Detection of Desmoglein-3 Auto-Antibodies in Patients with Lichen Planus

Abd-El Aziz El-Rifaie[a],Laila Ahmed Rashed [b],Mona El sayed Ahmed Abdellatif [a], Asmaa Abdel Ghany Mohamed [a].

[a] Dermatology Department-Faculty of Medicine, Beni-Suef University.

[b] Professor of Biochemistry-Faculty of Medicine, Cairo University.

Abstract:

There is controversy about the role of auto anti-bodies against desmoglein 3 (Dsg 3) in the pathogenesis of lichen planus (LP). The goal of this study is to detect desmoglein-3 auto-antibodies in lichen planus patients comparted to normal control persons via investigating its potential role in the pathogenesis of this disease. A case control study for detection of Desmoglein-3 autoantibodies in serum of 40 lichen planus patients and 40 healthy controls using enzyme-linked immunosorbent assay (ELISA) technique.The patients and healthy controls were recruited from Dermatology outpatient clinic at Beni-Suef University hospital. The measurement of anti Dsg3 antibodies in LP patients was significantly higher as compared to normal control persons. The mean scores were 1361.37 ± 300.5 in oral LP & 2118.70 ± 584.37 in cutaneous LP vs. 66.98 ±70.95 in controls (p-value < 0.001). Desmoglein-3 auto-antibodies in LP may have a role in the pathogenesis of the disease. Further investigations are needed for detection of Dsg 3 auto-antibodies in LP skin biopsy or blood samples by other methods such as Immunofluorescence.

Keywords: lichen planus, Dsg3, Autoantibody

1. Introduction

Lichen planus (LP) is an idiopathic inflammatory disease of skin, nails, mucous membranes and hair. It is considered a T-cell autoimmune damage to basal cells that express altered self-antigens on their surface and deposition of immunoglobulins, complements and fibrin in the basement membrane zone, but no specific antigen has been yet identified [1].

Cutaneous LP and oral LP are common variants of the disease; Cutaneous LP affects about 1% of adult population, whereas Oral LP have been observed in up to 1-4% of population [1]. The

aetiopathogenesis of LP is not fully understood; many theories are included as genetic predisposition, exposure to environmental triggers or immune dysregulation. A large body of these theories supports a role of immune dysregulation in the pathogenesis. The various mechanisms hypothesized to be involved in the immunopathogenesis (Antigen-specific cell-mediated immune response, non-specific mechanisms, autoimmune response or humoral immunity)[2].

These circulating antibodies to a lichen planus-specific antigen on the granular and deep epithelial prickle cells in the skin of lichen planus patients was suggested, but the antigen itself was infrequently demonstrated. The presence of antiepithelial antibodies was detected in patients with oral and cutaneous lichen planus associated with therapeutic drug intake, but the antibodies were generally present only in low concentrations [3].

Auto-antibodies to desmoglein (DSG) 1 and 3, desmosomal cadherins detected in stratified squamous epithelium and involved in cell-to-cell coherence, play a pathogenic role in autoimmune bullous diseases, causing detachment of desmosoms and consequent acantholysis [4].

DSGs are the main components of cellular coherence in the epidermis and mucosal surfaces. They are consisting of DSG1, DSG2, DSG3 and DSG4.The role of these autoantibodies against the epidermal desmogleins has been presumed in the autoimmune bullous diseases such as pemphigus vulgaris and bullous pemphigoid (BP) [5].

DSG3 antigen is expressed in the basal membrane zone of the epidermis keeping the cells from detachment.The autoantibodies against DSG3 are increased due to inflammatory damage to basal keratinocytes. This damage releases the DSG3 proteins which act as auto-antigens for auto-antibodies formation in the circulatory blood [6].

There is controversy about the exact role of these autoantibodies against Dsg3 in the pathogenesis and prognosis of lichen planus; in a previous study of the presence of the circulating levels of these antibodies in patients with different types of oral LP and the comparsion with healthy controls, the increased level of anti-Dsg3 antibodies in erosive oral LP was under cut-off values. Moreover, the pathogenic role of anti-Dsg3 antibodies in erosive oral LP is uncertain [6].

The aim of our study was detection of desmoglein-3 auto-antibodies in lichen planus patients as comparted to normal control persons to investigate the possible role of dsg-3 autoantibodies in the pathogenesis of this disease.

2. Subjects and Methods:

This study was a case control study included 40 Egyptian LP patients, their age ranged from (20 to70 years). They were recruited from Dermatology outpatient clinic

at Beni-Suef University hospital. Forty unrelated apparent healthy controls with similar demographic (matched age and sex) were taken during the period from (1st February 2019 to 31 of August 2019).

The studied subjects were divided into two groups as follows:

- Group I: (n = 40) LP patients.

- Group II: (n = 40) unrelated apparent healthy controls.

Cases were chosen randomly according to inclusion and exclusion critreria.

2.1 Inclusion criteria:

1. Age between 20 to 70 years.

2. Patients with acute or chronic LP.

3. Males and females were be included.

2.2 Exclusion criteria:

1. Age below 20 and above 70 years.

2. Patients with other autoimmune diseases or bullous diseases .

3. Patients with associated systemic or dermatological diseases.

4. Patients who are under treatment from recently detected infection.

Controls were chosen randomly from any other outpatient clinic.

Informed consent was obtained from the participants in this study after ethical committee approval from dermatology outpatient clinic at Beni-Suef University hospital. All cases were subjected to estimation of anti Dsg3 antibodies in serum of diseased patients.

2.3 All patients were subjected to:

1. History taking: Full personal and medical history was taken.

2. Clinical examination: To determine the site, extent, type of LP.

3. Estimation of the anti Dsg3 antibodies: Blood sample was taken from each participant and serum was separated and kept frozen at -80 °C till analysis of desmoglein 3 antibodies by enzyme-linked immunosorbent assay (ELISA) technique.

2.4 Principle of the Assay

The kit was based on sandwich enzyme-linked immune-sorbent assay technology. DSG3 was pre-coated on 96-well plates. The Biotin- labeled DSG3 was used to detect the antigen. The standards (test samples and Biotin- labeled DSG3) were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin Conjugate was firstly added then unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a specific blue colored product that changed into the yellow color after adding acidic stop solution. The density of the yellow color is proportional to the Anti-DSG3 antibody amount of sample captured in the plate. The O.D. absorbance was read at 450nm in a microplate reader, and then the concentration of Anti-DSG3 antibody can be calculated [6].

2.5 Calculation of Results

For calculation, (the relative OD.450) = (the OD.450 of each well) - (the OD.450 of

Zero well). That standard curve was plotted as the relative OD.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Anti-DSG3 antibody concentration of the samples was interpolated from the standard curve.

2.6 Statistical methodology

- Analysis of data was done by IBM computer using SPSS (statistical program for social science) as follows;

- Description of quantitative variables as mean, SD and range.

- Description of qualitative variables as number and percentage.

- We used the unpaired t-test to compare quantitative variables, in parametric data (SD < 50 % mean)

- P value > 0.05 insignificant

- P < 0.05 significant

- P < 0.01 highly significant [7].

3. Results:

3.1 Clinical characteristics of the cases and controls

The current study included 40 acute and chronic lichen planus (LP) patients from both sexes. They all presented to dermatology department at Beni-Suef University hospital.

The LP patients were 33 males and 7 females patients, their age ranged from 21 to 65 years. the average age was; 40.55 ±9.6. 40 healthy controls were taken, they were age and sex matched.

The disease duration varied from 1 month to 8 years, with a mean duration of 35.2 ±31.7 months.

Regarding the sites of lesions distribution; 10 cases (25%) had classic and generalized LP, 5 patients (12.5%) had Classic, generalised and oral LP, 1 patient (2.5%) had LP in the lower limb, 2 patients (5%) had LP in upper limbs, 20 patients (50%) had oral LP without cutaneous lesions, 1 patient (2.5%) had hypertrophic LP in shin of tibia,1 patient (2.5%) had classic LP in both upper and lower limbs.

The lichen planus course in oral cases was 15 cases with progressive course (75%), and 5 cases with stable course (25%), but the lichen planus course in cutaneous cases was 11 cases with progressive course (75%), and 9 cases with stable course (25%).

Table (1): Sex Distribution of the Cases and controls:

Description	N (%)			Total	p-value*
	Oral LP N= 20	Cutaneous LP N= 20	Controls N= 40		

Sex						
	Female	6 (30%)	1 (5%)	17 (42.5%)	24	0.620
	Male	14 (70%)	19 (95%)	23 (57.5%)	56	

Table (1): show no statistically significant difference between the cases and the control groups regarding sex (p-value> 0.05).

Table (2): Age Distribution the Cases (oral& cut.) and Controls; (N= 80):

	Mean	SD	Minimum	Maximum	median	p-value*
Oral LP	40.55	9.6	22	56	41.5	
Cutaneous LP	40.65	14.9	21	65	40	0.478
Controls	39	7.9	24	56	39	

Table (2): show no statistically significant difference between cases and control groups regarding to age (p-value> 0.05).

Table (3): Site and Distribution of LP Lesions in the studied Cases; (N=40):

Site (Type)	Cases	
	Count	%
Classic and Generalized	*10*	*25 %*
Classic, Generalized and Oral	*5*	*12.5%*
Lower limbs (Classic LP)	*1*	*2.5%*
Upper limbs (classic LP)	*2*	*5%*
Mucosa (Oral LP)	*20*	*50%*
Shin of tibia (hypertrophic LP)	*1*	*2.5%*
Upper and lower limbs (Classic LP)	*1*	*2.5%*

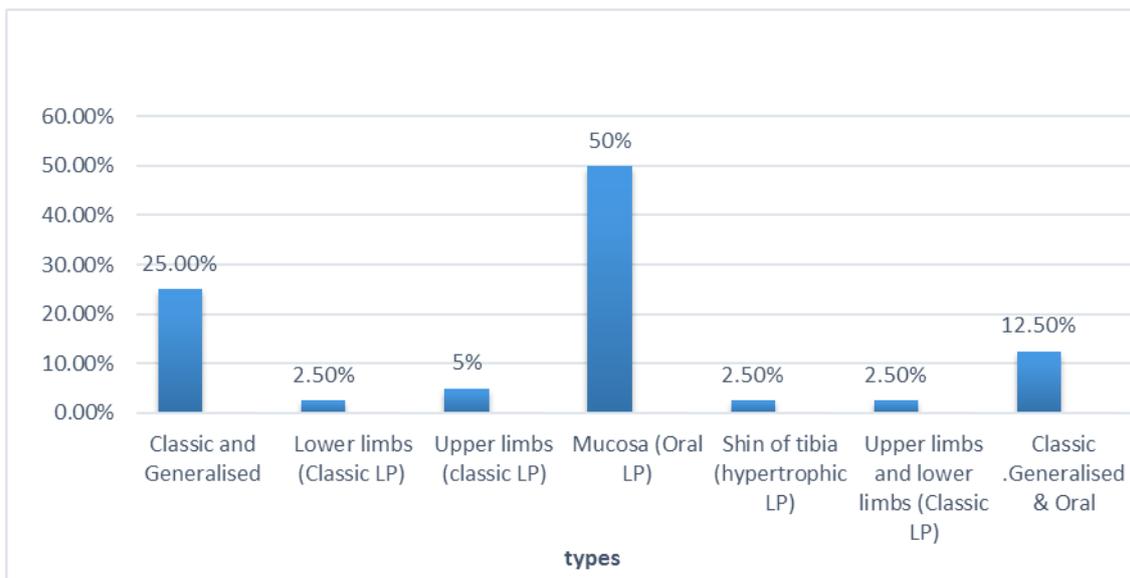**Figure (1): Site and Distribution of LP Lesions in the studied Cases**

Table (4): Anti Dsg3 Abs in the Serum of LP Patients (Oral LP &Cut. LP) as Compared to Normal Control Persons; (N= 80):

Anti Dsg3 Abs	Oral Cases N = 20	Cut. Cases N = 20	Controls N= 40	*p-value*
Mean	1361.37	2118.7	66.98	0.001*
SD	300.5	584.37	70.95	
median	1267.85	2105	51.2	
Minimum	1009.5	1268.4	0	
Maximum	2167.1	3316.4	182.4	

Table (4): show that Anti Dsg3 Abs in the Serum of LP patients (oral LP &cut. LP) was significantly higher as compared to normal control persons (p-value < 0.001)

Figure (2): Anti Dsg3 Abs in the Serum of LP Patients (Oral LP &Cut. LP) as Compared to Normal Control Persons
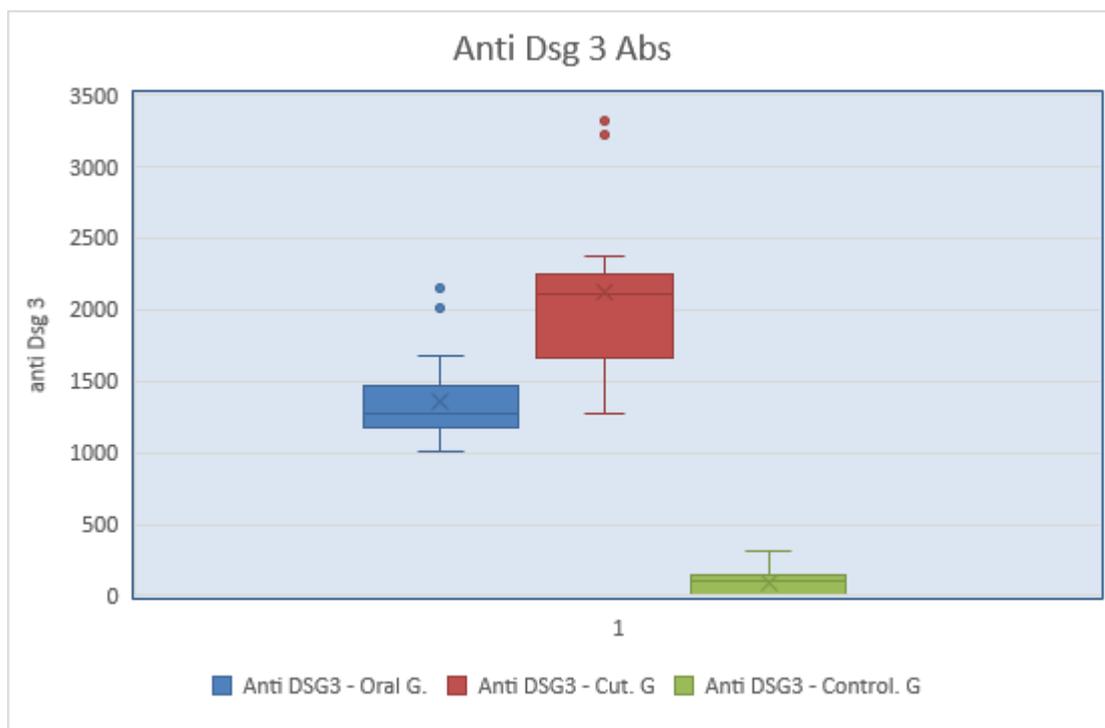

Table (4) and Figure (2) show that Anti Dsg3 Abs in the Serum of LP patients (oral LP &cut. LP) was significantly higher as compared to normal control persons (p-value < 0.001).

Table 5: Relation between Anti Dsg3 Abs in the Serum of LP Patients and the course of disease; (N=40):

	progressive N= 26	stable N= 14	*P-value*
Mean ± SD	1659.6 ± 543.8	1714.8 ± 690.6	0.784
Minimum	1009.5	1131.8	
Maximum	3217.1	3316.4	
Range	1444.1	1451.8	

Table (5): show that there was no detected relation between course of disease and anti Dsg3 Abs in the serum of LP patients; p-value >0.05.

4. Discussion:

Lichen planus (LP) is an idiopathic inflammatory disease of skin, nails, mucous membranes and hair. It is a T-cell mediated autoimmune damage to basal cell layer that express altered self-antigens on their surface and deposition of immunoglobulins, complements and fibrin in the basement membrane zone, but no specific antigen has been yet identified [8].

The aetiopathogenesis of LP is not fully understood; Many theories are included as genetic predisposition, exposure to environmental triggers or immune dysregulation. A large body of these theories supports a role of immune dysregulation in the pathogenesis. The various mechanisms hypothesized to be involved in the immunopathogenesis (Antigen-specific cell-mediated immune response, non-specific mechanisms, autoimmune response or humoral immunity) [2].

The presence of circulating antibodies to a lichen planus specific antigen on the granular and deep epithelial prickle cells in the skin lesions of lichen planus was suggested, but the antigen itself was only infrequently demonstrated. The presence of anti epithelial antibodies was reported in patients with oral and cutaneous lichen planus associated with drug therapy, but the antibodies were generally present only in low concentrations [9].

Auto-antibodies to Dsg1 and Dsg3, desmosomal cadherins expressed in stratified squamous epithelia and involved in cell-to-cell adhesion, play a pathogenic role in autoimmune bullous diseases, PV and BP, causing disruption of desmosoms and consequent acantholysis [10].

PV and BP share clinical similarities with LP. Mucosal lesions of patient with PV exhibit a similar phenotype as erosive mucosal LP [11]. In this present study we aimed to detect Dsg3 antibodies in LP patients serum of diseased patients as compared to normal control persons to investigate the possible role of these antibodies in the pathogenesis of LP. Serum samples were taken from the patients and also from the controls for measurement of anti Dsg3 antibodies by ELISA.

We performed this case-control study at dermatology outpatient clinic at Beni-Suef University hospital during the period from (1st february 2019 to 31 of august 2019) and the study included 80 participants; divided into two matched groups as 40 cases with LP and 40 normal controls.

The LP clinical types were variable; cutaneous LP with its subtypes (classic, hypertrophic and generalized) and oral LP with its subtypes (erosive and non-erosive). The disease course was variable between progressive and stable course.

Our study showed that The measurement of anti Dsg3 antibodies in serum samples from cases cutaneous and oral LP & controls reveled that; the anti Dsg3 abs in the serum of LP patients (oral LP &cut. LP) was

significantly higher as compared to normal control persons (p-value < 0.001); where the mean scores were 1361.37 ± 300.5 in oral LP & 2118.70 ± 584.37 in cutaneous LP vs. 66.98 ±70.95 in controls.

According to our results, we suggested that Dsg 3 auto-antibodies presence in the serum of LP patients may be a result of the degenerative demage of the keratinocytes rather than being a cause of the disease. The expression of self antigens (Dsgs) to the immunity will induce more T-cell mediated auto immune damage to the basal keratinocytes causing more inflammation (vicious circle).

There was no detected Correlation between anti Dsg3 Abs in The Serum of LP Patients (oral and cutaneous) and their age (p-value> 0.05). Similarly, there was no detected relation between sex and the level of anti Dsg3 Abs in the serum of LP patients (p-value >0.05). Moreover, it was found that no correlation with the duration of LP disease and the level of Dsg 3 auto-antibodies. Also no relation with the distribution of the LP lesions, no relation with the course of the disease.

Our findings were supported by that reported in a previous study done by ***Vahide et al.,*** to determine Autoantibodies to Dsg1 and 3 in patients with lichen planus. In their study; the research team examined the presence of anti-Dsg1 and 3 in patients with different types of oral LP patients and to compare them with cutaneous LP patients and healthy controls.

They found that; the serum levels of anti-Dsg3 antibodies in patients with erosive oral LP are significantly increased in comparison with healthy controls. This increase is not observed in reticular oral LP and cutaneous LP. Although the difference that they observed is statistically significant but it was under cut-off values not so clinically important [6].

Vahide et al. focused on the oral LP cases while our study included both oral (erosive and non-erosive) and cutaneous LP with its subtypes (classic, hypertrophic and generalized). However, both results concluded that the levels of anti-Dsgs antibodies in patient serum either oral or cutaneous were increased compared to the control cases.

In other study the author and colleagues studied 35 patients with oral lichen planus and 35 healthy control, serum autoantibody against Dsg3 showed significant increase in patients with OLP. Increased concentrations of anti Dsg3 autoantibodies, detected in the serum of patients with OLP, indicated that anti-keratinocytes autoantibodies may be involved in the pathogenesis of OLP [12].

In Croatia, other study included 57 Croatian patients with OLP and found that concentrations of serum antibodies against Dsg 1 and 3 in patients with non-erosive forms of lichen planus, erosive forms of lesions (reticular & bullous), and in a control group of patients with recurrent acute aphthous ulcers showed a significant increase. The authors showed that the humoral immunity against keratinocyte cadherins Dsg 1 and 3 seems to

play a role in OLP. They had chosen recurrent aphthous ulceration and OLP because these seemed to share the immunopathological features involving T-cell-mediated immunity [13].

They also included in the study patients who were taking medications. Medications such as nonsteroidal anti-inflammatory drugs (NSAIDs) can cause lichenoid reactions. These authors did not report whether concomitant cutaneous lichen planus was present. Therefore, the results of their research cannot be extrapolated [13].

Similarly, *Kinjyo et al.,* reported a case of erosive oral LP with anti-Dsg1 and 3 antibody levels of 49 and 36 U/ ml, respectively [14].

Before that, two Japanese cases of erosive oral LP were reported with higher serum levels of anti-Dsg1 antibodies, anti-Dsg1 and 3 antibody levels of 56.5 and 0.9 U/ml, respectively, for the first case and 40 and 24 U/ml, respectively, for the second one In these studies, the authors could not explain why serum levels of anti-Dsg1 antibodies were higher than anti-Dsg3 in oral LP [15].

Herrero et al., reported positive results for anti-Dsg3 antibodies in one case of clinically severe erosive oral LP out of 21 cases of oral LP (20 IU/ml; normal range \7 IU/ml). Therefore, they assumed that when the disease is more inflammatory, there is a higher probability for detecting circulating autoantibodies against epithelial antigens [11]. In contrast, in the case reported by *Kinjyo et al.,* anti- Dsg1 and 3 antibody levels were 49

and 36, respectively, on first examination, 35 and 27 at 4 months after the treatment with topical tacrolimus and 46 and 32 at 2 years after the treatment, respectively. In the follow-up visits, there was no lesion but antibody levels were consistently high [14].

Munde et al mentioned that geographic variance could affect the demographic and clinical presentation of OLP so that explained why serum levels of anti-Dsg3 antibodies were variable in oral LP according to geographic variance [16].

5. Conclusion and Recommendations:

Desmoglein-3 auto-antibodies in LP may have a role in the pathogenesis of the disease. This was detected as level of Dsg 3 auto-antibodies in serum of LP patients was significantly higher as compared to normal control persons. Further studies are warranted to better understand the certain mechanism of these auto-antibodies and how to prevent its role in the pathogenesis of LP disease.

Further investigations are needed for detection of Dsg 3 auto-antibodies in LP skin biopsy or blood samples by other methods as Immunofluorescence (direct and indirect).

There are limitations of this study, we are aware that ELISA has limitations as a diagnostic test (as do most protein assays) and is affected by interference.

Also in all the previous studies the authors focused on oral LP cases only. although there is awide range of LP types and distributions.

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