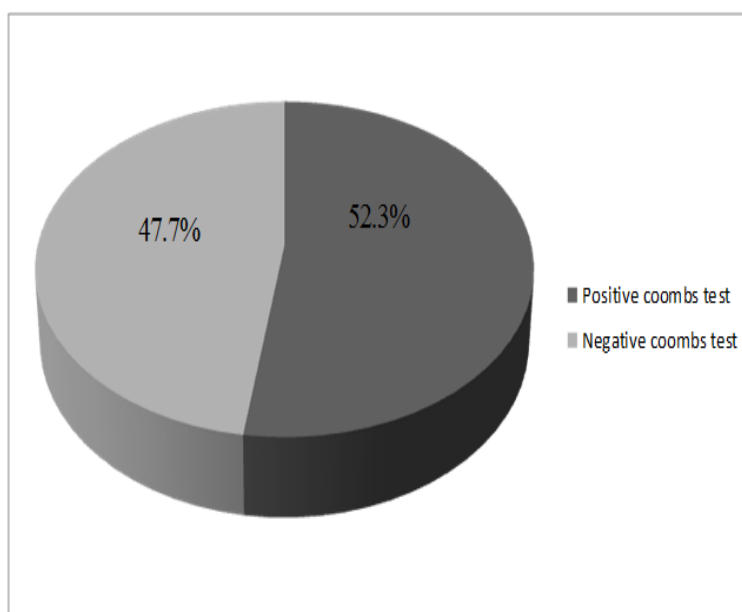
**Table 1: Demographic Data of the pSLE Patients (N=65).**

Characteristic	Patients (N=65)
Age (mean $\pm$ SD) yr	11.79 $\pm$ 2.77
<5 years, n (%)	2(3%)
5-10 years, n (%)	20(30.8%)
>10 years, n (%)	43(66.2%)
Gender, n (%)	
Female	56(86%)
Male	9(14%)
Female:Male	6:1
Disease duration (mean $\pm$ SD)months	5.42 $\pm$ 4.44

Data was expressed with number, percentage and mean $\pm$ SD



**Figure 1: Clinical manifestations of pSLE patients (N=65).**



**Figure 2: Frequency of positive coombs test in pSLE patients (N=65).**

**Table 2: Association of Positive Coombs Test with Severity of Anemia (N=65).**

Anemia	Coombs test		P value
	Positive (n=34)	Negative (n=31)	
Mild	2(5.8%)	1(3.2%)	0.382
Moderate	21(61.7%)	19(61.3%)	
Severe	9(26.4%)	5(16.1%)	

Data was expressed as number and percentage. Statistical analysis was done by **Mann-Whitney U test**

**Table 3: Association of Positive Coombs Test with Anti-cardiolipin (aCL) antibodies (N=65).**

aCL antibodies	Coombs test		P value
	Positive (n=34)	Negative (n=31)	
Positive	18(52.9%)	4(12.9%)	0.001
Negative	16(47.1%)	27(87.1%)	

Data was expressed as number and percentage. Statistical analysis was done by **Fisher's exact test**.

**Table 4: Association of Positive Coombs Test with Other Clinical Manifestations in pSLE patients (N=65).**

	Coombs test positive(n=34)	Coombs test negative(n=31)	P value
Renal manifestations (n=34)			0.262
Present	16(47.1%)	18(58.1%)	
Absent	18(52.9%)	13(41.9%)	
Neurological manifestations(n=15)			0.083
Present	5(14.7%)	10(32.3%)	
Absent	29(85.3%)	21(67.7%)	
Mucocutaneous manifestations(n=57)			0.408
Present	29(85.3%)	28(90.3%)	
Absent	5(14.7%)	3(9.7%)	
Musculoskeletal manifestations(n=33)			0.353
Present	16(47.1%)	17(54.8%)	
Absent	18(52.9%)	14(45.2%)	

Data was expressed as number and percentage. Statistical analysis was done by **Chi-square test**.

## DISCUSSION

Anemia is the most common hematological abnormality in pSLE patients. Among different etiologies of anemia, coombs positive hemolytic anemia is caused by the presence of several autoantibodies which may induce autoimmune destruction of erythrocytes.<sup>[16]</sup> Coombs positive pSLE patients may differ from others because they are vulnerable to sudden, severe, life-threatening anemia.<sup>[8]</sup> Several studies have found a significant association of positive coombs test with different clinical manifestations and autoantibodies like anti-cardiolipin (aCL) antibodies in pSLE patients.<sup>[17,18]</sup>

In this study, the patients' mean age was 11.7 years, which was almost similar to a previous Bangladeshi study where the mean age of pediatric SLE was found 11.6 years.<sup>[10]</sup>

Literature suggests that, in SLE patients, females are more affected than males and the ratio changes with age.<sup>[19,20]</sup> In the present study, the majority (86%) was female. In a cohort of pSLE patients in Bangladesh, female predominance was observed with the female: male ratio 7:1.<sup>[10]</sup> The possible explanation may be the influence of sex hormone (estrogen) and high level of interferon inducing more autoimmunity in female SLE patients.<sup>[21]</sup>

Among different systemic manifestations, the common manifestations in the present study were constitutional symptoms (92.3%) followed by mucocutaneous, renal, musculoskeletal, gastrointestinal and neurological manifestations. Similar findings were also observed in an American study where 75% of the patients had constitutional symptoms. Mucocutaneous, renal and neurological symptoms were observed in 90%, 55% and 27% patients respectively.<sup>[22]</sup> In an Egyptian study 81% of pSLE patients had renal involvement followed by constitutional symptoms(77%) and others.<sup>[23]</sup> This variation of presentations in different studies may be due to differences in the sample size, ethnic and geographical variations.

In this study, 52.3% of the pSLE had positive coombs test. Benseller and Silverman observed coombs test positivity in 30-40% of pSLE patients in their study whereas Bakr showed 51% pSLE patients with coombs positive hemolytic anemia in his study.<sup>[24,23]</sup> An Omani study found 60% cases with positive coombs test in their series.<sup>[20]</sup> All these findings correlate well with the present study.

In this study, 61% of coombs positive patients had moderate anemia and 26.4% had severe anemia and the association of positive coombs test with the degree of anemia was not statistically significant. In a multicenter study, 30 to 40% pSLE patients had positive coombs test, among them 10% were found to have overt hemolysis and presented with severe anemia.<sup>[8]</sup> Voulgarelis et al. found, only 18% coombs positive patients presented with severe anemia in their study.<sup>[25]</sup> Literatures suggest that positive coombs test may not always correlate with severity of anemia.<sup>[26,27]</sup> In a chronic illness like SLE, usually low grade hemolysis is present which may not manifest severe anemia in coombs positive patients.<sup>[28]</sup>

Anti-cardiolipin (aCL) antibodies were positive in 33.9% of cases in this cohort whereas it was positive in 52.9% of coombs positive and 12.9% in coombs negative patients. The association was highly significant between coombs positivity and the presence of aCL antibodies (P-value 0.001). Voulgarelis et al. found a significant association of coombs positive hemolytic anemia with presence of aCL antibodies.<sup>25</sup> Present study findings are also similar with the Assalie et al. study where a significant association was found between coombs positive anemia with positive aCL antibodies.<sup>[12]</sup> The possible explanation may be the cross-reactivity of aCL antibodies with cardiolipin antigen, the most abundant phospholipid of the erythrocyte membrane causing immune mediated hemolysis in SLE patients.<sup>[29]</sup>

Association of positive coombs test with renal and neurological manifestations were observed in few studies.<sup>[11,28]</sup> Aleem et al. found a significant association between renal involvement and coombs positivity in

pSLE patients.<sup>[11]</sup> Another multiethnic study showed that 36% of coombs positive pSLE patients had renal involvement which was not significant.<sup>[28]</sup> Jeffries et al. found a significant association between neurological symptoms and positive coombs test in a cohort of pSLE patients.<sup>[30]</sup> Fang et al. found that only 10% of patients with positive coombs test had neurological manifestations but the association was not significant.<sup>[30,31]</sup> In the present study, 47.1% of pSLE patients with renal involvement had coombs positive hemolytic anemia but it was not significant. In Aleem et al. study, 80% patients with renal involvement presented with microscopic hematuria whereas in the present study, only 34% patients presented with hematuria.<sup>[11]</sup> So, Hematuria could be a confounding factor for variation in this study. Neurological manifestations were observed in 23% of pSLE patients and of them 14.7% had positive coombs test in this study, but the association was not significant. This finding also differs from the Jeffries et al. study where 40% of pSLE patients presented with neurological manifestations and association was significant in their study.<sup>[30]</sup>

A number of our patients with mucocutaneous and musculoskeletal manifestations had positive coombs test but the association was not significant. This finding was comparable to a study conducted by Elezi et al. where patients with mucocutaneous and musculoskeletal involvement didn't show any significant difference between coombs positive and coombs negative group.<sup>[32]</sup>

## CONCLUSION

Coombs positive hemolytic anemia is common in pSLE patients. An association of positive coombs test with aCL antibodies was highly significant. However, significant association was not found between positive coombs test with different clinical manifestations.

## Key message

*What is already known about this subject?*

- Positive coombs test is common hematological finding in pSLE patients.

*What does this study add?*

- Coombs test positivity is common in pSLE patients and significantly associated with anticardiolipin (aCL) antibodies.

*How might this impact on clinical practice?*

- This study identified hematological abnormalities in pSLE patients, ensure effective treatment, and minimize this disease's morbidity.

**Competing interests:** None.

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