DISCUSSION

Systemic lupus erythematosus (SLE) is a prototypical systemic autoimmune disease in which tolerance against ubiquitous self-antigens is lost (Martin et al., 2014). The profound heterogeneity of SLE causes problems regarding diagnostic accuracy in clinical practice and particularly in clinical research (Ighe et al., 2015).

Clinical symptoms are heterogeneous and range from mild skin rashes to more severe multi-organ manifestations, primarily involving the kidney, the brain and blood (Liu and Davidson, 2012).

The clinical diagnosis of SLE relies on the identification of markers of systemic tolerance failure e.g. anti-nuclear and anti-double stranded DNA antibodies, associated to the presence of target organ involvement e.g. glomerulonephritis and skin rash (Crispín et al., 2011). Mechanisms of tissue damage in patients with SLE involve autoantibody and immune complex deposition, as well as infiltration of tissues by lymphocytes (Crispín et al., 2008).

This condition is characterized by both innate and adaptive immune dysregulation but the relative importance of cytokines operative in these systems is questionable (Vincent et al., 2013). The disease course is unpredictable, with episodes of flares and remissions, sometimes leading to permanent organ damage and preterm death (Ighe et al., 2015).

Chronic immune activation in SLE leads to the production of large amounts of inflammatory cytokines and contributes actively to local inflammation and tissue damage (Murphy et al., 2013).

These cytokines may exert either pro or anti-inflammatory effects, or both, depending on specific local microenvironment, thus contributing greatly
to SLE pathogenesis. Understanding these cytokine abnormalities may be beneficial in developing effective targeting therapy (Su et al., 2012).

Among those cytokines is IL-17. It is currently the focus of increasing attention. Indeed, from the past 10 years, numerous lines of evidence have suggested it could play a major role in the pathogenesis of SLE (Martin et al., 2014). IL-17 is produced by several cell subsets including CD4⁺ T cells, CD8⁺ T cells, γδ T cells, NK cells, neutrophils, dendritic cells, microglia, eosinophil, astrocytes and oligodendrocytes (Shabgah et al., 2014).

IL-17 has been associated with the pathogenesis of a range of autoimmune diseases, including rheumatoid arthritis (RA), systemic sclerosis, multiple sclerosis (MS) and SLE (Vincent et al., 2013), it promotes inflammation by inducing local chemokine and cytokine secretion and is essential for the clearance of certain pathogens such as bacteria and fungi. Moreover, IL-17 has been linked to the instigation of immune-mediated organ damage in the context of several autoimmune diseases (Su et al., 2012).

Multiple lines of evidence have identified IL-17 as a key player in the pathogenesis of SLE. Lupus mice deficient in IL-17 or IL-17 receptors were shown to be protected from lupus nephritis (Robinson et al., 2013).

Diverse cytokine abnormalities common in patients with SLE may skew the differentiation of T cells into IL-17 producing CD4⁺ and DN T cells. This phenomenon could promote the auto-immune process by increasing the activation of immune cells and stimulating B cell proliferation and antibody production (Crispín et al., 2011).

The objective of this study was to evaluate whether IL-17 is involved in autoimmune response in patients with SLE and to assess the proportion of intracellular IL17 in some T cell subpopulation.
The present study was carried out on 60 individuals, they were divided into: 40 SLE patients fulfilling the criteria of The American College of Rheumatology (ACR) (Hochberg, 1997), 37 females and 3 males (group A), and 20 apparently healthy individuals as controls, clinical examinations as well as routine laboratory investigations confirm their healthy state (group B) the selection was according to the inclusion and exclusion criteria set.

Patients were subjected to full history taking and complete clinical examination including general, locomotor system, skin, and cardiovascular chest, neurological and vascular examination.

Investigations included were complete blood picture, ESR, 24 hour protein in urine, serum creatinine, ANA, anti-dsDNA, and flow cytometric examination of CD3⁺, CD4⁺ and CD8⁺ cells and intracellular IL17 in each of them. For flow cytometric examination all blood samples were processed within 24-72 hours of collection. Three colours flow cytometric acquisition was performed using Cell Quest software program (BD).

In our study, the mean age of SLE cases was 31±8.7 years (range: 18 to 48) compared to the mean age of the controls 29.1±7.8 years (range: 18 to 40) was not statistically significant (P=0.425) but Cabrera and coworkers (2013) found that the mean age of SLE cases was 43.67±13.81 years while in controls was 38±12 years.

The female predominance was similar among cases (92.5%) and controls (90%) in this study. Khan and Ahmed (2016) showed that estrogen enhances severity and flares of disease in females. Jeleniewicz and colleagues (2015) reported that 80% of SLE patients were females.

It was observed in our study, that the most frequent clinical variables among SLE patients at the time of taking samples were arthritis 72.5%, malar
rash 70%, alopecia 62.5% and oral ulcers 62.5%, and the least frequent clinical variable was neurologic disorders 0%. But in Zhao and colleagues (2010) study 45% of SLE patients had arthritis, 49% had malar rash, 22% had alopecia, 17% oral ulcer, and 8% had nervous system disorders.

However, Jeleniewicz and colleagues (2015) reported that 50% of SLE patients had arthritis, 40% had rash, 30% had oral ulcers and 30% had photosensitivity.

In our work, the laboratory investigations among SLE group and control group showed a highly statistically significant decrease in haemoglobin concentration in SLE patients when compared with healthy controls (p<0.001), about 82.5% of SLE patients had anemia, 10% had leucopenia and 15% had thrombocytopenia.

This was somewhat in accordance with the study done by Elewa and coworkers (2014) which found 60% of SLE patients had anemia, 25% had leucopenia while 22% had thrombocytopenia. Alarcon and colleagues (2015) also reported that 66% of SLE patients had anemia, 56.4% had leucopenia and 16.3% with thrombocytopenia.

However Sasidharan and colleagues (2012) found that 63% of SLE patients had anemia, 16% had leucopenia and 40% had thrombocytopenia.

In the present study there was a highly statistically significant increase in ESR in SLE group when compared with healthy controls (p<0.001). These results were in agreement with Hyoun and colleagues (2013) and Stojan and researchers (2013).

In our study, none of the healthy controls exhibited autoantibodies. However, all SLE patients in our study had positive ANA, while 95% of them had positive anti-dsDNA. These results are in agreement with Elewa and
coworkers (2014) who reported that patients with positive ANA is 100% of SLE patients while 80% had positive anti-dsDNA, however 62% had proteinuria, while it was not in accordance with Zhao and researchers (2010) who found that only 92% of SLE patients with positive ANA, while 36% had positive anti-dsDNA.

It was also observed that 75% of SLE patients had proteinuria. These results agreed with Elewa and coworkers (2014) who reported that 62% of SLE patients had proteinuria.

The current study also investigates the flow cytometric proportion of CD3⁺, CD4⁺ and CD8⁺ T cells in SLE patients and healthy controls. The mean proportion of CD3⁺ T cells in SLE patients was 59.1 (13.8-88.3) while the mean proportion was 65 (47.1-83.2) in controls. While the mean proportion of CD4⁺ T cells in SLE patients was 36.15 (11.0-53.6) and in controls was 39.4 (22.7-56.4). However the mean proportion of CD8⁺ T cells in SLE patients was 31.3 (10.4-61.4) while in controls 31.6 (10.5-63.7).

There was no statistical significant difference in the percentage of CD3⁺, CD4⁺ and CD8⁺ T cells between SLE patients and controls. This comes in accordance with Liu and Wang (2015) who found that there was no statistical significant difference in the frequency of CD4⁺ cells between SLE cases and control subjects.

On the contrary, Wu and colleagues (2014) found that the proportion of CD4⁺ T cells in SLE patients were significantly lower than those in the controls while the proportion of CD3⁺ and CD8⁺ T cells in SLE patients were significantly higher than those in the controls.

In the current work, IL-17 expressing cells were measured by intracellular IL-17 staining using flow cytometry. We observed a higher
percentage of intracellular IL-17 in peripheral blood cells in SLE patients (mean = 92.7 %) than healthy controls (mean = 90.5 %), however this did not reach significance (p= 0.594).

These results were supported by Dolff and colleagues (2011) who found that the proportion of IL-17 producing cells was not significantly increased in SLE patients when compared with healthy controls. However, Crispin and researchers (2008) reported that the fraction of IL-17 expressing T cells was significantly higher in SLE patients than controls.

It was observed in our work that, in SLE patients, most of IL17⁺ lymphocytes were present within CD3⁺T cells (mean=57), while CD4⁺ T cells (mean=35) and CD8⁺T cells (mean=23.4) in SLE patients.

To evaluate frequency and cellular source of IL-17 in SLE, CD3⁺IL17⁺ T cells were compared in SLE patients and healthy controls. Most of IL17⁺ lymphocytes were present within the CD3⁺T cells subset in both SLE patients (mean = 57 %) and healthy controls (mean = 53%), however there was no statistical significant difference in the proportion of CD3⁺IL17⁺ T cells between them (p= 0.210).

The obtained results showed that the proportion of CD3⁺IL17⁺ cells positively correlated with the proportion of IL17⁺ expressing cells in SLE patients (p<0.001).

Dolff and colleagues (2011) reported that the proportion of IL-17 expression in CD3⁺ cells tend to be higher in SLE patients, however this did not reach statistical significance. However Shah and researchers (2010) noticed a significant increase of IL-17 producing cells CD3⁺ CD4⁻ T cells that include DN T cells in patients with SLE compared with controls.
Our study provides a new insight into understanding of intracellular IL17 intensity in CD3+, CD4+ and CD8+ T cells. IL-17 intensity was calculated by using the percentage of cells which were present in peripheral blood sample and the percentage of the same cells containing intracellular IL-17.

By this, the true and actual cells positive for IL-17 could be assessed clearly. As far as our knowledge, this is the first time this idea of understanding is submitted.

In the present study the mean of the intensity of IL-17 in CD3+ T cells in SLE patients was 94.6% while in control subjects was 81.2% there was a highly significant difference (p < 0.001). The proportion of the intensity of IL-17 in CD3+ T cells was significantly positively correlated with both the percentage of IL17+ T cells in SLE patients (p < 0.001) and percentage of CD3+ IL17+ T cells in SLE patients (p < 0.001).

CD3+ AUC failed to discriminate between SLE and controls, CD3+IL17+ showed poor AUC, while intensity of IL-17 in CD3+ showed excellent AUC for discrimination between SLE and controls. Intensity of IL-17 in CD3+ showed significantly higher AUC when compared to CD3+ and CD3+IL17+ (p<0.001, =0.001 respectively). When the sensitivity and specificity of intensity of IL-17 in CD3+ for discrimination between SLE patients and controls using ROC curves were evaluated the sensitivity and specificity were 87.5% and 95% with a cut off value 90.2.

This study also investigate the proportion of CD4+IL17+T cells in cases and control subjects, the proportion of CD4+IL17+ T cells was higher in patients with SLE (mean= 35.16 ) than in controls (mean=31.14 ) however it was not statistically significant (p= 0.207).
Puwipirom and researchers (2010) found that the percentage of CD4^+IL17^+ cells was not different between control subjects and SLE patients, in fact some control subjects had higher percentage of CD4^+IL17^+ cells than SLE patients. Jilin and colleagues (2010) who also agreed with our results found that the percentage of CD4^+T cells in patients with active SLE was not significantly different from that in patients with inactive SLE and control subjects.

Wong and coworkers (2008) and Yang and coworkers (2009) found evidence suggesting that the Th17 subset is abnormally expanded in patients with SLE. Accordingly, a higher fraction of CD4^+ T cells produce IL-17 in these patients. Also, Shah and coworkers (2010) and Dolff and coworkers (2011) reported an increased frequency of CD4^+IL17^+T cells in SLE patients than in controls.

Henriques and coworkers (2011) found a trend for a higher frequency of Th17 among CD4 T cells in both active and inactive SLE, but did not reach statistical significance. However, analysis of the amount of IL-17 produced at single cell level showed a significant decreased expression of IL-17 in Th17 cells of both SLE groups compared to normal controls.

In the present study, the mean of the intensity of IL-17 in CD4^+ T cells in SLE patients was 96.9% while in control subjects was 79%, there was a highly significant difference (p <0.001).The proportion of the intensity of IL-17 in CD4^+ T cells significantly negatively correlated with total leucocytic count in SLE patients (p= 0.005), while significantly positively correlated with the percentage of CD4^+IL17^+ cells in SLE patients (p <0.001).

The intensity of IL-17 in CD4^+ T cells showed significantly higher AUC when compared to CD4^+ and CD4^+IL17^+ (p<0.001 for both). CD4^+ and
CD4⁺IL17⁺ AUCs did not differ significantly. CD4⁺ AUC failed to discriminate between SLE and controls, CD4⁺IL17⁺ showed poor AUC, while intensity of IL-17 in CD4⁺ showed excellent AUC for discrimination between SLE and controls. Intensity of IL-17 in CD4⁺ showed significantly higher AUC when compared to CD4⁺ and CD4⁺IL17⁺ (p<0.001, =0.001 respectively).

When the sensitivity and specificity of intensity of IL-17 in CD4⁺ for discrimination between SLE patients and controls using ROC curves were evaluated the sensitivity and specificity were 100% and 100% with a cut off value 89.7.

In the current study, CD8⁺IL17⁺T cells were also evaluated in SLE cases and controls. The SLE patients constitutively had a high statistically significant proportion of CD8⁺IL17⁺T cells (mean=23.4) than the healthy controls (mean=15.4) (p=0.003), inspite of similar frequencies of CD8⁺T cells fractions among SLE cases (mean=31.3) and controls (mean=31.6).

Wong and colleagues (2008); Yang and colleagues (2009), Crispin and colleagues (2011) found that a higher percentage of T cells from peripheral blood express IL-17 in patients with SLE when compared to healthy individuals, IL-17 is produced by neutrophils, NK cells, and several T cell types including CD4⁺, CD8⁺, double negative (DN; CD4⁻CD8⁻), and TCR-γδ cells.

However, Puwipirom and colleagues (2010) when assessing IL17⁺ T cells found that the percentage of IL17⁺ CD8⁺ cells were not different between control subjects and SLE patients.

On the contrary, Yang and researchers (2009) and Dolff and researchers (2011) found that very few CD8⁺ T cells produced IL17 from patients with SLE than controls. Crispin and researchers (2008) reported that CD8⁺ T cells produced minimal amounts of IL-17 compared with those produced by CD4⁺ cells.
The mean of the intensity of IL-17 in CD8⁺ T cells in our study in SLE patients was 73.9% while in control subjects was 50.6% there was a highly significant difference (p < 0.001). An increase in the proportion of the intensity of IL-17 in CD8⁺ T cells was demonstrated which correlated positively with the percentage of CD8⁺IL17⁺ cells in SLE patients (p < 0.001).

CD8⁺ showed poor AUC, CD8⁺ IL17⁺ showed far AUC, while intensity of IL-17 in CD8⁺ showed excellent AUC for discrimination between SLE and controls. Intensity of IL-17 in CD8⁺ showed significantly higher AUC when compared to CD8⁺ and CD3⁺IL17⁺ (p<0.001, =0.007). CD8⁺ and CD8⁺IL17⁺ AUCs did not differ significantly.

The present study evaluated sensitivity and specificity of intensity of IL-17 in CD8⁺ for discrimination between SLE patients and controls using ROC curves the sensitivity and specificity were 92.5 % and 95% with a cut off value 62.7.

In this study, a statistical significant negative correlations were obtained between the proportion of both CD3⁺IL17⁺ T cells and CD4⁺IL17⁺ T cells and total leucocytic count (p= 0.018, 0.005 respectively) and proteinuria (p= 0.033, 0.012 respectively) in SLE group, while positive correlations were obtained between the proportion of CD3⁺IL17⁺ T cells and haemoglobin concentration in SLE group (p=0.005).

Crispin and Tsokos (2010) reported in their studies that the IL-17 production is increased in patients with SLE, elevated IL-17 levels probably contribute to the recruitment and activation of immune cells (e.g., neutrophils and T cells) to target organs and thus amplify an ensuing immune response. The immune environment in patients with SLE is ideally suited for the generation of IL-17 producing T cells. Produced IL-17 probably has broad effects on the immune system that include B cell stimulation.
Taken together, the elevation of IL-17 intensity among SLE patients in CD3⁺, CD4⁺ and CD8⁺ T cells leading to elevation of the proinflammatory cytokine IL-17 which trigger the inflammatory process in SLE. Our study provides evidence of the role of IL-17 in the pathogenesis of SLE disease regardless of whether it is a cause or a consequence.