SEEING SKIN IN CUTANEOUS VASCULITIDES THROUGH PATHOLOGIST'S EYES ROLE OF HISTOPATHOLOGY AND DIF IN DIAGNOSIS

Pathology

Singh Deeksha*	MD Pathology, Lady Hardinge Medical College, New Delhi.*Corresponding Author
Agarwal Shilpi	Director Professor, Department of Pathology, Lady Hardinge Medical College, New Delhi.
Chander Ram	Director Professor & Head, Department of Dermatology and STD, Lady Hardinge Medical College, New Delhi.
Agarwal Kiran	Director Professor, Lady Hardinge Medical College, New Delhi.
Singh Preksha	MD Dermatology and STD, Lady Hardinge Medical College, New Delhi.

ABSTRACT

Vasculitides is a group of disorders characterized by inflammation of vessel wall. Because of its rich vasculature, the skin manifestations are frequently seen in vasculitis. Vasculitis is a clinico-pathologic diagnosis. Histopathologic study of skin biopsy is confirmatory while direct immunofluorescence (DIF) examination is adjunctive to this and helps to categorize the vasculitis. Although the percentages of cases of Cutaneous Vasculitis diagnosed on histopathology & direct immunofluorescence separately were found to be comparable in this study, both techniques have their own place in the confirmation & classification of cutaneous vasculitis particularly in HSP with appropriate clinical correlation.

KEYWORDS

cutaneous vasculitis; direct immunofluorescence; perivascular inflammation; fibrinogen

INTRODUCTION

Vasculitides is a group of disorders characterized by inflammation of vessel wall. Cutaneous involvement in vasculitis may be primary or associated with other systemic manifestations e.g. Henoch Schonlein Purpura (HSP) which is characterized by a purpuric rash, often with arthritis, abdominal pain & hematuria. Cryoglobulinaemic vasculitis (CV) involves mainly skin, joints, kidneys and peripheral nerves. Cryoglobulinaemic vasculitis usually involves skin in the form of purpura on the lower legs, which may develop after cooling.

Vasculitis is a clinico-pathologic diagnosis. Histopathologic study of skin biopsy is confirmatory while direct immunofluorescence (DIF) examination is adjunctive to this and helps to categorize the vasculitis.[1] A lesion of 18–24 hours' duration shows the most diagnostic features.[2] However, in practice, biopsies from much older lesions are sent for DIF, especially in resource poor settings like India where patient usually seeks specialist's help later in the course of disease.[3]

MATERIALS AND METHODS

A cross-sectional study was undertaken in a tertiary care hospital in Delhi after obtaining clearance from the institutional ethical committee. Aims were to correlate the histopathology & DIF results of lesional skin biopsy in clinically diagnosed cases of Cutaneous Vasculitides. Twenty-eight cases having palpable purpura on skin, with or without systemic features, who gave informed written consent presenting in the Department of Dermatology, Venereology & Leprosy, over a 2-year period; were studied in the Department of Pathology. Patients with bleeding diathesis or history of systemic steroids/ immunosuppressive therapy/ anticoagulants in last 1 month were excluded. A 4 mm punch biopsy of lesion was taken. One bisected half was sent for histopathological examination in 10% Neutral Buffered Formalin (NBF) and other half in Michel's medium for DIF examination. Five µm thick frozen tissue sections were taken for DIF. Slides were stained with fluorescein isothiocyanate (FITC) conjugated antibodies (Dako) directed against IgG, IgM, IgA, C3 and fibrinogen by a standardized method and viewed under the fluorescence microscope fitted with a UV-light source. Each slide was assessed for presence or absence of deposits, site of deposition, pattern as well as intensity (1+ to 4+) of fluorescence.

Table 1: Key features of various cutaneous vasculitides in the study

Histopathology	DIF
Leucocytoclastic vasculitis (LCV) & Henoch Schonlein Purpura (HSP)	Immunoglobulins & complement in dermal blood vessels wall (in LCV & CV), IgA in HSP
Cryoglobulinemic vasculitis (CV)	
• Svasation of RBCs	
• Acute vasculitis	

No vasculitis, perivascular mononuclear cells infiltrate

- Extravasation of RBCs in the dermis
- Homogeneous, eosinophilic, PAS positive material in superficial dermal blood vessels

Final diagnosis was made after considering histopathology and DIF findings in relation to clinical manifestations & relevant investigations wherever necessary.

RESULTS

Out of 28 cases included in the study, histopathologic diagnosis was made in 25 (89.3%) cases on the basis of key features (all diagnosed as leucocytoclastic vasculitis). [Table 1, Figure 1] Whereas 3 (10.7%) cases showed non-specific findings (like perivascular acute / chronic inflammatory infiltrate only) and could not be diagnosed on histopathology alone.

Figure 1: (a) Histopathological picture of a case of LCV [H&E, 400X] (b) deposits of IgA in a case of HSP [400X]

On DIF, 23/28 (82.1%) cases showed immunoreactant deposition in dermal blood vessel walls. Of these, 20/28 (71.4%) cases were diagnosed as LCV, 2/28 (7.1%) cases as HSP & 1/28 (3.6%) case as immunologically mediated vasculitis. [Table 2]

Table 2: Distribution of immunoreactants (n=28)

Final Diagnosis (n)	Histopath diagnosis (n)	Number of DIF positive cases (n)	Type of Immune Deposits (n)	Site of Immune Deposit
LCV (25)	25	20	Fibrinogen (16), C3 (1), IgG (1), C3+Fibrinogen (1), IgG+Fibrinogen (1)	Superficial dermal blood vessels wall

HSP (2)	0	2	IgA (2)	
Immunologically mediated vasculitis (1)	0	1	IgM (1)	

Overall, in cutaneous vasculitides, either alone or in combination, the most common deposit seen in superficial dermal blood vessels wall, was fibrinogen seen in 18/28 (64.3%) cases. In LCV, the most common deposit seen in superficial dermal blood vessels wall was fibrinogen seen in 16/25 (64%) cases followed by C3, IgG, combination of C3 with fibrinogen & combination of IgG with fibrinogen in 1/25 (4%) case each.

Both cases of HSP showed IgA deposits while single case of CV showed IgM deposits in superficial dermal blood vessels wall.

Overall, a histo-immunological concordance was seen in 19/28 (67.8%) cases. Nine (32.1%) out of 28 cases of cutaneous vasculitides showed histo-immunological discordance including 6 cases of LCV, 2 cases of HSP & 1 case of CV. Out of 6/25 (24%) histopathologically diagnosed cases of LCV, 5/28 (17.8%) cases were found to be DIF negative while 1 (3.6%) case was finally diagnosed as HSP after correlating DIF findings with clinical features (purpura, arthralgia & hematuria). While 3/28 (10.7%) cases with non-specific histopathology were finally diagnosed as one case of HSP, LCV & CV each, after correlating DIF with other clinico-investigative findings. The patient of cryoglobulinemic vasculitis presented with purpura & cyanosis in acral parts, precipitated by cold & hematologic abnormalities (presence of RBC rouleaux formation on peripheral blood smear & cryoglobulins in serum). [Table 3]

Table 3: Nine cases showing histopathological & DIF discordance in clinically diagnosed cases of cutaneous vasculitides

Clinical diagnosis	Histopathological diagnosis	DIF result	Final Diagnosis (no. of cases)
LCV	LCV	Negative	LCV (5)
LCV	NS	Suggestive of LCV	LCV (1)
HSP	LCV	Suggestive of HSP	HSP (1)
HSP	NS	Suggestive of HSP	HSP (1)
CV	NS	Suggestive of Immune mediated vasculitis	CV (1)

Histopathology was diagnostic/suggestive (96%) in more cases than DIF (80%) particularly in LCV. However, DIF played a crucial role in establishing the diagnosis of HSP & CV in conjunction with clinico-histopathological findings. [Table 4]

Table 4: Comparison of positive results of DIF and histopathology

Final Diagnosis (n=28)	Diagnosed on histopathology (%)	Positive on DIF (%)
LCV (25)	96% (24/25)	80% (20/25)
HSP (2)	50% (1/2)	100% (2/2)
CV (1)	0	100% (1/1)
Total (28)	89.3% (25/28)	82.1% (23/28)

DISCUSSION

A histopathological diagnosis of LCV was made in 25/28 (89.3%) whereas 3/28 (10.7%) cases showed non-specific histopathology. Bagai et al [7] & Gupta S [8] reported features of vasculitis on histopathology in 94.4% & 84% cases respectively. Kumar et al [9] reported 75% cases of LCV & Nandeesh BN et al [3] found LCV (66%), lymphocytic vasculitis (18%) & perivascular inflammation (16%) on histopathology. Khetan P et al [6] reported leukocytoclasia in 84.9% cases of cutaneous vasculitis.

In the present study, 23/28 (82.1%) cases showed positive findings on DIF, out of which, 20/28 (71.4%) cases were diagnosed as LCV, 2/28 (7.1%) cases as HSP & 1/28 (3.6%) case as immunologically mediated vasculitis (finally diagnosed as cryoglobulinemic vasculitis) on DIF.

Table 5: Comparison of overall positivity of DIF in cutaneous vasculitides in a few studies

Authors	Overall positivity (%)
Grunwald et al. 1997 (n=40)	92
Barnadas et al. 2004 (n=50)	82
Kulthanan et al. 2004 (n=30)	76
Gupta et al. 2009 (n=23)	74
Nandeesh et al. 2013 (n=198)	39
Present study (n=28)	82.1

Kulthanan et al [14] found DIF positivity of 76% in LCV, however the positivity varied from 82% in 1 day old lesions to 74% in 2-7 day old lesions at the time of biopsy. Nandeesh BN et al [3] reported overall DIF positivity of only 39% with significantly higher positivity of 85% in <7 days old lesions. Kumar et al [9] & Nandeesh BN et al [3] reported positive DIF in 50% & 90% cases of HSP respectively.

Overall, in cutaneous vasculitides, the most common deposit was fibrinogen seen in 18/28 (64.3%) followed by IgG, IgA & C3 in 2/28 (7.1%) cases each in concordance with Bagai A et al [7] who also reported fibrinogen (97.2%) as the most common immunoreactant in cutaneous vasculitides followed by C3 (86.1%), IgA (77.8%), IgM (61.1%) & IgG (16.7%). Gupta S et al [8] reported IgM & complement as the most common deposits (52.2%) in cutaneous vasculitides followed by IgA in (43.5%) & IgG (27.1%). Mackel S et al [4] reported C3 (81%) & fibrinogen (78%) to be the commonest immunoreactants Khetan P et al [6] also found C3 to be the commonest deposit followed by IgG, IgA & IgM in vasculitides.

In LCV also, fibrinogen alone was the most common deposit seen at dermal blood vessel walls in 16/25 (64%) cases of LCV. Grunwald MH et al [12] also reported fibrinogen (67%) to be the predominant deposit in LCV.

Both cases of HSP showed IgA deposits while single case of immunologically mediated vasculitis (which later was diagnosed as CV) showed IgM deposits at dermal blood vessel walls. Kumar L et al [9] reported IgA in dermal capillaries on DIF in 50% cases while Nandeesh B et al [3] reported IgA in 90% cases of HSP.

All 23/23 (100%) DIF positive cases including 20/20 (86.9%) cases of LCV, 2/2 (100%) of HSP & 1 case of CV showed immunoreactant deposition in superficial dermal blood vessels. Kulthanan K et al [14] found that the most common site of immunoreactant deposits was superficial blood vessel walls alone (47%), followed by a combined pattern of DEJ & blood vessel wall deposits (39%), a combination of superficial & deep dermal blood vessel deposition (26%) & deep blood vessel deposits alone (3%). Gupta S et al [8] reported blood vessel deposits in 73.9% & additional cytooid bodies & lupus band in 4.3% cases each.

A histo-immunological concordance was seen in 19/28 (67.8%) cases. Mackel SE et al [4] reported that immune deposit may be absent in older lesions of vasculitis. Ryan TJ et al [15] also emphasized the timing of biopsy as a biopsy specimen taken too late (i.e. >48 hrs) may show more of pathology of repair than injury & negative DIF because immune deposits are degraded rapidly. Kumar L et al [9] reported histo-immunological correlation in 30.5% cases of HSP, histopathology alone diagnosed vasculitis in 44.4% patients while DIF alone clinched the diagnosis in 7/36 (19.4%) cases.

CONCLUSION

Although the percentages of cases of Cutaneous Vasculitis diagnosed on histopathology & direct immunofluorescence separately were found to be comparable in this study, both techniques have their own place in the confirmation & classification of cutaneous vasculitis particularly in HSP with appropriate clinical correlation. However, the timing of biopsy is important & it should be done at an early stage of lesion so as to avoid non-specific features on histopathology & false-negative results on direct immunofluorescence.

REFERENCES

- Palit A, Inamdar AC. Vasculitis: Approach to diagnosis and therapy. Indian J Dermatol Venereol Leprol. 2006; 72: 334-45.
- Sams Jr WM. Hypersensitivity angitis. J Invest Dermatol 1989; 93:78-81.
- Nandeesh BN, Tirumala R. Direct immunofluorescence in cutaneous vasculitis: experience from a referral hospital in India. Ind J Dermatol. 2013; 58 (1): 22-25.
- Mackel SE, Jordon RE. Leukocytoclastic vasculitis. A cutaneous expression of immune complex disease. Arch Dermatol 1982; 118: 296-301.
- Hodge SJ, Callen JP, Ekenstam E. Cutaneous leukocytoclastic vasculitis correlation of histopathological changes with clinical severity and course. J Cut Pathol 1987; 14: 279-84.
- Khetan P, Sethuraman G, Khaitan BK, Sharma VK, Gupta R, Dinda AK, et al. An aetiological & clinicopathological study on cutaneous vasculitis. Indian J Med Res. 2012 January; 135(1): 107-113.
- Bagai A, Albert S, Shenoi SD. Evaluation and therapeutic outcome of palpable purpura. Ind J Dermatol Venereol Leprol. 2001; 67: 320-3.
- Gupta S, Handa S, Kanwar AJ, Radotra BD, Minz RW. Cutaneous vasculitides: clinicopathological correlation. Ind J Dermatol Venereol Leprol. 2009; 75: 356-62.
- Kumar L, Singh S, Goraya JS, Uppal B, Kakkar S, Walker R, et al. Henoch-Schönlein purpura: The Chandigarh experience. Indian Pediatrics. 1998; 35: 19-25.
- Bagga A, Kabra SK, Srivastava RN, Bhuyan UN. Henoch-Schönlein purpura in Northern Indian children. Indian Pediatr 1991 Oct; 28(10): 1153-57.

11. Dispenzieri A, Gorevic PD. Cryoglobulinemia. *Hematol Oncol Clin North Am* 1999; 13: 1315-49.
12. Grunwald MH, Avinoach I, Amichai B, Halevy S. Leukocytoclastic vasculitis – correlation between different histologic stages and direct immunofluorescence results. *Int J Dermatol* 1997; 36: 349-352.
13. Barnadas MA, Perez E, Gich I, Llobet JM, Ballarin J, Calero F, et al. Diagnostic, prognostic & pathogenic value of the direct immunofluorescence test in cutaneous leukocytoclastic vasculitis. *Int J Dermatol* 2004; 43: 19-26.
14. Kulthanan K, Pinkaew S, Jiamton S, Mahaisavariya P, Suthipinitarm P. Cutaneous leukocytoclastic vasculitis: the yield of direct immunofluorescence study. *J Med Assoc Thai.* 2004 May; 87(5):531-5.
15. Ryan TJ. Cutaneous vasculitis. *J Cutan Pathol* 1985; 12:381-387.