Altered CD19/CD22 Balance in Egyptian Children and adolescents with Systemic Lupus Erythematosus

[1]Zeinab A. El-Sayed, [2]Seham M. Ragab, [3]Khaled A. Khalifa, [3]Ramy A, El- Ashmawy

[1]Department of Pediatrics, Faculty of Medicine, Ain Shams University, [2]Department of Pediatrics, Faculty of Medicine, Menoufiya University, [3]Department of Clinical Pathology, Faculty of Medicine, Menoufiya University, [4]Department of Clinical Pathology, Shibin El-Koom Teaching Hospital, Egypt.

B cells from systemic lupus erythematosus (SLE) patients display signalling defects that may underlie disease pathogenesis activity.CD19 and CD22 play a major role as regulators of B-cell response. The aim of this study was to clarify the relationship between B cell surface markers namely CD19, CD20 and CD22 expression and clinical and laboratory indices of SLE activity. The study included 33 SLE patients and 20 healthy children and adolescents as controls. Flowcytometric assay of dual markers, CD19/CD20, and CD20/CD22 was done. SLE disease activity was assessed by SLEDAI score. CD22% was significantly higher while CD20% was significantly lower in the study compared to the control group. No significant difference was observed in both groups with respect to CD19% or CD19/CD22% ratio. The level of CD22 expression was significantly lower in high and very high active cases than in mild and moderate cases and negatively correlated with SLDEAI score and ESR. Results obtained showed that, B cell surface receptors CD20 and CD22 are significantly affected in patients with SLE, pointing to their possible involvement in the aetiopathogenesis of the disease and in the regulatory mechanisms in response to the immune disturbance.

Systemic lupus erythematosus (SLE) is an extremely complicated disease with considerable heterogeneity in clinical manifestations and disease course, characterized by pathogenic autoantibody formation, immune complex deposition, and end organ damage (Looney *et al.,* 2004). The precise reason for the abnormal autoimmunity that causes lupus is not known. Inherited genes, viruses, ultraviolet light, and certain medications are all factors that may play a role in the development of disease (Mok and Lau, 2003). Although other immune cells play a role in SLE, B cells from SLE patients display signalling defects that may underlie pathogenesis and explain the characteristic hyperactivity of B cells in active disease (Pugh-Bernard and Cambier, 2006). Humoral immune responses and the production of auto antibodies are regulated in part by signalling through B cell Ag receptors. Autoimmunity and immune responses are further regulated or "fine tuned"-by signal transduction molecules that amplify or inhibit Ag receptor signalling during responses to self and foreign Ags. These regulatory molecules include a subset of functionally interrelated cell surface receptors, such as CD19 and CD22, and their intracellular signalling components. An excess in CD19 cell surface expression results in increase in antinuclear antibodies production thus small changes in CD19 expression can induce autoantibody production (Sato *et al.,* 2000). The absence of CD22 expression lowers the signalling threshold for B cell receptor (BCR)-crosslinking and can thus influence the fate of the B cell (Nitschke *et al.,* 1997).

B cell dysfunction has been thought to be critical in the pathogenesis of systemic lupus erythematosus (SLE), with evidence for direct pathogenic roles for at least some autoantibodies, most notably anti-double-stranded (ds)DNA (Gorman *et al.,* 2003).

CD19 is a B-cell-specific member of the Ig superfamily expressed by early pre-B cells

from the time of heavy chain rearrangement until plasma cell differentiation. The CD19 cell surface density is tightly regulated during B-cell differentiation (Yasutomo, 2003). CD22 is a transmembrane sialoglycoprotein and a member of the immunoglobulin super family. Its expression is restricted to lymphocytes of the B cell lineage and is highly developmentally regulated. Cell surface expression is lost during terminal differentiation into plasma cell and after B cell activation (Carnahan *et al.,* 2003). CD19 and CD22 alter signalling through membrane immunoglobulin of B cells by binding cytosolic proteins. Biochemical and genetic studies have shown that these receptors enable B cells to amplify responses to certain T-cell-dependent antigens (CD19), to restrict their response to T-cell zones of secondary lymphoid organs (CD22) (Doody *et al.,* 1995). CD19 and CD20 markers, appear early in the process of B cell development. They remain present until the stage of the mature B cell in the periphery, where conversion to a plasma cell is associated with loss of CD20, although the CD19 marker is still detectable. The role of CD20 in B cell physiology remains uncertain. Possible roles include its functioning as a calcium channel subunit (Gorman *et al.,* 2003).

The aim of this study was to clarify the relationship between B cell surface markers namely CD19/CD20 and CD22/CD20 expression and clinical and laboratory indices of SLE activity.

Subjects and Methods

This study was carried out at the Paediatric Allergy and Immunology unit, Children's Hospital, Ain Shams University and Paediatric Department, Menoufiya University Hospital. The study was conducted on 3 children (age, female <1- >11; male <1- >12 year) and 30 adolescents (age, female >11-18 year; male >12-18 year) with SLE fulfilling the revised classification criteria for SLE of the American College of Rheumatology "ACR" (Gladman *et al.,* 2002 and Klein-Gitelman, 2008). Four normal children (age, female <1- >11; male <1- >12 year) and 16 normal adolescents (age, female >11-18 year; male >12-18 year) of matched age and sex were involved as a control group. The Ethics Committee approved the study and informed written consents were obtained from the caregivers of each patient and healthy child before enrolment in the study.

Patient group: included 33 patients with SLE; 28 females (84.8%) and 5 males (15.2%), age ranged between 6 and 18 years. Duration of illness ranged between 6 months and 17 years.

As controls, 20 children were included in the study. 12 Females (70.59%) and 8 males (29.41%) whose age ranged between 9 and 18 years. They had no evidence of rheumatic disease or such a history in any of their family members.

Methods

Full history taking was obtained from patients or their caregivers including personal history, social history, family history and disease duration. Study children were then subjected to thorough clinical examination including general and abdominal examination and assessment of disease activity.

In SLE group, the disease activity was assessed by Systemic lupus erythematosus disease activity index [SLEDAI] (Gladman *et al.,* 2002; Klein-Gitelman, 2008). Activity categories was defined on the basis of SLEDAI score as: no activity (SLEDAI=O), mild activity (SLEDAI =1-5), moderate activity (SLEDAI=6-10), high activity (SLEDAI=11-19) and very high activity (SLEDAI>20) (Cook *et al.,* 2000). Accordingly, 13 children were mild active 2 were moderate active patients, 14 were high active patients and 4 were very high active patients. All patients were receiving: Oral NSAID, prednisolone, calcium, and vitamin D. Moreover, five patients were receiving pulsed cyclophosphamide together with monthly pulsed methylprednisolone. Two patients died of severe activity with multiple organs involvement and were excluded from the study.

Laboratory investigations performed only for SLE patient included complete Blood count (CBC): using ABX-Pentra-80, hematology analyzer (HORIBA medical France), complete urine analysis for lupus patients (Medi-Test Combi 10 SGL; Germany), and erythrocyte sedimentation rate (ESR): using Westergren method (Westergren, 1921). Antinuclear antibodies (ANA) were assessed in the two groups by indirect immunoflourescent microscopy (IMMCO Diagnostics; USA) according to manufacture's instructions.

Flowcytometric assay of dual colour analysis for CD20/CD19 and CD20/CD22 was performed using

flow cytometry (FACSCalibar BD, USA), with FITC conjugated CD20; PE conjugated CD19 and CD22 monoclonal antibodies (PharMingen BD Co. USA).

Venous blood (2 ml) was collected from each studied subject in a vacutainer tube containing EDTA. A full blood count was done to determine the leucocytes count.

Tubes were labelled with the used monoclonal antibodies CD20/CD19 or CD20/CD22 and 10 µl of appropriate conjugated monoclonal antibodies were added. FITC and PE were used as negative control. Two tubes were labelled by single monoclonal antibody to adjust the compensation. Following the addition of blood, tubes were vortexed and incubated for 30 min. at room temperature. (DAKO® Uti-lyseTM) lysing reagent A (100 ųl) was added for 10 min. followed by, 1 ml of lysing reagent B for another 10 min.

After washing twice by PBS, the samples were re-suspended in PBS.

Statistical Methods

Data collected was statistically analyzed using an IBM personal computer and a statistical package for the social sciences (SPSS) version 11. Chi-square test (X^2) :-was used to study the relation between two qualitative variables, Student t-test:- for comparison between two groups having parametric quantitative variables, Mann-Whitney test(U) for non parametric quantitative variables, ANOVA (f) test :- was used for comparison between three or more groups having quantitative variables, and Pearson correlation (r) was used to detect association between quantitative variables. A p-value of <0.05 was considered statistically significant (Rockette, 1999).

Results

The demographic data of the study group, which consisted of 33 patients, reflected the well known age (mean age of 14.48 years ± 2.94) and sex distribution of SLE. SLE affects predominantly females. Studies have demonstrated that estrogens enhance anti-DNA antibody formation and increase the severity of renal disease (Madhok and Wu, 2007). Results revealed no significant difference ($p>0.05$) in the mean age between the study (14.48 years ± 2.94) and the control groups (13.42 years ± 2.87). Regarding family history of SLE or autoimmune disease, it was found that one patient (3.03%) had positive family history, while 32 patients (96.97%) had negative family history, and there is no significant difference between study and control groups. Malar rash a characteristic skin lesion of SLE was encountered in 20 patients out of 33 patients (61%). Discoid rash, however, was found in 2 out of 33 patients (6%) only. Photosensitivity was positive in 19 patients (57.57%). Mouth ulcers were detected in 20 patients (61%) out of the 33 patients. Ten of the studied SLE patients (30%) had cardiac involvement in the form of pericarditis with or without pericardial effusion. As regards renal manifestations (nephritis); it was found that 20 patients (61%) had lupus nephritis. Pleural effusion was diagnosed in 5 patients out of the 33 studied patients(15%). Anaemia was seen in 51.5%. Thrompocytopenia was found in 20 patients (61%). SLEDAI ranged between one and thirsty six (mean 9.73 ±2.53) with 61% of patients exhibiting moderate to very high disease activity (Table 1).

Table 1. Demographic and Clinical SLE Cases and Controls.

Parameter	Study (n =33)	Control (n=20)	*P value
Age			
($\bar{x}$ ±SD)	14.48 ± 2.93	13.42 ± 2.87	NS
Range (yrs)	6-18	7-18.2	
Gender n (%)			
Male	5 (15.15)	12 (60)	0.00001
Female	28 (84.85)	8 (40)	
Family History [n (%)]			
Positive	1 (3.03)	0 (0.0)	NS
Negative	32 (96.97)	20 (100.0)	
Weight percentile [n (%)]			
<5th	0 (0)		
5-95th	31 (93.94)		
>95th	2 (6.06)		
Height percentile [n (%)]			
<5th	5 (15.15)		
5-95th	28 (84.85)		
>95th	0 (0)		
Malar rash [n (%)]	20 (60.6)		
Discoid rash [n (%)]	2 (6.06)		
Photosensitivity [n (%)]	19 (57.57)		
Mouth ulcer [n (%)]	20 (60,6)		
Arthritis or arthralgia[n (%)]	33 (100)		
Cardiac manifestations [n (%)]	10 (30.3)		
Nephritis [n (%)]	20 (60.6)		
Pleural manifestations [n (%)]	5 (15.15)		
Seizures [n (%)]	3 (9.09)		
ANA [n (%)]	20 (60.6)		
Anemia [n (%)]	17 (51.51)		
Leucopenia [n (%)]	23 (69.69)		
Thrompocytopenia [n (%)]	20 (60.6)		
SLEDAI			
($\bar{x}$ ±SD)	9.73 ± 2.53		
Range	1-36		

*<0.05 is significant. NS= not significant

As expected ESR level was significantly higher in the study group than the control group (Table 2).

T cell regulation markers, the percentage of CD22 was observed to be significantly higher in SLE cases, while CD20% was significantly lower than the control group. There were no significant differences between SLE cases and controls regarding the CD19 % or CD19/CD22 ratio (Table 2). According to the classification used, we found that 13 SLE cases were with mild active disease, two moderate active, 14 high active and 4 very high active disease; mean duration of disease was 4.52 ± 0.57 years. To determine a relation between the different parameter measured and disease manifestations, we compared SLE cases with or without nephritis, positive and negative ANA.

SLEDAI and ESR levels were significantly higher in patients with nephritis than those without nephritis (Table 2). Lymphocytic count was significantly lower in patients with nephritis than patients without nephritis. Expression of CD19% and CD20%, were higher while CD22% expression was lower in patients with nephritis than those without nephritis. However, the differences were not statistically significant (Table 2). SLEDAI, ESR and CD19% were significantly higher in patients with positive ANA than patients with negative ANA., while, lymphocytic count was significantly lower in patients with positive ANA than patients with negative ANA:, CD20% was lower in patients with positive ANA than patients with negative ANA yet the difference was not significant(Table 2). Comparing drug regimen in SLE cases, we observed that SLEDAI and ESR were significantly higher in patients receiving steroids only than patients receiving cytotoxic drug plus steroid. On the other hand lymphocytic count was significantly lower in patients receiving steroids only than patients receiving cytotoxic drug plus steroid. Expression of studied CD markers studied was similar in both grous (Table 2). Comparing the stages of disease activity:- SLEDAI and ESR were significantly elevated in high and very high active cases than mild and moderate cases. The lymphocyte count and CD22% was significantly lower in high and very high active cases than in mild and moderate cases. CD20% was insignificantly elevated in high and very high active than in mild and moderate cases (Table 2). Notably a significant negative correlation between ESR and CD22%, was obtained (Figure 1), however no significant correlation was observed with CD19%, CD20%, or the CD19/CD22 ratio in patients with SLE. A negative significant correlation was also observed between disease activity estimated by SLEDAI and CD22% (Figure 2). In addition, SLEDAI correlated positively with ESR.

Table 2. Comparison of SLEDAI and Laboratory Investigations between SLE Cases and Control and between Different Entities of SLE Patients.

Groups	SLEDAI	ESR mm3/hour	Lymphocytic count (1000/m m^3)	CD19%	CD20 %	CD22%	CD19/CD22 ratio
Controls (n=20)	ND	6.0 ± 1.91	ND	1.61 ± 0.00	4.05 ± 0.02	0.59 ± 0.00	2.54 ± 0.22
All SLE cases (n=33)	9.73 ± 2.53	41.0 ± 26.42	3.5±0.75	1.87 ± 0.20	1.49 ± 0.05	1.49 ± 0.05	5.57 ±3.36
P value	ND	0.0001	ND	NS	0.001	0.036	NS
SLE with nephritis (n=20)	18.95±6.79	53.80±27.25	1.07 ±0.22	1.88± 0.22	1.71 ± 0.10	0.91±0.01	1.3 ±0.83
SLE without nephritis (n=13)	1.77± 0.44	21.62± 2.22	2.4 ± 0.49	1.87± 0.21	1.18 ±0.01	2.21 ± 0.11	1.73 ± 1.49
P value	0.0001	0.0001	0.001	NS	NS	NS	NS
Patients with +ve ANA (n=20)	16.55±9.78	53.80±27.25	1.07 ±0.22	0.079± 0.26	0.012 ±0.012	0.015±0.019	1.39 ±0.86
Patients with –ve ANA (n=13)	5.46±5.88	25.23± 7.61	2.43 ± 0.49	0.011± 0.014	0.012 ±0.012	0.016 ± 0.015	1.29 ± 1.49
P value	0.0001	0.0001	0.001	0.04	NS	NS	NS
Patients receiving cytotoxic drug plus steroid (n=)	1.6±0.55	20.20±1.09	2.65 ± 0.25	0.01 ± 0.004	0.019 ±0.016	0.17±0.35	1.1 ±0.65
Patients receiving steroid only (28)	14.07± 9.70	44.86 ± 27.04	1.33 ± 0.53	0.017 ±0.019	0.059 ± 22	0.016 ± 0.018	1.4 ± 1.8
P value	0.001	0.001	0.01	NS	NS	NS	NS
Mild and patients moderate active (n=15)	2.78 ± 0.55	23 ± 4.34	2.22 ± 0.69	0.013 ± 0.011	0.016 ± 0.017	0.07 ± 020	1.29 ± 1.4
High and very high active patients (n=18)	21.85 ± 8.77	66.22 ± 27.69	1.05 ± 0.24	0.018 ± 0.021	0.082 ± 0.27	0.013 ± 0.017	1.4 ± 0.84
P value	0.0001	0.001	0.01	NS	NS	0.01	NS

ND =Not determined. *P*<0.05 is significant. NS= not significant

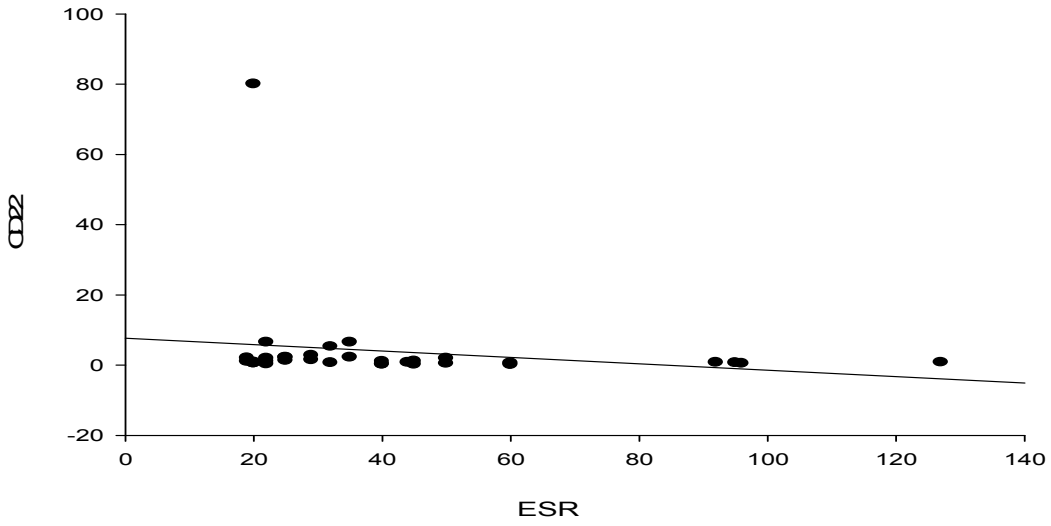

Figure 1. Correlation between ESR and CD22% in cases with systemic lupus erythematosus.

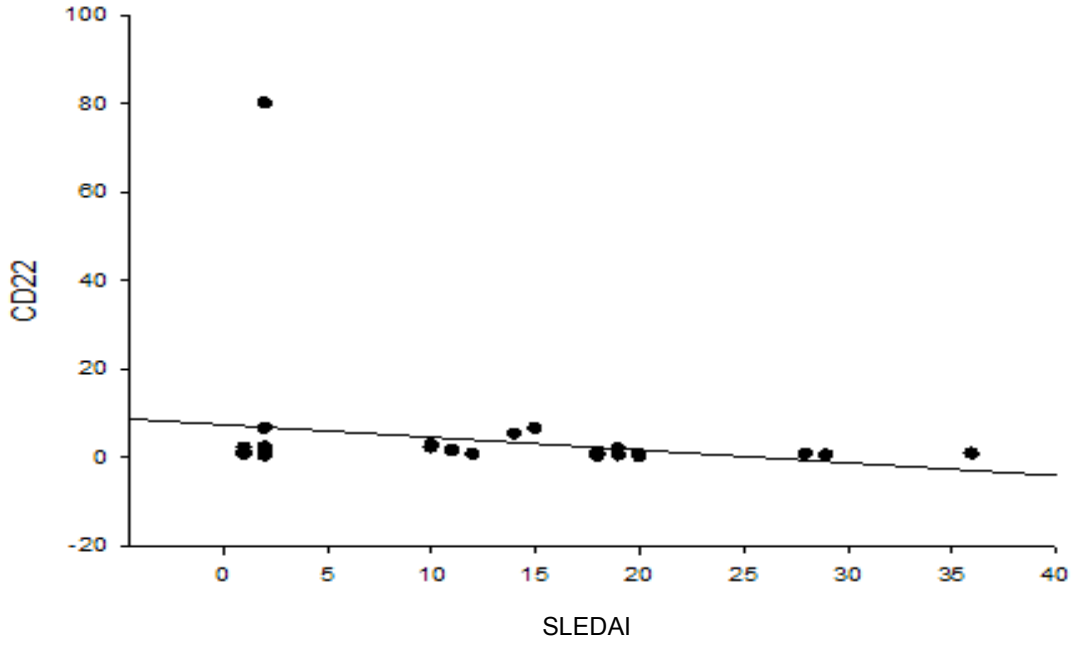

Figure 2. Correlation between SLEDAI and CD22% in cases with systemic lupus erythematosus.

Discussion

Recently the efficacy of B cell depletion therapy supports the key role of the B cell in SLE pathogenesis. B-cell dysfunction in SLE have been defined; include abnormal expression or function of key signaling molecules, dysregulation of cytokines with key B cell effects, and perturbations in B cell developmental subsets. Many of these defects may contribute to or be reflective of abnormalities in B cell tolerance. Both antibodydependent and antibody-independent mechanisms of B cells are important in SLE. Autoantibody-independent B cell functions have been postulated to include antigen-presentation, T-cell activation and polarization, and dendritic cell (DC) modulation (Renaudineau, *et al.,* 2004; Anolik, 2007).

In our study the mean age of study-cases reflects disease onset be around adolescent age in addition, to the increased female to male ratio. It was previously reported that lupus is predominantly a female disease being attributed to estrogens hormonal effect, while in children the female-to-male ratio is lower, since sex hormone effects are presumably minimal (Madhok & Wu 2007; Tirumani & Leber, 2008).

Although history of lupus in parents or siblings was associated with an increased risk for SLE (Cooper *et al.,* 2002) most of our study-cases did not have a family history of SLE or autoimmune disease

Definition of renal involvement varies but overt renal disease is found in at least one-third of our SLE patients, with up to 80% of children developing lupus nephritis. Clinical presentation has been found to bear little relationship to renal biopsy findings (Cross & Jayne, 2005). Different scoring systems were put to assess activity of the disease process in SLE patients. In the present work, SLEDAI was the one used to follow disease status. Disease activity was more prominent with younger age and shorter duration of the disease. This was embodied in the significant negative correlation of these latter two with SLEDAI (P=0.015 and P=0.035 respectively). ESR is a simple investigational tool that helps to detect disease activity. It was elevated in 100% of patients with active disease as defined by SLEDAI. The mean value of patients in high activity was significantly higher than in those in mild activity.

Vilá and coworkers, (2005) demonstrated that mild, moderate, and marked ESR elevations are strongly associated with disease activity in SLE. Moderate and marked ESR elevations are also associated with accrual damage. Data suggest that ESR could be used to assess disease activity and predict organ/system damage in a relatively rapid and inexpensive manner in SLE.

Autoimmunity and immune responses are regulated or "fine tuned" by signal transduction molecules that amplify or inhibit Ag receptor signalling during responses to self and foreign Ags. These regulatory molecules include a subset of functionally interrelated cell surface receptors, such as CD19 and CD22, and their intracellular signaling components signalling including Lyn, Btk, Vav, and the SHP1 protein tyrosine phosphatase (Sato *et al.,* 2000).

CD19 and CD22 alter signalling through membrane immunoglobulin of B cells by binding cytosolic proteins. Biochemical and genetic studies have shown that these receptors, namely CD19 enable B cells to amplify responses to certain T-cell-dependent antigens, and CD22 restricts their response to T-cell zones of secondary lymphoid organs (Doody *et al.,* 1995). As regards CD markers, CD22% was significantly higher in the study group than the control group. These results are in agreement with those of Lajaunias *et al.,* (2003) who found that CD22 % was increased on circulating B cells in parallel with progression of SLE. So high expression of CD22 on circulating B cells is a marker for development of SLE, suggesting a role for

CD22 in the regulation of humoral immune response in SLE. Hence, it can be a deduced that high levels of CD22 expression is an adaptation to overcome the excessive auto antigen receptor signalling and to restrict B cells responses and autoantibody production.

In favour of this deduction is the finding in the present work that CD22% was significantly higher in mild and moderately active group (0.07 ± 020 unit) than in high and very high active groups in which CD22 expression was not enough to prevent severe disease (0.013 ± 0.017 unit) (P=0.01). Improvement of SLE was recognized among patients with an increased proportion of CD22-positive cells (Suzuki et al., 2006). This is further supported by our finding of a significant negative correlation between CD22 % and both of SLEADAI and ESR in SLE patients.

Eisenberg, (2006) demonstrated that anti-CD22 is a humanized monoclonal antibody that recognizes a pan-B-cell marker. It potentially down regulates B cell activity through negative signalling, as well as depleting B cells. Poe *et al., (*2000) demonstrated that CD22 is involved in a negative regulatory effect on BCR signal transduction.

The expression of CD19 was higher yet insignificant in the patient than the control groups of the present work. This is in agreement with Suzuki et al (2006). The reason that the difference was minor in the present study might be the consequence of therapy, as all of them have already been maintained on immunosuppressant therapy. Therefore, it can be hypothesized that CD19 expression is high in SLE, being incriminated as a probable the basic stimulus that initiates the autoimmune process and then tend to normalize with treatment. Measurement of its level of expression in newly diagnosed SLE before starting therapy will decide on the validity of this hypothesis.

Kuroki *et al.,* (2002) found that CD19 regulates the signalling for B lymphocyte development, activation and proliferation. CD19 deficiency and over expression were shown to result in hypogammaglobulinemia and autoantibody production, respectively. Also, Inaoki *et al.,* (1997) demonstrated that the CD19 cell surface molecule regulates signal transduction events critical for B lymphocyte development and humoral immunity. Increasing the density of CD19 expression renders B lymphocytes hyper-responsive to transmembrane signals, Thus, CD19 over expression shifts the balance between tolerance and immunity to autoimmunity by augmenting antigen receptor signaling.

The level of expression of CD19 varied slightly with disease activity; the mean value was insignificantly higher in patients with high to very high activity and the levels did not correlate neither with ESR nor with SLEDAI. This reflects its incrimination in the autoimmune inflammation and in support of this nation is its higher expression in patients with positive antinuclear antibodies.

In the present study, there was no significant difference between cases and controls regarding the CD19/CD22 ratio and this came in agreement with Suzuki *et al.,* (2006). The unchanged balance between CD19 and CD22 as represented by the CD19/CD22 ratio in SLE patients is a reflection of the higher insignificant expression of the numerator CD19. In the present study also, the mean CD19/CD22 ratio was insignificantly higher in patients with higher grades of active disease reflecting the lower CD22 in the former group as compared to patients having mild to moderately active disease. However, it did not correlate with ESR or SLEDAI and did not differ with respect to specific disease entities as nephritis. Fujimoto and Sato, (2007) demonstrated that CD19 and CD22 do not merely regulate B-cell receptor (BCR) signals

independently, but they have their own regulatory network. Walker and Smith, (2008) demonstrated that CD22 is an inhibitory coreceptor of the (BCR), and plays a critical role in establishing signalling thresholds for B-cell activation.

The recognition of B cell dysfunction as central to SLE pathogenesis and the large body of evidence implicating abnormalities in the B-cell compartment in SLE, led to a recent therapeutic focus to develop interventions that target the B-cell compartment by multiple mechanisms. Anti CD20, a mouse-human chimeric monoclonal antibody against CD20 that specifically depletes B cells, It appears to have the potential to induce clinical remission in severe, refractory SLE. B-cell depletion has the potential to induce disease amelioration by inhibiting autoantibody production and/or by interfering with other B-cell pathogenic functions (Sabahi & Anolik, 2006; Chehab *et al.,* 2007). This corroborates the fact that SLE is a B-cell mediated disease and that B-cell targeted antibodies may be active in the treatment of this debilitating illness.

In this study B cell of SLE patients exhibited highly significantly decreased expression of CD20 as compared to controls ($P< 0.001$). This low expression in SLE patients points to a probable inherent defect that could be understood as to cause rapid differentiation of B cells into plasma cells with loss of CD20 expression as plasma cells are known to be devoid of CD20 expression. Enhanced frequency and cell number of peripheral plasma blasts that lack the expression of CD20 is characteristic of active lupus in both children and adults (Odendahl *et al.,* 2003). Another different explanation is relevant to the fact that our patients were chronically treated SLE patients with a mean disease duration of (4.52±0.57 years). They were maintained on steroid therapy with or without IV pulses of methyprednisolone and cyclophosphamide (both drugs exert a B cell depleting effect).

CD20 expression did not correlated with SLEDAI. Furthermore, it did not correlate with ESR and it was also insignificantly different between mild and moderate active cases compared to high and very high active ones. This further stresses the absence of a relation between CD20 expression and the grade of disease activity. In the mean time, the decreased CD20% is not a consequence of targeting of lymphocytes by autoantibodies, otherwise, it would have fluctuated with different states of the disease.

In conclusion, B cell surface receptors namely CD22 and CD20 are significantly affected in patients with SLE pointing to their possible involvement in the aetiopathogenesis of the disease and the regulatory mechanisms evoked in response to the immune disturbance. The role of CD19 could not be decided exactly owing to the fact that all patients were under immunosuppressant medications however there is a tendency for an increase in its expression in patients with SLE especially those with higher grades of activity.

References

1. Anolik JH (2007). B cell biology and dysfunction in SLE. Bull NYU Hosp Jt Dis. 65(3):182-6.
2. Carnahan J, Wang P, Kendall R, Chen C, Hu S, Boone T, Juan T, Talvenheimo J, Montestruque S, Sun J, Elliott G, Thomas J, Ferbas J, Kern B, Briddell R, Leonard JP, Cesano A.(2003). Epratuzumab, a humanized monoclonal antibody targeting CD22: characterization of *in vitro* properties. J Cancer Research; 9:3982-90.
3. Chehab G, Sander O, Fischer-Betz R, Schneider M. (2007). Anti-CD20 therapy for inducing and maintaining remission in refractory systemic lupus erythematosus. J Rheumatol 66(4):328, 330-6.
4. Cook RJ, Gladman DD, Pericak D & Urowitz MB (2000). Prediction of short term mortality in systemic lupus erythematosus with time dependent measures of disease activity. J Rheumatol; 27(8):1892–1895.
5. Cooper GS, Dooley MA, Treadwell EL, St Clair EW, Gilkeson GS.(2002). Risk factors for development of systemic lupus erythematosus:

allergies, infections, and family history. J Clin Epidemiol. 55(10):982-9.

6. Cross J, Jayne D. (2005). Diagnosis and treatment of kidney disease. Best Pract Res Clin Rheumatol. 19(5):785-98.
7. Doody GM, Justement LB, Delibrias CC, Matthews RJ, Lin J, Thomas ML, Fearon DT (1995). A role in B cell activation for CD22 and the protein tyrosine phosphatase SHP Science. 14; 269(5221):242-4.
8. Eisenberg R. (2006). Targeting B cells in systemic lupus erythematosus: not just déjà vu all over again. Arthritis Res Ther; 8(3):108.
9. Fujimoto M, Sato S. (2007). B cell signaling and autoimmune diseases: CD19/CD22 loop as a B cell signaling device to regulate the balance of autoimmunity. J Dermatol Sci. 46(1):1-9.
10. Giannouli S, Voulgarelis M, Ziakas PD, Tzioufas AG. (2006). Anaemia in systemic lupus erythematosus: from pathophysiology to clinical assessment. Ann Rheum Dis. 65(2):144-8.
11. Gladman DD, Ibanez D, Urowitz MB. (2002). Systemic lupus erythematosus disease activity index 2000. J Rheumatol; 29(2): 288–291.
12. Gorman C, Leandro M, Isenberg D (2003). B cell depletion in autoimmune disease. Arthritis Res Ther; 5 Suppl 4:S17-21.
13. Inaoki M, Sato S, Weintraub BC, Goodnow CC, Tedder TF (1997). CD19-regulated signaling thresholds control peripheral tolerance and autoantibody production in B lymphocytes J Exp Med. 1; 186(11):1923-31.
14. Klein-Gitelman MS (2008). Systemic Lupus Erythematosus. Cited on http://emedicine.medscape.com/article/ 1008066-overview.
15. Kuroki K, Tsuchiya N, Tsao BP, Grossman JM, Fukazawa T, Hagiwara K, Kano H, Takazoe M, Iwata T, Hashimoto H, Tokunaga K.(2002): Polymorphisms of human CD19 gene: possible association with susceptibility to systemic lupus erythematosus in Japanese. Genes Immun. 3 Suppl 1:S21-30
16. Lajaunias F, Ida A, Kikuchi S, Fossati-Jimack L, Martinez E, Moll T, Law C, Izui S. (2003). Differential control of CD22 ligand expression on B and T lymphocytes, and enhanced expression in murine systemic lupus: Arthritis Rheum. 48(6):1612-21.
17. Looney R, Anolik J, Sanz I. (2004). B lymphocytes in systemic lupus erythematosus: lessons from therapy targeting B cells. Lupus; 13(5):381-90.
18. Madhok R, Wu O. (2007). Systemic lupus erythematosus. Am Fam Physician. 1; 76(9):1351-3. Review.
19. Mok C, Lau C. (2003). Pathogenesis of systemic lupus erythematosus. J Clin Pathol. 56(7):481-90.
20. Nitschke L, Carsetti R, Ocker B, Kohler G, Lamers M. (1997). CD22 is a negative regulator of B-cell receptor signalling. J Curr Biol. 1; 7(2): 133-43.
21. Odendahl M, Keitzer R, Wahn U, Hiepe F, Radbruch A, Dörner T (2003). Perturbations of peripheral B lymphocyte homoeostasis in children with systemic lupus erythematosus. Ann Rheum Dis; 62(9):851-8.
22. Poe JC, Fujimoto M, Jansen PJ, Miller AS, Tedder TF (2000). CD22 forms a quaternary complex with SHIP, Grb2, and Shc. A pathway for regulation of B lymphocyte antigen receptor-induced calcium flux. J Biol Chem. 9; 275(23):17420-7.
23. Pugh-Bernard AE, Cambier JC. (2006). B cell receptor signaling in human systemic lupus erythematosus. Curr Opin Rheumatol. 18(5):451-5. Review.
24. Renaudineau Y, Pers JO, Bendaoud B, Jamin C, Youinou P. (2004). Dysfunctional B cells in systemic lupus erythematosus. Autoimmun Rev. 3(7-8):516-23
25. Rockette H E. (1999) "Occupational biostatistics" in Rockette H E, ed. Environmental occupational Medicine 3rd ed. Chapter 6: 57-66.
26. Sabahi R, Anolik J. (2006). B-cell-targeted therapy for systemic lupus erythematosus. Drug; 66(15):1933-48.
27. Sato S, HasegawaM, Fujmoto M, Tedder TF, Takehara K (2000). Quantitative Genetic Variation in CD19 Expression Correlates with Autoimmunity. J Immunol; 165:6635-43.
28. Suzuki J, Nakano S, Nakairi Y, Mitsuo A, Amano H, Morimoto S, Tokano Y, Takasaki Y. (2006). CD19/22 balance relates to improvement of disease activity in systemic lupus erythematosus. J Mod Rheum; 16(4): 235-8.
29. Tirumani A, Leber M J. (2008). Last update of Systenic lupus erythematosus. Cited on http://emedicine. medscape.com/article/809378-overview.

30. Vilá LM, Alarcón GS, McGwin G Jr, Bastian HM, Fessler BJ, Reveille JD.(2005). LUMINA Study Group. Systemic lupus erythematosus in a multiethnic cohort (LUMINA): XXIX. Elevation of erythrocyte sedimentation rate is associated with disease activity and damage accrual. J Rheumatol. 32(11):2150-5.

31. Walker JA, Smith KG. (2008). CD22: an inhibitory enigma. Immunology. 123(3): 314-25. Westergren A. (1921). Studies of the suspension stability of the blood in pulmonary tuberculosis. Acta Medica Scadinavica 54: 247-282.

32. Yasutomo k. (2003). Pathological lymphocyte activation by defective clearance of self-ligands in systemic lupus erythematosus. Rheumatology (Oxford). 42(2):214-22.