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# The antinuclear antibodies fluorescent patterns in patients with connective tissue diseases

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**SUMMARY**

**Objectives:** Identification of the indirect immunofluorescent (IIF) patterns of antinuclear antibodies (ANAs) and anti-double stranded DNA (anti-dsDNA) antibody profiles in patients with connective tissue diseases (CTDs).

**Material and Methods:** The study included 50 patients with CTDs. They were recruited from Ibn-Sina Teaching Hospital in Mosul City for the period from January 2013 to April 2014. From each patient ten milliliters (ml) of blood were collected. Two milliliters were collected in tubes containing EDTA for performing complete blood count and ESR. In addition, relevant laboratory tests including liver function test, renal function tests, and urinalysis were also conducted. The remaining of the separated serum was kept in clean tubes and stored at -20$^{o}$ C until use for IIF tests. The tested autoantibodies by IIF technique included ANA and anti-dsDNA autoantibodies.

**Results:** Constitutional symptoms, skin rashes, and haematological manifestations were found to be the most common clinical characteristics reported among patients with CTDs. The ANA and anti-dsDNA autoantibodies were detected in 45/50 (90%) and18/50 (36%) patients with CTDs respectively. Eleven ANA-IIF patterns were detected in patients with CTDs. The homogenous fluorescent pattern was found to be the most common pattern and detected in 17/50 (56.7%) patients with CTDs. The anti-dsDNAautoantibodies were detected specifically in patients with systemic lupus erythematosus (SLE).

**Conclusions:** Connective tissue diseases are associated with different ANA-IIF patterns. The anti-dsDNA antibodies are specificautoantibodies of patients with SLE.

**Key words:** antinuclear antibodies, indirect immunofluorescent, connective tissue diseases.

## INTRODUCTION

Connective tissue diseases include spectrum of systemic autoimmune disorders characterized by autoimmune phenomena including circulating autoantibodies and autoimmune-mediated organ damage [1].The IIF pattern reflects the topographic distribution of the target autoantigens and therefore may convey significant information about theirnature.The observedpattern may provide preliminary information on the natureof the autoantibody in a given sample and even indicate whichadditional and specific tests should be conducted [2].

In fact, several IIF patterns have been shown to bear tight association with certain autoantibody specificities[3]. It is important to note that over 90% of ANA detected in HEp-2 cells at least two combined nuclear and/or cytoplasmic patterns [4].The higher titers of ANA are more clinically significant; ANA in healthy individual is generally low titers. There is some evidence that clinical manifestations associated with certain autoantibodies are more evident among patients with high titer of that specificity [5].

The anti-dsDNA antibodies were discovered in 1957 and since then have been well recognized as diagnostic markers of SLE [6]. They are highly specific for SLE and represent one of the criteria of the American College of Rheumatology (ACR) for the classification of SLE [7,8]. However, some patients with other rheumatic diseases or chronic active hepatitis may have mildly or moderately elevated serum titers.

Since theIIF on HEp-2 cells is considered as the gold standard screening method for the detection of ANA [9]. Therefore, the current study is aimed to identify the various immunofluorescent patterns of ANAsand study the anti-dsDNAautoantibody profiles of patients with CTDs.

## MATERIALS AND METHODS

Fifty patients suffering from different types of CTDs belonging to systemic lupus erythematosus (SLE), systemic sclerosis (SSc), mixed connective tissue diseases (MCTD), polymyositis-scleroderma (PM-Scl ) overlap syndrome, and idiopathic inflammatory myopathies (IIMs). They were recruited from Ibn-Sina Teaching Hospital in Mosul City for the period from January 2013 to April 2014. All patients were first interviewed and examined clinically according to a special Questionnaire Form.

### Materials

All laboratory kits and reagents used throughout this study were purchased from Euroimmun company, Medizinische,Labordiagnostika AG, Lubeck, Germany.The slides used for the detection of ANAs were consisted of 2 Biochips per field (HEp-2 and primate liver). Furthermore, slides with single Biochip containing flagellates (*Crithidialuciliae*) were used for the detection of anti-dsDNA autoantibodies.

### Sample collection and processing

A sample of 10 ml blood was obtainedfrom anti-cubital vein by sterile disposable syringes. Two milliliters were collected in tubes containing ethylenediamine tetraacitic acid (EDTA) to be used for performing complete blood count and ESR. The remaining blood was poured into plane tubes without anticoagulant and left for about half an hour, then centrifuged and serum was separated by micropipette and kept in clean tubes and stored at -20$^{o}$C until use for IIF tests. In addition, relevant laboratory tests including liver function test, renal function tests, and urinalysis were also conducted. For ANA-IIF test, serum to be investigated have been diluted 1:100 in PBS-tween. Therefore, 10.1 µl of samplewere diluted in 1000µl of PBS-tween in a polystyrene tube and mixed thoroughly. Furthermore, for anti-dsDNAIIF test, serum to be investigated have been diluted 1:10 in sample buffer 2. Therefore, 11.1 µl of the sample were diluted in 100µl of sample buffer 2 in a polystyrene tube and mixed thoroughly.

### Titer plane Technique

Samples have been applied to the titer plane. The Biochip slides were then placed into therecesses of the titer plane, where all Biochip's ofthe slides come into contact with the samples, andthe individual reactions commence simultaneously.

The fluorescence was read with the microscope initially in objective 20X and then focused using 40X (excitation filter: 488 nm, color separator: 510 nm, blocking filter: 520 nm, blue light) for both autoantibodies.

For the detection of antinuclear antibodies (ANAs),substratecombination of HEp-2 cells and primate liver were incubated with diluted patient's sample. If positive reaction is obtained, specific antibodies of classes IgA, IgG and IgM attach to the antigens. In a second step, the attached antibodies are stained with fluorescein-labeled anti-human antibodies and made visible with fluorescence microscope. In case of positive samples, the cell nuclei showed a distinct fluorescence, which was characterized by certain patterns.

For detection of anti-dsDNA autoantibodies, astandard substrate flagellates*Crithidialuciliae* was incubated with patients' sera. If a positive reaction is obtained, specific antibodies of classes IgA, IgG and IgM attach to the antigens. In a second step, the attached antibodies are stained with fluorescein-labeled anti-human antibodies and made visible with a fluorescence microscope. In the case of a positive result, a distinct, homogenous, in parts circular fluorescence of the kinetoplast was identified. Reactions of the cell nucleus, the basal body (base of the flagellum) or the cytoplasm were not taken into account.

### Statistical analysis

Basic descriptive statistics, including means, SDs, range, and percentages were used to characterize the study case [10].

## RESULTS

The demographic data, clinical characteristics and antibody profiles of patients with connective tissue diseases are shown in Table 1, 2 and Figure 1. Fifty patients (4 males and 46 females) were reported to have CTDs. The age of patients was ranged from 7 to 65 (31 ± 14.2) years. The duration of the disease was ranged from 2 to 276 (60 ± 18) months. Constitutional symptoms were the most clinical manifestations and were reported in 40/50(80%) patients.Moreover, hematological manifestations were detected in 31/50 (62%) patients. Skin rashes were found to be the 3$^{rd}$ most common clinical characteristics and were reported in 28/50(56%) patients. In addition, five out of fifty (10%) patients were reported to have genital ulcer.Raynaud's phenomenon was detected in 20(40%) cases. Renal involvement in form of microscopic haematuria and proteinuria, red blood cell (RBC) cast and/or impaired renal function was reported in 12/50 (24%) patients.

The ANAs were detected in 45/50(90%) patients with CTDs. The anti-dsDNAautoantibodies (*Crithidialuciliae*) were detected 18/50 (36%) patients.A homogenous circular fluorescence of kinetoplast or of both nucleus and kinetoplast was regarded as positive reaction(Figure2).

The ANAs were found to have to eleven different immunofluoresecent patterns. Theimmunofluorescent patterns of ANA on HEp-2 and primate liver Biochip and their associated autoantibodies and diseasesare shown in Table 3. Four cell nuclear fluorescent patterns were identified including, cell nucleus homogenous, cell nucleus granular, cell nucleus dotted (5-20) and nucleus nucleolar. The most common fluorescent pattern was cell nuclei homogeneous, showed an associationwith dsDNA, nucleosomes and histones and was detected in 17 patients with SLE (Figures 3-5).
The nucleoplasm coarse granular pattern (anti-nRNP/Sm) was found in 3 cases with mixed connective tissue disease (MCTD) and 2 with SLE. The HEp-2 cellsshowedcoarse granular, which is distributed over the entire cell nuclei, leaving the nucleoli free (Figure 6). Autoantibodies against Ku were associated with nucleoplasmic fine granular pattern and were found in 2 cases with SLE and 1 with IIM. The cell nuclei of both HEp-2 and primate liver showed fine granular fluorescence and the nucleoli were partly fluoresce (Figure 7). Moreover, the cell nucleousfine granular pattern was seen in one case with SLE. It was associated with SSA, SSB andin this fine granules were observed and thecytoplasmic area was negative (Figure 8).

The nucleus nucleolar (homogenous-fine granular) fluorescent patterns were seen in 6 patients with SSc. The associated autoantibodies (anti-Scl-70) were directed against DNA Topoisomerase 1(Figure 11). In addition, one case with SSc was found to have nucleusnucleolar (granular fluorescence of nucleoli) and showed an association with anti-fibrillarinantibodies (Figure 12). The anti-PM-Sclautoantibodies (directed against PM/Scl75 and PM/Scl100) were detected in 2 patients with PM-SSc overlap syndrome. The cell nucleoli of both HEp-2 andprimate liver showed homogenous homogeneous fluorescent pattern (Figure 13). The cytoplasm granular fluorescent pattern was detected in 2 patients with IIM. The autoantibodies were directed against Jo-1 (Figure 14).The cytoplasmic fine granular (smooth) fluorescent pattern was detected in 5 SLE patients. The hepatocytes of primate liver showed a full cytoplasmic fluorescence with patchy accentuation (Figure 15).

**Table 1: Demographic data, clinical characteristics and antibody profiles Ofpatients with connective tissue diseases.**

| **Demographic** | |
|---|---|
| **Number of patients** | 50 |
| Age, range (mean) years | 7- 65 (31 ± 14.2) |
| Disease duration range (mean) months | 2–276 (60 ± 18) |
| Sex (M/F) | 4/46 |
| **Clinical characteristics** | **No. (%)** |
| Constitutional symptoms (fever, fatigue, arthralgia, myalgia) | 40(80) |
| Raynaud's phenomenon | 20(40) |
| Skin rash | 28 (56) |
| Oral ulcer | 8(16) |
| Genital ulcer | 5(10) |
| Skin ulcer | 3(6) |
| Arthritis | 24(48) |
| Serositis | 2(4) |
| Myositis | 15(30) |
| Lung manifestations | 13(26) |
| Cardiovascular diseases | 6(12) |
| Gastrointestinal manifestations | 10(20) |
| Neurological manifestations | 3 (6) |
| Renal complications | 12 (24) |
| Hematological manifestations (leucopenia, anemia, lymphopenia, thrombocytopenia) | 31 (62) |
| Abortion | 4 (8) |
| **Autoantibody profile** | |
| ANA positivity (HEp-2/primate liver) | 45 (90) |
| Anti-dsDNA positivity (*Crithidialuciliae*) | 18 (36) |

**Table 2: Demographic data, clinical characteristics and antibody profiles of patients with SLE.**

| **Demographic data** | |
|---|---|
| Number of patients | 30 |
| Age, range (mean) years | 9-55 (32± 2.4) |

| | |
|---|---|
| Disease duration range (mean) months | 12–250 (55 ± 16) |
| Sex (M/F) | 1/29 |
| **Clinical characteristics** | **No. (%)** |
| Constitutional symptoms (fever, fatigue, arthralgia, myalgia) | 20 (66.7) |
| Raynaud's phenomenon | 6 (20) |
| Skin rash | 19 (63.3) |
| Oral ulcer | 5 (16.7) |
| Arthritis | 16 (53.3) |
| Myositis | 2 (6.6) |
| Serositis | 2 (6.6) |
| Neurological manifestations | 3 (10) |
| Renal complications | 10 (3.33) |
| Hematological manifestations (anemia, leucopenia, lymphopenia, thrombocytopenia) | 18 (60) |
| Abortion | 2 (6.6) |
| **Autoantibody profile** | |
| ANA positivity (HEp-2/primate liver) | 30 (100) |
| **Immunofluorescent patterns** | |
| Cell nucleus homogenous (nucleosomes, histones, dsDNA) | 17 (56.6) |
| Nucleus granular (U1-nRNP, Sm) | 2 (6.6) |
| Nucleus granular (Ku) | 2 (6.6) |
| Nucleus granular (SS-A, SS-B) | 1 (3.3) |
| Nucleus granular (PCNA) | 1 (3.3) |
| Nucleus dotted (5-20 dotts) | 2 (6.6) |
| Cytoplasm fine granular (Rib-P-Protein) | 5 (16.7) |
| dsDNA positivity (*Crithidialuciliae*) | 18 (60) |

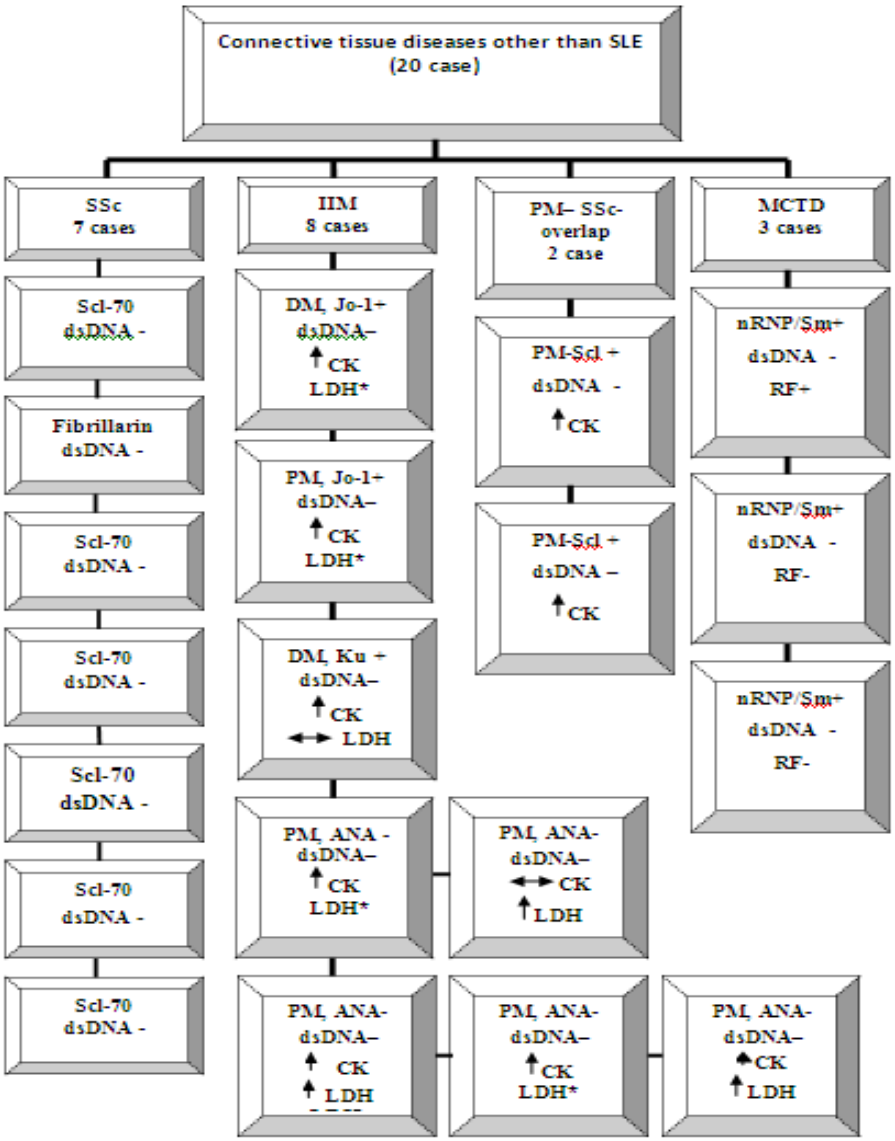


**Figure 1: Thedifferent profiles of the patients with connective tissue diseases other than SLE. *= not available. CK: creatinin kinase, LDH: lactate dehydrogenase, RF: rheumatoid facror.**

**Table 3: The immunofluorescentpatternsof ANAs on HEp-2 and primate liver Biochips and their associated diseases.**

| Fluorescent pattern | Antigens | Antibodies | Disease | n=45 |
|---|---|---|---|---|
| | | | | No.(%) |
| Cell nucleus homogenous | Deoxyribose phosphate backbone of the DNA. | dsDNA | SLE | 17 (37.7) |
| | Antibodies bind to conformational epitopes of the dsDNA histone complex | Nucleosomes | | |
| | Histones | Histones | | |
| Nucleus granular (coarse) | U1-nRNP, Sm | nRNP/Sm | SLE<br>MCTD | 2 (4.4)<br>3 (6.6) |
| Nucleus granular (fine) | p70, p80 | Ku | SLE<br>IIM | 2 (4.4)<br>1 (2.2) |
| Nucleus granular (fine) | SS-A, SS-B | SS-A, SS-B | SLE | 1 (2.2) |
| Nucleus granular (fine) | PCNA | PCNA | SLE | 1 (2.2) |
| Nucleus dotted | Sp100, PML, SUMO-1, SUMO-2. | NA* | SLE | 2 (4.4) |
| Nucleus nucleolar (homogenous-fine granular) | DNA topoisomerase 1 | Scl-70 | SSc | 6 (13.3) |
| Nucleus nucleolar (granular fluorescence of nucleoli | U3-nRNP | Fibrillarin | SSc | 1 (2.2) |
| Nucleus nucleolar (homogenous-granular) | PM/Scl75, PM/Scl100 | PM-Scl | PM/SSc-overlap | 2 (4.4) |
| Cytoplasm granular (fine granular to homogenous) | Histidyl-tRANS-Synthetase | Jo-1 | IIM | 2 (4.4) |
| Cytoplasmic fine granular (smooth) | Rib-P-Protein | Rib-P-Protein | SLE | 5 (11.1) |

*** NA**: not applicable.

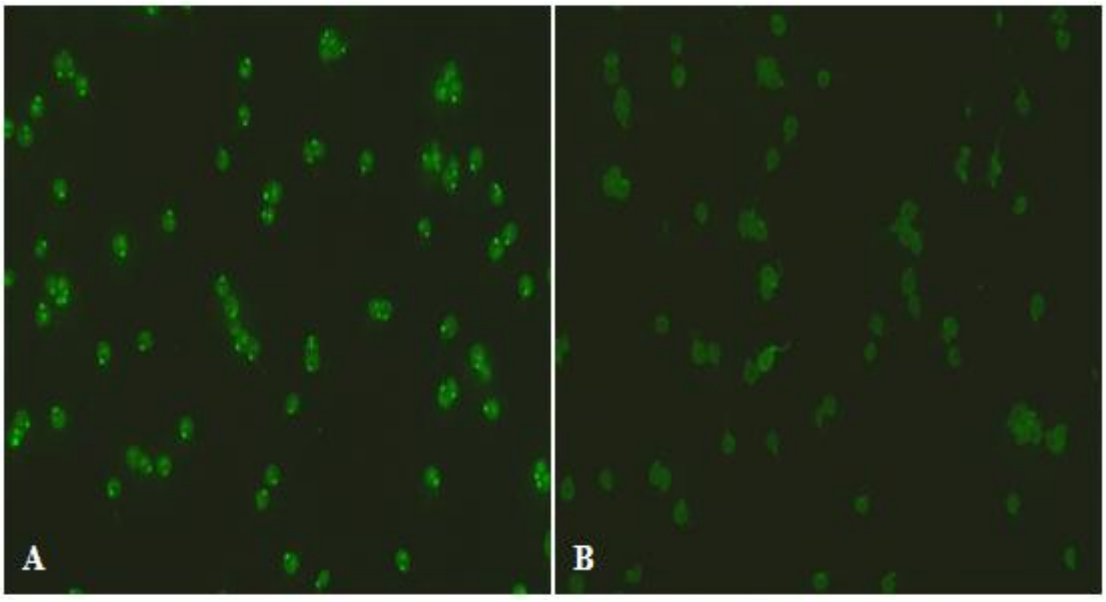


**Figure 2: *Crithidia luciliae*: autoantibodies against dsDNA. A distinct, homogenous, in parts circular fluorescence of kinetoplast can be identified in positive samples (A). The kinetoplast shows no staining in negative samples (B).**

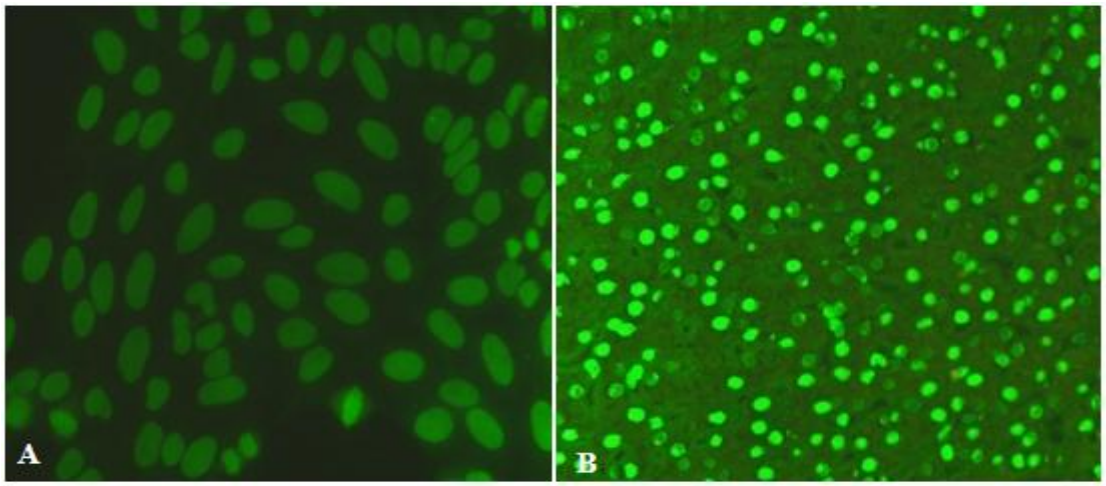


**Figure 3: Nucleus homogenous: antibodies against dsDNA. HEp-2 cells (A): Homogenous fluorescence of cell nuclei. Antibodies react with epitopes in the deoxyribose phosphate backbone of the DNA. Mitosis: Condensed chromosomes of mitosis are positive; area surrounding the chromosomes is dark. Primate liver (B): Homogenous fluorescence of cell nuclei.**

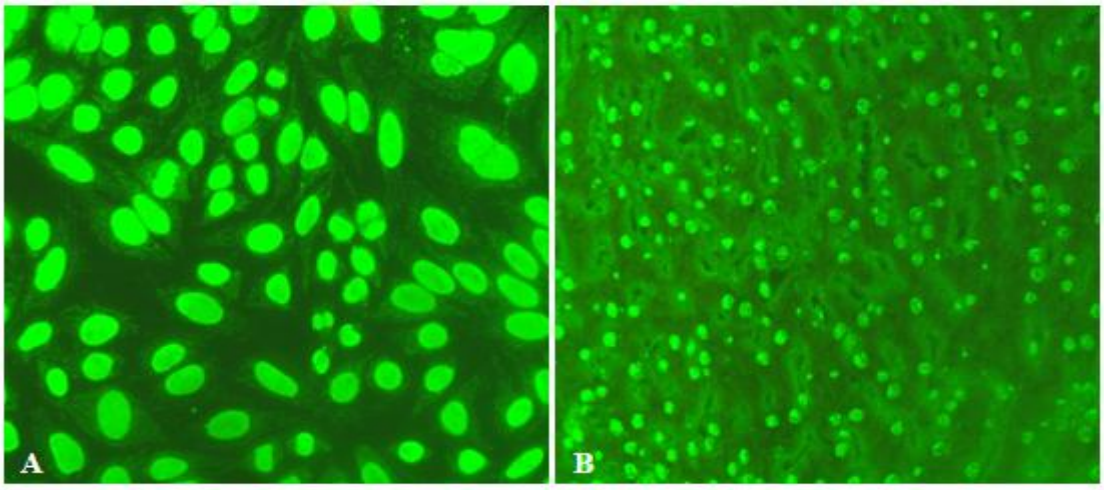


**Figure 4: Nucleus homogenous: antibodies against nucleosomes. HEp-2 cells (A): Homogenous fluorescence of cell nuclei. Mitosis: Condensed mitotic chromosomes are positive; area surrounding the chromosomes is dark. Primate liver (B): Homogenous fluorescence of the cell nuclei. Antibodies bind to the conformational epitopes of the dsDNA and histones.**

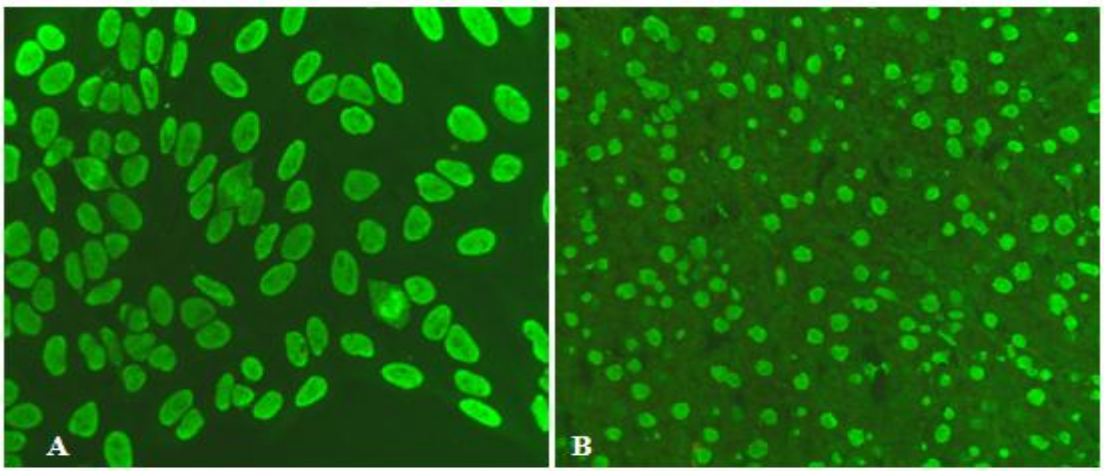


**Figure 5: Nucleus homogenous: antibodies against histones. HEp-2 cells (A): Homogenous fluorescence of cell nuclei. Mitosis: Condensed mitotic chromosomes are positive. Primate liver (B): Homogenous, partly coarse to fine clumpy fluorescence of cell nuclei.**

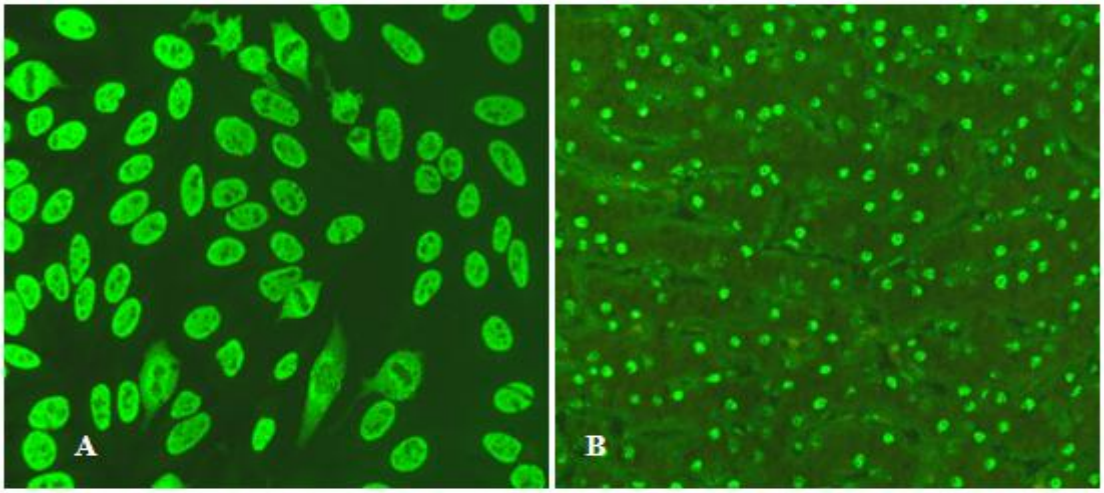


**Figure 6: Nucleus granular: antibodies against nRNP/Sm. Antigens: U1-nRNP, Sm. HEp-2 cells (A): Coarse granular, sometimes medium to fine granular fluorescence, which is distributed over the entire cell nuclei, leaving the nucleoli free. Mitosis: Condensed chromosomes are dark. The periphery shows a fine granular to granular fluorescence. Primate liver (B): granular fluorescence of the hepatocyte nuclei, the nucleoli are excluded.**

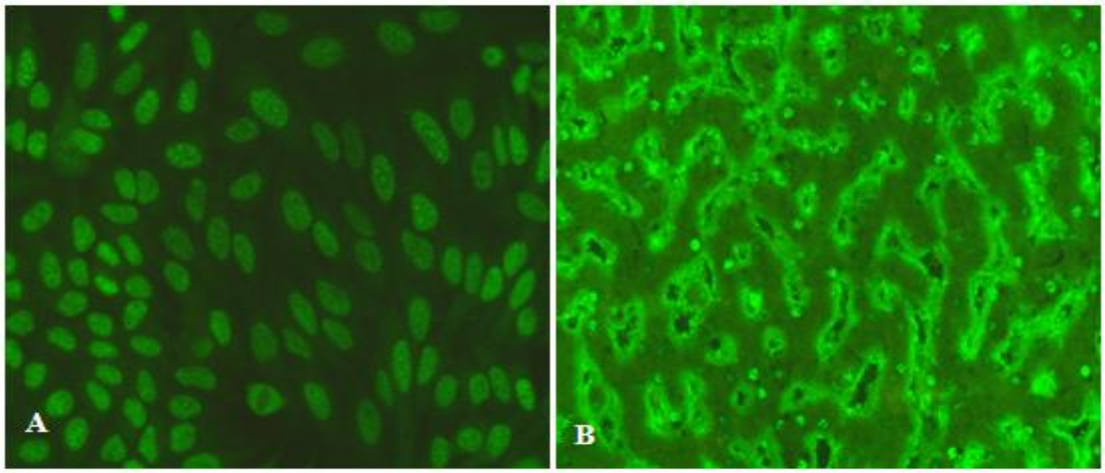


**Figure 7: Nucleus granular: antibodies against Ku. Antigens: p70, p80. HEp-2 cells (A):Fine granular fluorescence of the cell nuclei, the nucleoli partly fluoresce. Mitosis: Condensed chromosomes are dark. The periphery shows a fine granular to granular fluorescence. Primate liver (B): Typical granular clumpy fluorescence of the cell nuclei.**

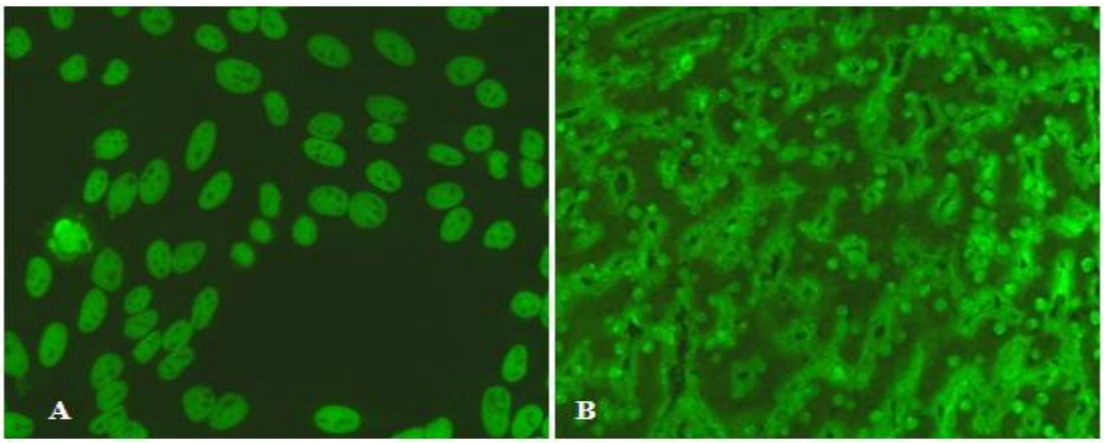


**Figure 8: Nucleus granular: Autoantibodies against SS-A, SS-B. HEp-2 cells (A): Fine granular fluorescence of cell nuclei. The nucleoli also fluoresce, but are partly excluded. Mitosis: condensed chromosomes are dark the periphery shows a fine granular to granular fluorescence. Primate liver (B): Smooth fluorescence of the nucleoli.**

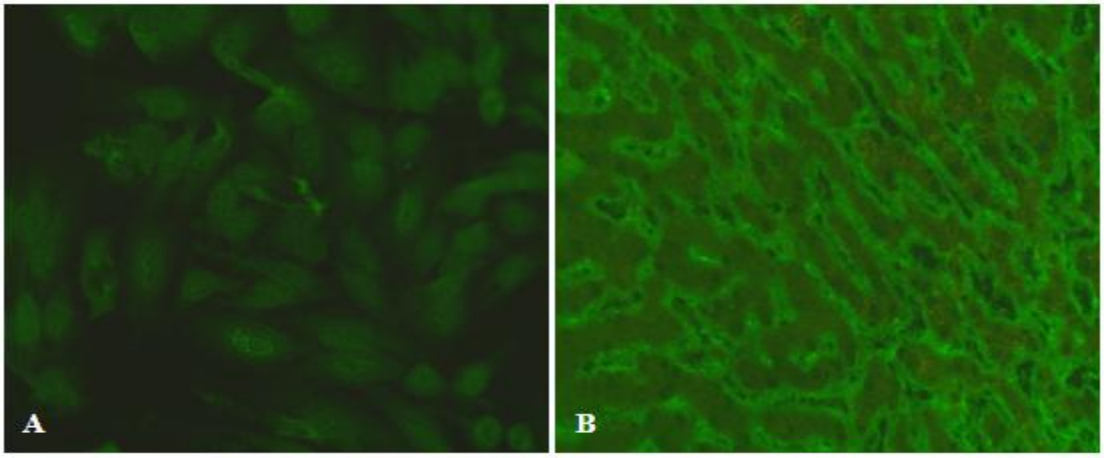


**Figure 9: Nucleus granular: antibodies against proliferating cell nuclear antigen (PCNA). Antigen: PCNA. HEp-2 cells (A): fluorescence pattern depend on the cell cycle. Half of the interphase cell nuclei show a bright, fine by rough granular base fluorescence, the nucleoli are excluded. Mitosis: Negative perimichrosomal area dark. Primate liver (B): negative.**

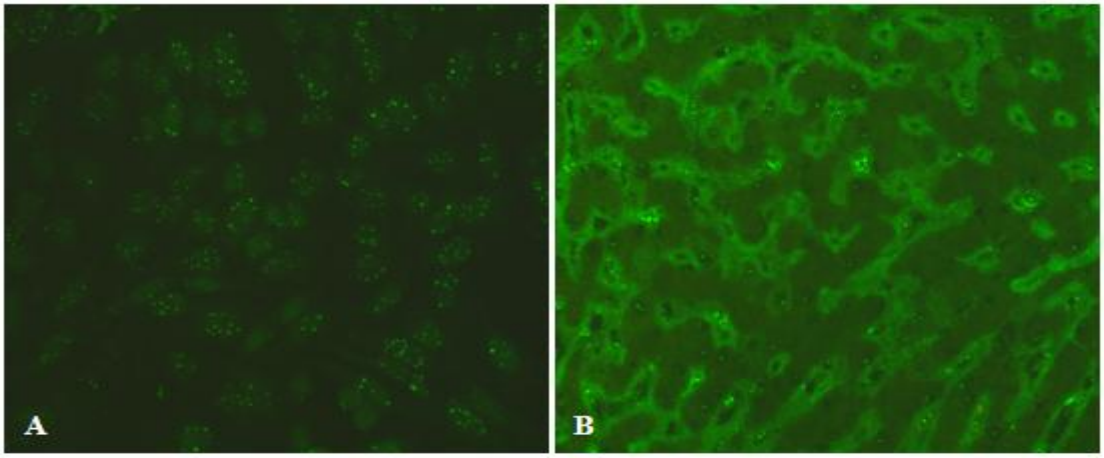


**Figure 10: Nucleus dotted. Antigen: Sp100, PML, SUMO-1, SUMO-2.HEp-2 cells (A):Interphase cell nuclei: Five to twenty splinter-like granula of varying size, which are distributed diagonally over the cell nucleus (nuclear dots). The cytoplasm is dark. Mitosis: PML nuclear dots are dissolved, only a few granula fluoresce outside the unstained chromosomes. Primate liver (B): the same strong reaction on primate liver as on HEp-2 cells.**

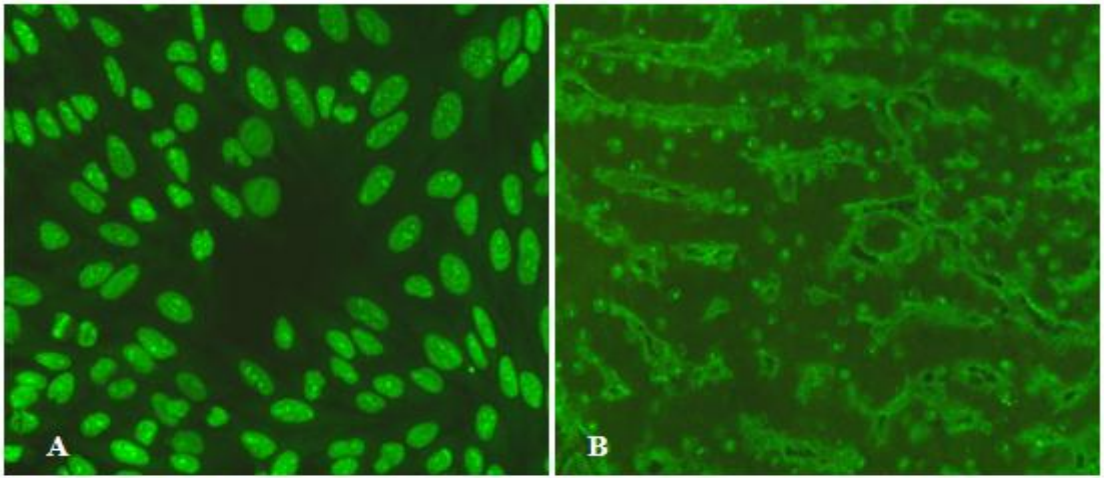


**Figure 11: Nucleus nucleolar: Autoantibodies against Scl-70. Antigen: DNA Topoisomerase 1.HEp-2 cells (A): Homogenous-fine granular fluorescence of interphase cell nuclei. The nucleoli are accentuated and also show a homogenous fine granular fluorescence. The cytoplasm is dark. Mitosis: Perichromosomal fluorescence. Primate liver (B): Almost homogenous fluorescence in the interphase. The nucleoli are homogenous, but show a weaker fluorescence than on HEp-2 cells.**

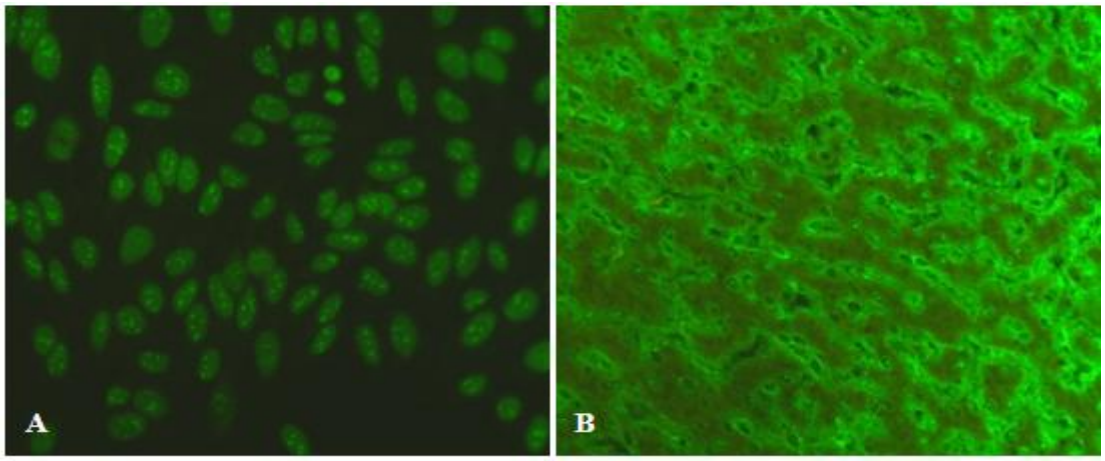


**Figure 12: Nucleus nucleolar: Autoantibodies against fibrillarin. Antigen:U3-nRNP.HEp-2 cells (A):Granular fluorescence of the nucleoli.Primate liver (B): Homogenous fluorescence of the nucleoli.**

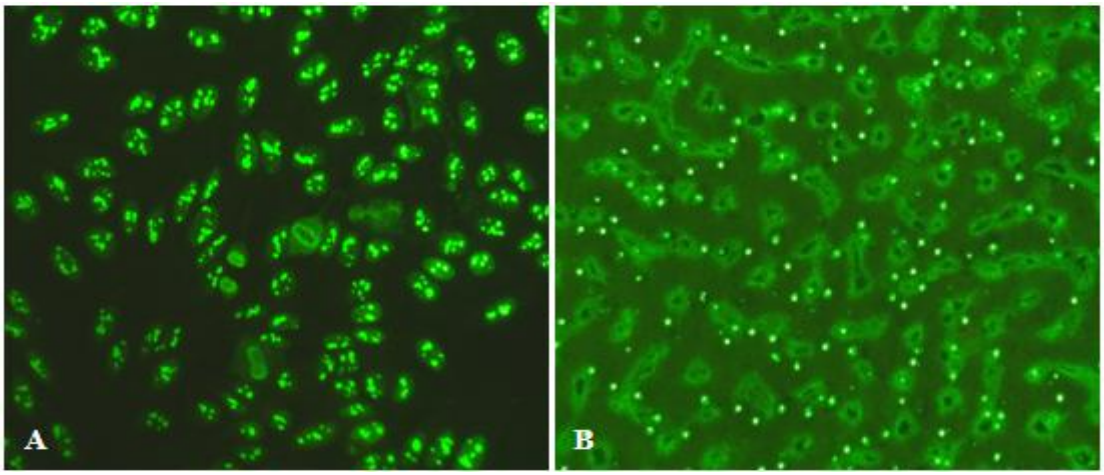


**Figure 13: Nucleus nucleolar: antibodies against PM-Scl. Antigens: PM/Scl75, PM/Scl100. HEp-2 cells (A): Homogenous fluorescence of the nucleoli accompanied by a weaker, fine granular reaction of the karyosome. Mitosis: Condensed chromosomes are excluded, a fine granular fluorescence can be seen outside the chromosomes. Primate liver (B): Homogenous fluorescence of the nucleoli and a very weak fine granular to reticular fluorescence of the cell nuclei.**

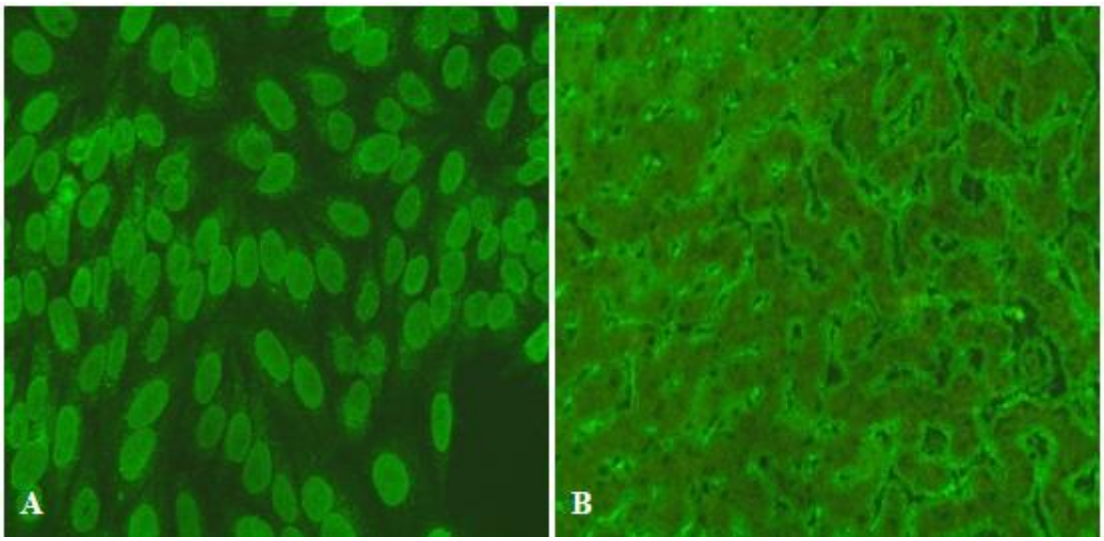


**Figure 14: Cytoplasm granular: Autoantibodies against Jo-1. Antigen: Histidyl-tRANS-Synthetase.HEp-2 cells (A): Fine granular to homogenous, cytoplasmic fluorescence. Mitosis: Chromosome region negative. Primate liver (B): Very fine granular fluorescence of the tissue.**

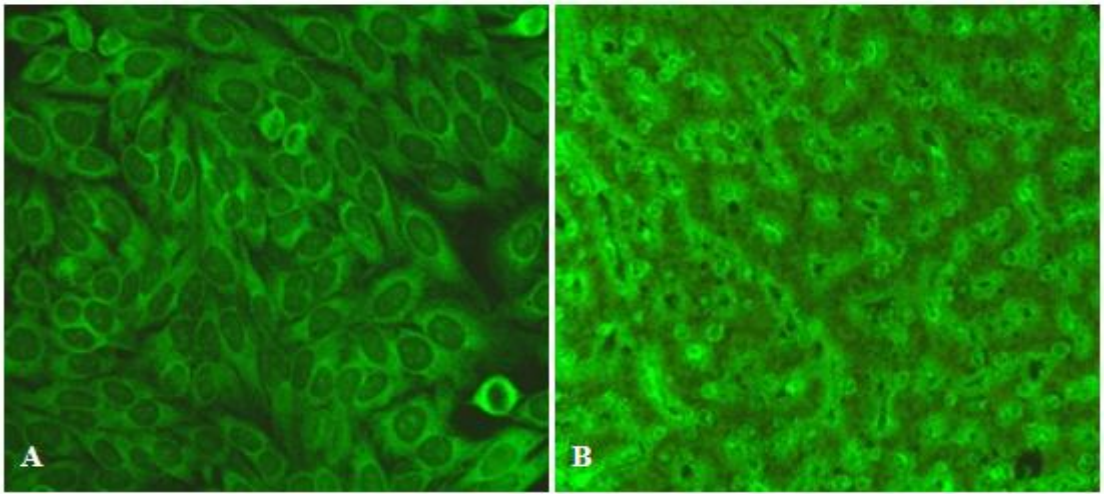


**Figure 15: Cytoplasmic fine granular: Autoantibodies against Rib-P-Protein. HEp-2 cells (A): Smooth cytoplasmic fluorescence. Mitosis: Chromosome region negative. Primate liver (B): Hepatocyte shows a full-surface cytoplasmic fluorescence with patchy accentuation.**

## DISCUSSION

In clinical practice, the IIF is the golden standard among laboratory tests detecting a presence and localization of ANAs [11].Furthermore, evaluation of patients' sera using more than one tissue substrate may be clinically valuable. Therefore, in the current study, ANA-global tests and *Crithidialuciliae*were used to assess the ANA-IIF and anti-dsDNAautantibody profiles in patients with CTDs respectively.

In the present study, fifty patients (4 males and 46 females) were reported to have CTDs. The mean age of patients was 31 ± 14.2 years.TheCTDs were found to be more common in females (46/50).These findings may indicate that CTDs are more common among females especially during the child bearing age. Hormonal factors and genetic predisposition may play a role in this female predominance[12].Furthermore, females generally have more robust humoral and cellular immune responses than males [12]. Estrogens appear to play a central role in the immune response and immune-mediated diseases. Moreover, estrogen receptors are present on the cells involved in the immune response, namely thymocytes, macrophages and endothelial cells. The influence of estrogens on cytokine production by target cells, through interference with their transcriptional activity. The effect of estrogens on the expression of the protooncogenes and oncosuppressor genes involved in programmed cell death (apoptosis) might also be relevant to human autoimmunity [13].

In the currentwork, the ANAs were detected in 45/50(90%) patients with CTDs. Furthermore, these ANAs were found to have to eleven different IIF patterns. These patterns were specified according to the binding site of the cellular antigens of the tissue substrate. These findings may indicate that these systemic autoantibodies of different specificities are commonly detected in patients with CTDs. Moreover, the association of these autoantibodies (specific IIF pattern) with specific type of CTDs may suggest a pathogenic role of these autoanibodies in the immunopathogenesis of CTDs. These findings are in accordance Mariz and Coworkers (2011) who found that 138/153 (90.2%) patients with autoimmune rheumatic diseases were positive for ANAs[3]. However, the ANAs can be found in the absence of obvious signs of rheumatic disease [14]. Furthermore, the ANAs can be detected in up to 12.9% healthy individuals [3]. Therefore, testing the patients' sera for ANAs by IIF technique should be applied for patients with high clinical suspicion of systemic autoimmunity including CTDs and interpreted by an experienced examiner.

The cell nucleus homogenous fluorescent pattern was found to be the common ANA pattern. It was demonstrated in 17 out of 45 (37.7%) patients with positive results of ANAs. The homogenous pattern showed an association with three antigens dsDNA, nucleosomes and histones. Differentiation between these three subspecificities was difficult under fluorescent microscope (minor fluorescent differences). However, the use of two tissue substrates (i.e., HEp-2 human cells and primate liver) perfield may help in the characterization and identification of these subspecificities. For further confirmation of the type of autoantibodies (detected in this pattern) other immunological tests may be needed.These findings are in agreement with Sebastian and Coworkers (2010) who had found that homogenous pattern was the most common ANA pattern that detected among patients with different types of rheumatic diseases. It was evident in 46 (45.5%) of the positive 101 samples[15].

The anti-dsDNAautoantibodies (*Crithidialuciliae*) were detected specifically in patients with SLE. They were reported in 18/30 (60%) patients with SLE. These findings are in line with Villalta and Coworkers (2013) who had detected anti-dsDNA autoantibodies in 134/200 (67%) patients with SLE[16]. In contrast, Wichainun and Colleagues (2013)

reported anti-dsDNAautoantibodies in 37/100 (37%) patients with SLE[17]. The anti-dsDNA autoantibodies are the only antibodies that may be used to monitor the disease activity of SLE [18]. Furthermore, the levels of anti-dsDNA autoantibodies in patients' sera correlate with disease activity [19]. Therefore, differences in the disease activity may play a role in these variations.

Failure of detection of the anti-dsDNA antibodies in patients with CTDs other than SLE (SSc, PM-Scl-overlap syndrome, MCTD, IIM) may indicate that these autoantibodies are not disease specific. Moreover, anti-dsDNA antibodies are highly specific for the diagnosis of SLE and are included in the classification criteria for SLE [18]. However, Váncsa and Coworkers (2010) had detected the anti-dsDNA autoantibodies in 13/130 (10%) patients with PM/DM[20]. Moreover, Sen and Coworkers (2014) had detected the anti-dsDNA autoantibodies in only 2/23 (8.7%) patients with MCTD[21]. Differences in the samples size and/or variations between ethnic groups may play a rolein these variations. Furthermore, the anti-dsDNA autoantibodies positivity should not be considered an exclusion criterion for this disease but as a warning of a possible evolution into SLE [22].

## IN CONCLUSION

The CTDs are associated with different ANA-IIF patterns. Furthermore, the detection of these different ANA-IIF patterns indicates that these diseases have different phenotypes. The anti-dsDNA antibodies are specific antibodies of patients with SLE. Moreover,the use of primate liver in addition to the HEp-2 human cells (two Biochips) in the same field may provide an additional benefit in the characterization and specification of the type of ANA-IIF patterns and associated autoantibodies. Therefore, this combination may replace the standard protocol of using HEp-2 human cells in ANAs screening that currently used in the routine clinical practice. However, larger studies are needed to assess these findings.

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