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Original Research Article

Utility of Immunofluorescence in Cutaneous Vasculitis

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Abstract

Vasculitis may be due to various causes, including connective tissue disorders, medications, and infections. Histopathology shows similar features in different diseases so DIF (direct immunofluorescence) helps to categorize these vasculitic lesions on basis of positivity of different immunoglobulins. In this study, 40 cases of suspected vasculitis were confirmed by histopathology. Females were more commonly affected and the age range was from 9 to 71 years. Among these cases, 21 were immune complex mediated vasculitis of which c3 and IgG were the most commonly found immunoglobulins. Two of these cases were IgA mediated vasculitis. DIF was of great importance for the diagnosis of the disease for appropriate treatment.

Keywords: Skin biopsy, cutaneous vasculitis, immunofluorescence, immunoglobulins.

BACKGROUND

Systemic vasculitis may be divided into primary and secondary vasculitic syndromes. The etiology is unknown in primary syndrome leading to blood vessel inflammation. On the other hand, secondary vasculitis can be due to various causes which include connective tissue disorders, infections, drugs, immunization and neoplasia. Most of these patients are diagnosed with leukocytoclastic vasculitis [1].

These various immune complex mediated vasculitides can be confirmed by DIF (direct immunofluorescence) examination. Histopathology in such cases may show only features of vasculitis, therefore DIF is required for diagnosis [2].

Clinically, leukocytoclastic vasculitis presents with palpable purpura which may be seen on pressure areas. Histopathologically, small vessel vasculitis show features of vessel damage including fibrin, thrombi, endothelial cell damage, neutrophils and nuclear dust. DIF shows positivity in vessel walls for IgG and c3 in leukocytoclastic vasculitis and IgA deposits are seen in Henoch Schonlein Purpura [3].

Timing of biopsy samples is extremely important, and lesions should be sampled for biopsy within 18-36 hours and for DIF within 6 hours after onset of the skin lesions. Failure to do so may lead to lack of characteristic findings and difficulties in diagnosis [4].

MATERIALS AND METHODS

Retrospective analysis of all skin biopsies sent for assessment of vasculitis over a period of 3 years was done (2018-2021). Samples were received in 10% formalin for routine histopathology, and normal saline for DIF studies. Sections were cut and stained with routine Hematoxylin and Eosin. For DIF, frozen sections were cut and stained with respective antibodies to IgG, IgA, IgM, c3 and c1q. Scoring for DIF positivity was done on a scale of 0 to 4+ with scores more than 2+ recorded as positive. Slides were analyzed in their respective microscopic configurations and diagnoses were made.

RESULTS

A total of 135 skin biopsies were received at the Department of Pathology and Laboratory Medicine, Grande International Hospital over the period of 2018 to 2021 for histopathology and immunofluorescence studies. Among these, 40 cases showed microscopic features of vasculitis, of which 23 were female and 17 were male. Age of these patients ranged from 9 years to 71 years of age. Figure 1 shows the presenting features of these patients.

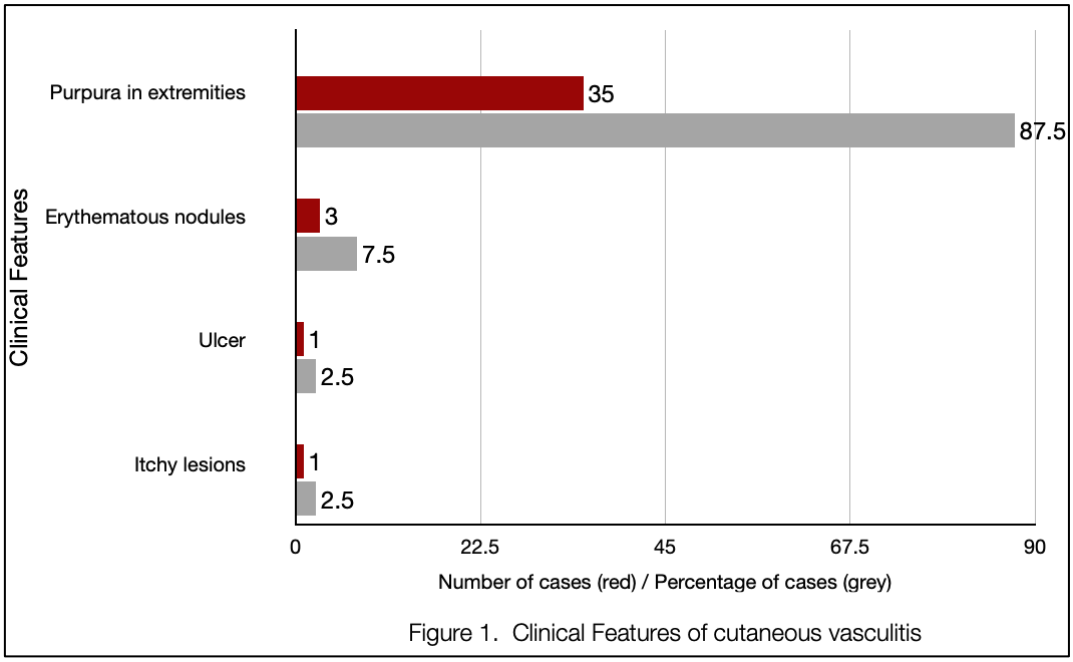

Figure 1. Clinical Features of cutaneous vasculitis

Clinically, almost all lesions presented with purpura in extremities. Other findings of vasculitis were erythematous nodules and rarely ulcers and itchy lesions.

The following table (Table 1) shows a comparison of immune complex mediated and non-immune mediated cases according to age and sex. All the cases showed features of vasculitis on histopathology.

Table 1: Age and sex distribution of immune vs non-immune mediated vasculitis

Age Group	Number of Cases	Immune Complex Mediated Vasculitis		Non-Immune Complex Mediated	
		M	F	M	F
Up to 10 years	1	0	1	0	0
11-20	5	1	1	1	2
21-30	14	2	5	4	3
31-40	8	2	3	1	2
41-50	3	1	1	1	0
51-60	5	0	1	1	3
61-70	3	1	1	0	1
71-80	1	0	0	1	0

Among these cases, 21 were non-immune mediated leukocytoclastic vasculitis and 19 showed immune complex deposits. Most of the cases were seen in the age group of 21 to 40 years of age. Immune complex mediated vasculitis was more commonly seen in women 13/23 cases (56.5%) compared to men accounting for 7/17 cases (41.2%).

The following table (Table 2) shows positivity of different immunoglobulins in immune complex mediated vasculitis.

Table 2: Percentage of positivity of different immunoglobulins

Immunoglobulin	Number of Positive Cases	Percentage of Positive Cases (N=19)
IgG	14	77.8
IgA	2	11.1
IgM	2	11.1
c3	15	83.3
c1q	4	22.2

Among these positive cases, c3 and IgG were seen in most of the cases. IgA mediated vasculitis was seen in two cases, accounting for 11.1% of all cases. C1q was also seen in 4 cases and follow up for lupus was advised, as c1q is typically associated with lupus cases. An example of immune complex mediated

vasculitis is seen in figure 2 which showed leukocytoclastic vasculitis on histopathology and positivity for IgG and c3 in immunofluorescence.

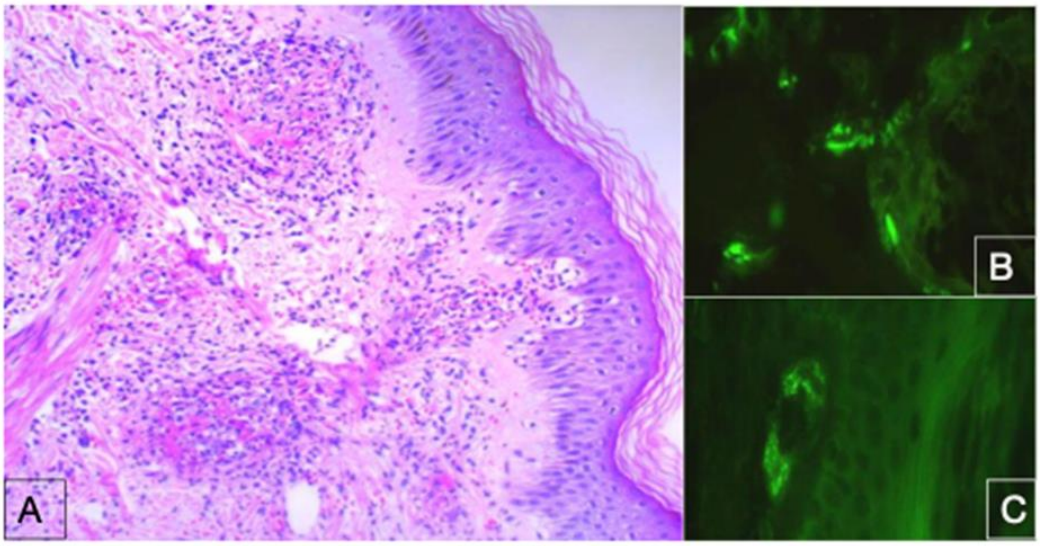

Figure 2: (A) Hematoxlyin and eosin (100x) section showing skin with upper dermis showing features of leukocytoclastic vasculitis including neutrophilic infiltrate, fibrin, and RBC extravasation. (B) and (C). Direct immunofluorescence findings showing perivascular positivity for IgG and c3 in upper dermal vessels.

DISCUSSION

In leukocytoclastic vasculitis, skin lesions may be the only manifestations of the disease. Clinically they present with macules or papules (palpable purpura). Sometimes, though, lesions may be pustular, hemorrhagic or ulcerate [5]. Our cases mostly presented with purpura in extremities with 1 case presenting with ulcer, and 2 with rashes.

C3 and IgM or IgA was found in most patients presenting with vasculitis in the study by Sams *et al.*, However, they were also present in normal skin about half of the patients so careful interpretation is required with histopathology and clinical features. Early biopsies are required as the neutrophils will ingest and destroy the antigen within 24 to 48 hours [6].

In a study by Kulthanan *et al.*, 76% cases showed immune deposits in vessel walls, mostly superficial. Most common reactants were c3 (71%) followed by IgM (35%), IgA (12%) and IgG (8%). One day old lesions yielded 82% positivity which dropped to 74% on biopsies sampled on days 2-7 [7]. In our study, c3 (83.3%) was the most common immune deposit followed by IgG (77.8%) and c1q (22.2%) followed by IgA (11.1%). Presence of c1q is typically associated with lupus in the kidney, so further evaluation for lupus was advised. In addition, deposition of c1q at dermoepidermal junction has been associated with lupus [8].

For biopsy for vasculits, erythematous or active border of new lesion less than 24 hours is recommended. Ulcers and old lesions should be avoided if possible [9].

Henoch-Schonlein purpura is a disease commonly seen in childhood which presents with leukocytoclastic vasculitis, abdominal pain, kidney disease and arthralgia. It is rare in adults [10]. Peri- or intravascular positivity for IgA is considered positive in these cases [11, 12]. Two cases showed IgA positivity in our study, which may be due to lower number of overall samples.

In a study by Nandeesh *et al.*, DIF showed overall positivity of 39% with c3 and IgA being the most common immunoglobulins. Male to female was 1: 1.6. Lower limbs are preferentially involved possibly due to gravitational vascular stasis. Non-specific trapping of immunoglobulins may also lead to false positive interpretation. Because of this, sites other than lower limbs are preferred for obtaining biopsies [13]. In our study, 47.5% of the cases showed immune deposits and male: female ratio was 1:1.35 which is similar to the aforementioned study. In addition, sites other than lower limbs were preferably sampled by the clinicians unless there were no lesions elsewhere available to be sampled.

CONCLUSION

Direct immunofluorescence is an invaluable tool for diagnosis of vasculitis and can be instrumental in the investigation and specific treatment of different types of vasculitis. Larger number of cases should be studied to get a better picture of the patterns of immunofluorescence in these cases.

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