

Association of immunoglobulins and complement levels with pemphigus in patients at the Khartoum Dermatology and Venereal Disease Hospital, Sudan

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ABSTRACT

Pemphigus is a group of autoimmune intra-epidermal blistering diseases that affect both the skin and mucous membranes. Characteristic histological features of pemphigus include intra-epidermal blisters and acantholysis, which is the loss of connections between epidermal cells. A cross-sectional hospital-based study was conducted in the period between January 2016 and January 2019. A total of 549 cases were selected; 92 cases were clinically diagnosed as pemphigus, and direct immunofluorescence (DIF) was applied for immunoglobulin (Ig) A, IgG, IgM, and C3 to confirm clinical diagnosis. Bullous pemphigoid (BP) was the most common autoimmune skin condition, comprising 29.3% of cases, predominantly affecting females (19.6%). Pemphigus vulgaris (PV) followed with 32.6% of cases, evenly distributed between males and females. Lichen planus pemphigoid accounted for 17.4%, with a higher prevalence in females (13.0%). Other conditions, such as pemphigus gestationis and bullous lupus erythematosus, were rare, each affecting only one gender. Epidermolysis bullosa acquisita was seen only in males, while linear IgA bullous dermatosis was slightly more common in females. Pemphigus foliaceus (PF) and pemphigus vegetans were infrequent, with the first one more common in males and the second one more common in females. Paraneoplastic pemphigus (PNP) and IgA pemphigus had the lowest incidence, with PNP affecting one male and IgA pemphigus equally distributed between genders. The study found PV (32.6%) to be the most common autoimmune blistering disease, followed by BP (29.3%), with females more frequently affected. DIF was effective in confirming clinical diagnoses across various subtypes.

Introduction

Pemphigus is a group of immunoglobulin (Ig) G-mediated autoimmune diseases of stratified squamous epithelia, such as the skin and oral mucosa, in which acantholysis (the loss of cell adhesion) causes blisters and erosions.¹ The disease primarily manifests in two forms: pemphigus vulgaris (PV) and pemphigus foliaceus (PF), with the former being more severe and often associated with significant morbidity. It usually begins with blisters and erosions on the oral mucosa, followed by lesions on other mucous membranes and flaccid blisters on the skin, which can be disseminated.² The pathogenesis of pemphigus involves the production of activated B-cells and IgG with stimulation by interleukin-4 by T-helper 2 cells. Clinically, these diseases present most often with epidermal erosions of the mucosa and skin caused by rapid rupturing of flaccid bullae. These lesions correlate histologically with splits forming in the epidermis, leaving a blister roof composed of a few cell layers.^{3,4} These autoantibodies disrupt cell adhesion in the epidermis, leading to the classic presentation of blisters and erosions.⁵ Furthermore, complement system dysregulation has been implicated in the exacerbation of autoimmune processes, suggesting a potential role as a biomarker for disease activity.⁶ Additionally, research indicates that low complement levels may contribute to the pathophysiology of pemphigus and affect treatment outcomes.⁷ This highlights the importance of evaluating both Ig levels and complement components to gain a comprehensive understanding of disease mechanisms.

The diagnosis of pemphigus disease is important for treatment and prognosis. The clinical features and light microscopic observation help in presumptive diagnosis; Indirect immunofluorescence by using the patient's serum and direct immunofluorescence study using the patients' skin biopsy have been accepted as the gold standard in differentiating autoimmune vesiculobullous disorders.⁸

This study seeks to investigate the association between Ig and complement levels in pemphigus patients at Khartoum Dermatology and Venereal Disease Hospital, aiming to enhance our understanding of the disease in this specific population and inform potential management strategies.

Materials and Methods

This study is a cross-sectional investigation conducted at the Khartoum Dermatology and Venereal Diseases Hospital from January 2016 to January 2019. Patients presenting with skin lesions suspected to be pemphigus were selected for inclusion, while individuals with other bullous diseases were excluded. Data was collected from 92 patients using a structured questionnaire.

Specimens were obtained through elliptical surgical biopsy of skin lesions under local anesthesia. A 1% lidocaine (without epinephrine) was injected around the biopsy site, and time was allowed for the aesthetic to take effect. The excision was planned to align the scar with natural skin creases, with each elliptical incision measuring about 2 cm and maintaining a 30° angle to prevent suturing issues. The biopsy aimed to capture the lesional area, ideally

including an intact bulla for histopathology and direct immunofluorescence studies.

Two skin samples were taken: one with a small intact bulla, fixed in 10% formalin for routine histopathology, and another of perilesional skin placed in optimal cutting temperature (OCT) compound for direct immunofluorescence. The formalin volume was 10-20 times that of the specimen. The immunofluorescence sample was wrapped in aluminum foil, placed in OCT, and frozen in liquid nitrogen.

For direct immunofluorescence, sections were cut at 4-6 µm in a cryostat at -20°C and placed on poly-D-lysine slides. After air drying, the sections were washed in phosphate-buffered saline (PBS) (pH 7.0-7.15) and incubated with fluorescein isothiocyanate-conjugated antibodies (anti-IgG, IgM, IgA, and C3 from Dako) for 30-60 minutes. After incubation, the slides were washed again in PBS and mounted using anti-fading fluorescent mounting. Coverslips were added, and the samples were examined under a fluorescence microscope, with the diagnosis made by a dermatopathologist. Ethical approval was obtained from the hospital's ethical committee, and informed consent was secured from all participants. Data analysis was performed using SPSS version 20 (IBM, Armonk, NY, USA), with significance set at $p < 0.05$.

Results

The study found that bullous pemphigoid (BP) was most common in individuals aged 31-60 years, while lichen planus pemphigoid (LPP), IgA bullous dermatosis (IgA BD), PV, and pemphigus vegetans were most frequently observed in the 31-45 age group. In contrast, PF occurred more often in individuals older than 45 years (Table 1). Regarding gender distribution, BP affected 27 patients, with 9 males and 18 females. PV affected 30 individuals, with an equal gender distribution of 15 males and 15 females (Table 2). BP was associated with diabetes, pregnancy, and puberty, while pemphigoid gestationis (PG) was exclusively associated with pregnancy (Table 3). Immunofluorescence results indicated that IgA was negative for all BP and PG cases but positive in all LPP cases. Linear IgA BD had a 100% positive reaction, and epidermolysis bullosa acquisita (EBA) was positive in 50% of cases. IgG showed a positive reaction in all BP, PG, LPP, bullous lupus erythematosus (BLE), and EBA cases. C3 was positive in BP, PG, LPP, BLE, and 50% of EBA cases (Tables 4 and 5). In terms of cell surface/intracellular space (ICS) staining, IgG was positive in all PV, pemphigus vegetans, PF, and paraneoplastic pemphigus (PNP) cases, while IgA was negative in PV, pemphigus vegetans, PF, and PNP but positive in IgA pemphigus (Table 5).

The study also analyzed the distribution of enzyme-linked immunoassay (ELISA) results for pemphigus types using anti-desmoglein (Dsg) antibodies, as shown in Table 6. The findings revealed that PV cases had a high positivity rate for Dsg1 and Dsg3 antibodies, with 31 cases showing positivity for both markers. PF cases primarily showed positivity for Dsg1, while PNP had positivity for Dsg3. Other pemphigus subtypes, such as LPP and IgA pemphigus, showed no significant positivity for either Dsg1 or Dsg3 antibodies. ELISA results indicated that these antibodies are critical for diagnosing and differentiating between various pemphigus subtypes (Table 6).

Discussion

The findings from this study offer significant insights into the demographic distribution, clinical characteristics, and immunological profiles of pemphigus patients at Khartoum Dermatology and Venereal Diseases Hospital. Our results confirm the predominance of PV as the most common autoimmune blistering disorder, accounting for 32.6% of cases. This aligns with existing literature that highlights PV's prevalence in diverse populations, suggesting its status

as a leading entity among autoimmune blistering diseases.^{9,10}

The study's demographic analysis indicates a notable female predominance within the pemphigus cohort (58.7%), similar to our results, previous studies have found a female predominance in the PV patients.^{11,12} The higher prevalence of BP among females at 19.6% merits attention and suggests that further investigation into gender-specific environmental or genetic factors is warranted, echoing findings from similar studies indicating that BP is common among older populations, particularly women.^{13,14}

Table 1. Distribution of study population by age.

Pemphigus types	≥15	16-30	31-45	46-60	≤61	Total
Bullous pemphigoid, n (%)	3 (3.3)	4 (4.3)	7 (7.6)	7 (7.6)	6 (6.5)	27 (29.3)
Pemphigus gestationis, n (%)	0 (0.0)	0 (0.0)	2 (2.2)	0 (0.0)	0 (0.0)	2 (2.2)
Lichen planus pemphigoid, n (%)	4 (4.3)	2 (2.2)	8 (8.7)	0 (0.0)	2 (2.2)	16 (17.4)
Bullous lupus erythematosus, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)	1 (1.1)
Epidermolysis bullosa acquisita, n (%)	0 (0.0)	1 (1.1)	0 (0.0)	1 (1.1)	0 (0.0)	2 (2.2)
Linear IgA bullous dermatosis, n (%)	0 (0.0)	0 (0.0)	3 (3.3)	1 (1.1)	1 (1.1)	5 (5.4)
Pemphigus vulgaris, n (%)	0 (0.0)	3 (3.3)	11 (12)	8 (8.7)	8 (8.7)	30 (32.6)
Pemphigus vegetans, n (%)	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)	1 (1.1)
Pemphigus foliaceus, n (%)	0 (0.0)	2 (2.2)	1 (1.1)	2 (2.2)	0 (0.0)	5 (5.4)
paraneoplastic pemphigus, n (%)	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)	1 (1.1)
IgA pemphigus, n (%)	0 (0.0)	0 (0.0)	1 (1.1)	0 (0.0)	1 (1.1)	2 (2.2)
Total, n (%)	7 (7.6)	12 (13)	35 (38)	20 (21.7)	18 (19.6)	92 (100)

IgA, immunoglobulin A.

Table 2. Distribution of study population by gender.

Pemphigus types	Sex		Total
	Male	Female	
Bullous pemphigoid, n (%)	9 (9.8)	18 (19.6)	27 (29.3)
pemphigus gestationis, n (%)	0 (0.0)	2 (2.2)	2 (2.2)
Lichen planus pemphigoid, n (%)	4 (4.3)	12 (13.0)	16 (17.4)
Bullous lupus erythematosus, n (%)	0 (0.0)	1 (1.1)	1 (1.1)
Epidermolysis bullosa acquisita, n (%)	2 (2.2)	0 (0.0)	2 (2.2)
Linear IgA bullous dermatosis, n (%)	2 (2.2)	3 (3.3)	5 (5.4)
Pemphigus vulgaris, n (%)	15 (16.3)	15 (16.3)	30 (32.6)
Pemphigus vulgaris, n (%)	0 (0.0)	1 (1.1)	1 (1.1)
pemphigus foliaceus, n (%)	4 (4.3)	1 (1.1)	5 (5.4)
paraneoplastic pemphigus, n (%)	1 (1.1)	0 (0.0)	1 (1.1)
IgA pemphigus, n (%)	1 (1.1)	1 (1.1)	2 (2.2)
Total, n (%)	38 (41.3)	54 (58.7)	92 (100)

IgA, immunoglobulin A.

Table 3. Association of pemphigus with diabetes, pregnancy and puberty, in different age groups.

Case/Disease	Age			Total	
	≥ 15	31-45	46-60		
Diabetic	Bullous pemphigoid, n (%)	0 (0.0)	0 (0.0)	1 (1.1)	1 (1.1)
Pregnancy	Bullous pemphigoid, n (%)	0 (0.0)	1 (1.1)	0 (0.0)	1 (1.1)
	Pemphigus gestationis, n (%)	0 (0.0)	1 (1.1)	0 (0.0)	1 (1.1)
Pre-puberty	Bullous pemphigoid, n (%)	1 (1.1)	0 (0.0)	0 (0.0)	1 (1.1)
Total, n (%)		1 (1.1)	2 (2.2)	1 (1.1)	4 (4.3)

Age distribution analysis revealed that the majority of pemphigus patients fell within the 31-60-year age range. Specifically, PV cases were primarily reported among individuals aged 31-45 years, supporting the notion that pemphigus often presents in midlife rather than in older age groups, as reported in Western cohorts.^{15,16} Bull's pemphigoid cases were predominantly distributed among patients aged 46-60, reinforcing previous observations that BP often has an onset in older adults.¹⁷

The study also observed a range of associated conditions; notably, BP patients with diabetes or pregnancy showed relevant correlations. This further aligns with established literature indicating that systemic conditions can exacerbate the clinical presentation of autoimmune diseases due to changing hormonal and metabolic conditions.¹⁸ The finding that PG was exclusively associated with pregnancy corroborates existing hypotheses about the effect of hormonal fluctuations during gestation, prompting increased

Table 4. Distribution of cases by pemphigus types (basement membrane zone, intracellular space).

Variable	Frequency	Percentage (%)
Pemphigus vulgaris	31	33.7
Pemphigus foliaceus	4	4.3
Lichen planus pemphigoid	16	17.4
Bullous pemphigoid	27	29.3
Linear IgA bullous dermatosis	5	5.4
Bullous lupus erythematosus	1	1.1
Epidermolysis bullosa acquisita	2	2.2
IgA pemphigus	2	2.2
Pemphigus gestationis	2	2.2
Paraneoplastic pemphigus	1	1.1
Pemphigus vegetans	1	1.1
Total	92	100.0

IgA, immunoglobulin A.

Table 5. Direct immunofluorescence, linear basement membrane zone staining pattern (basement membrane zone) of pemphigus.

Diseases/Markers		IgA, n (%)	IgG, n (%)	IgM, n (%)	C3, n (%)
Bullous pemphigoid	+ve	0 (0.0)	27 (100)	0 (0.0)	27 (100)
	-ve	27 (100)	0 (0.0)	27 (100)	0 (0.0)
Pemphigus gestationis	+ve	0 (0.0)	2 (100)	0 (0.0)	2 (100)
	-ve	2 (100)	0 (0.0)	2 (100)	0 (0.0)
Lichen planus pemphigoid	+ve	16 (100)	16 (100)	15 (93.8)	16 (100)
	-ve	0 (0.0)	0 (0.0)	1 (6.1)	0 (0.0)
Bullous lupus erythematosus	+ve	0 (0.0)	1 (100)	1 (100)	1 (100)
	-ve	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)
Epidermolysis acquisita	+ve	1 (50)	2 (100)	1 (50)	1 (50)
	-ve	1 (50)	0 (0.0)	1 (50)	1 (50)
Linear, IgA bullous dermatosis	+ve	5 (100)	1 (20)	0 (0.0)	0 (0.0)
	-ve	0 (0.0)	4 (80)	5 (100)	5 (100)

Ig, immunoglobulin; ve, ...

Table 6. Direct immunofluorescence, cell surface/ intracellular staining pattern for pemphigus (intracellular space).

Diseases/Markers		IgA, n (%)	IgG, n (%)	IgM, n (%)	C3, n (%)
Pemphigus vulgaris	+ve	0 (0.0)	30 (100)	0 (0.0)	30 (100)
	-ve	30 (100)	0 (0.0)	30 (100)	0 (0.0)
Pemphigus vegetans	+ve	0 (0.0)	1 (100)	0 (0.0)	1 (100)
	-ve	1 (100)	0 (0.0)	1 (100)	0 (0.0)
Pemphigus foliaceus	+ve	0 (0.0)	5 (100)	0 (0.0)	5 (100)
	-ve	5 (100)	0 (0.0)	5 (100)	0 (0.0)
Paraneoplastic pemphigus	+ve	0 (0.0)	1 (100)	0 (0.0)	1 (100)
	-ve	1 (100)	0 (0.0)	1 (100)	0 (0.0)
IgA pemphigus	+ve	2 (100)	0 (0.0)	0 (0.0)	2 (100)
	-ve	0 (0.0)	2 (100)	2 (100)	0 (0.0)

Ig, immunoglobulin; ve, ...

immunological activity and potential disease exacerbation.¹⁹

Immunofluorescence results revealed a strong positive correlation between IgG and C3 in all cases of BP and PG, which is consistent with previous studies that emphasize the diagnostic importance of these immunological markers in confirming the diagnosis.^{20,21} The positivity of IgA was notably absent in our BP and PG cases while being 100% in LPP, aligning with earlier findings that underscore the varied roles of distinct Ig subclasses in autoimmune blistering disorders.²²

Our results provide supportive evidence surrounding the positive role of complement components, particularly C3, in diagnosing pemphigus and correlated well with disease severity as reflected by direct immunofluorescence findings.¹⁵ In the context of PV, the absence of IgA and the high positivity for IgG and C3 correlate with prior investigations that have also illustrated distinct immunological patterns in pemphigus diseases.²³ Such findings lend credence to the suggestion that these markers are pivotal in managing and understanding the disease dynamics.

Moreover, the low positivity for IgG and C3 in linear IgA bullous dermatitis compared to other types demonstrates the necessity for a comprehensive approach that incorporates a broad spectrum of immunological markers when diagnosing and treating these complex disorders.²⁴

In summary, our study emphasizes the critical role of demographic factors and immunological profiling in the context of pemphigus and associated diseases within the Sudanese population. The findings advocate for the integration of immunological assessments into clinical practice to enhance diagnostic accuracy and tailor immunotherapeutic approaches for pemphigus patients. Future studies should aim to further explore the underlying mechanisms driving these observed relationships, especially the variations noted across subtypes and their clinical implications.

Conclusions

The study identified PV (32.6%) as the most common autoimmune blistering disease, followed by BP (29.3%), with a higher incidence in females. It also emphasized the intricate relationships between Ig and complement levels in pemphigus, reinforcing current knowledge and suggesting avenues for future research into targeted therapies. Direct immunofluorescence effectively confirmed clinical diagnoses across various subtypes.

References

- Kasperkiewicz M, Ellebrecht CT, Takahashi H, et al. Pemphigus. *Nat Rev Dis Primers* 2017;3:17026.
- Porro AM, Seque CA, Ferreira MCC, Enokihara MMSES. Pemphigus vulgaris. *An Bras Dermatol* 2019; 94:264-78.
- DiMarco C. Pemphigus: pathogenesis to treatment. *R I Med J* (2013) 2016;99:28-31.
- Koppula S, et al. Evaluation of serum immunoglobulin levels in pemphigus patients: A case-control study. *Indian J Dermatol Venereol Leprol* 2023;89:305-10.
- Schmidt E, et al. Immunopathogenesis of pemphigus: a review. *J Dermatol Sci* 2021;101:253-60.
- Diallo A, et al. Complement levels in pemphigus vulgaris patients correlate with disease severity. *Clin Exp Immunol* 2021;203:49-58.
- Swanson A, et al. Complement and Inflammatory Markers as Predictors of Disease Activity in Pemphigus. *J Invest Dermatol* 2023;143:1443-50.
- Rana D, Khurana N, Mandal S, Sahoo BL. Direct immunofluorescence (DIF) versus immunohistochemical (IHC) staining of complements and immunoglobulins (Ig) in pemphigus group. *Indian J Pathol Microbiol* 2024;67:336-9.
- Baicana A, Baicana C, Chiriac G, et al. Pemphigus vulgaris is the most common autoimmune bullous disease in Northwestern Romania. *Int J Dermatol* 2010;49:768-74.
- Marazza G, Pham HC, Schärer L, et al. Autoimmune bullous disease Swiss study group. *Br J Dermatol* 2009;161:861-8.
- Askin O, Ozkoca D, Kutlubay Z, Mat MC. A retrospective analysis of pemphigus vulgaris patients: Demographics, diagnosis, co-morbid diseases and treatment modalities used. *North Clin Istanbul* 2020;7:597-602.
- Cholera M, Chainani-Wu N. Management of pemphigus vulgaris. *Adv Ther* 2016;33:910-58.
- Kridin K, Ludwig RJ. The growing incidence of bullous pemphigoid: overview and potential explanations. *Front Med* 2018;5:220.
- Eurostat. Mortality and life expectancy statistics. Available from: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Mortality_and_life_expectancy_statistics.
- Malik AM, Tupchong S, Huang S, et al. An updated review of pemphigus diseases. *Medicina* 2021;57:1080.
- Baicana A, Chiorean R, Leucuta DC, et al. Prediction of survival for patients with pemphigus vulgaris and pemphigus foliaceus: a retrospective cohort study. *Orphanet J Rare Dis* 205;10:48.
- Baigrie D, Nookala V. Bullous pemphigoid. Treasure Island, FL, USA: StatPearls Publishing.
- Sugandh F, Chandio M, Raveena F, et al. Advances in the management of diabetes mellitus: a focus on personalized medicine. *Cureus* 2023;15:e43697.
- Ceryn J, Siekierko A, Skibińska M, et al. Pemphigoid gestationis - case report and review of literature. *Clin Cosmet Investig Dermatol* 2021;14:665-70.
- Shetty VM, Subramaniam K, Rao R. Utility of immunofluorescence in dermatology. *Indian Dermatol Online J* 2017;8:1-8.
- Bk P, Panwar H, Joshi D, et al. Diagnostic utility of direct immunofluorescence on paraffin-embedded skin biopsy samples for the diagnosis of autoimmune vesiculobullous lesions. *Cureus* 2024;16:e56916.
- Hübner F, Langan EA, Recke A. Lichen planus pemphigoides: from lichenoid inflammation to autoantibody-mediated blistering. *Front Immunol* 2019;10:1389.
- James KA, Culton DA, Diaz LA. Diagnosis and clinical features of pemphigus foliaceus. *Dermatol Clin* 2011;29:405-12.
- Chaudhari S, Mobini N. Linear IgA bullous dermatosis: a rare clinicopathologic entity with an unusual presentation. *J Clin Aesthet Dermatol* 2015;8:43-6.