Pathergy Test in Thai Patients with Behçet's Disease : A Clinical, Histopathological and Direct Immunofluorescence Study

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Abstract : To evaluate the clinical, histopathological and immunopathological findings of the pathergy test in Thai patients with Behçet's disease.

Methods : A pathergy test using the needle prick method with a 20G disposable needle after cleaning with 70% alcohol was performed in 5 patients (4 females and 1 male), with Behçet's disease according to International Study Group (ISG) criteria. The skin was pricked making a 45°angle with the skin surface to a depth of 3 mm at an avascular and hairless point of both forearms symmetrically. After 4 and 48 hours the test sites were evaluated clinically and skin biopsies were performed at a prick site on the right forearm after 4 hours for direct immunofluorescence study and after 48 hours at the prick site on the left forearm for histopathological study.

Results : Clinical pathergy tests were positive (a single papular lesion at the needle prick site) in 3 out of 5 patients at 48 hours. No response was observed at 4 hours. Direct immunoflurorescence study showed no deposition of immnoglobulins (IgG, IgM, IgA) and C_3 in any case. The histopathological findings 48 hours after the test revealed definite neutrophilic infiltration around a superficial vascular plexus in 4 cases, in addition, one of these had endothelial cell swelling with red blood cell extravasation. The rest (1 case) showed nonspecific finding.

Conclusion : We conclude that the pathergy test is a useful tool for the diagnosis of Behçet's disease. The intradermal needle prick method using 20G disposable needle after cleaning the skin with 70% alcohol, yields results of moderately high sensitivity. The results should be evaluated at 48 hours both clinically and histopathologically.

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คณะผู้วิจัยได้ทำการศึกษาผลการทดสอบ pathergy ทางคลินิก ทางจุลพยาธิวิทยา และอิมมูโนพยาธิวิทยา ในผู้ป่วยไทยโรค Behçet จำนวน 5 ราย เป็นหญิง 4 รายและชาย 1 ราย โดยวิธี needle prick ใช้เข็มฉีดยาใหม่ (ชนิดใช้ครั้งเดียวทิ้ง) ขนาด 20G แทงเอียงทำมุม 45° ลึก 3 มม. ที่แขนสวนปลายข้างละ 1 ครั้ง ในตำแหน่งใกล้เคียงกัน ภายหลังการเช็ดทำความสะอาดผิวหนังด้วยแอลกอฮอล์ 70% อ่านผลทางคลินิกภายหลังการทดสอบ 4 และ 48 ชั่วโมง ทำการตัดผิวหนังตรงตำแหน่งที่ทดสอบภายหลังอ่านผลทางคลินิกแล้วเมื่อ 4 ชั่วโมง จากแขนขวาเพื่อตรวจทางอิมมูโนพยาธิวิทยา และเมื่อ 48 ชั่วโมงจากแขนซ้าย เพื่อตรวจทางจุลพยาธิวิทยา

ผลการตรวจทางคลินิกพบว่าผู้ป่วย 3 ใน 5 ราย ให้ผลบวกโดยมี papular lesion ขึ้นตรงตำแหน่งเข็มสะกิด เมื่อ 48 ชั่วโมงหลังทดสอบ แต่เมื่อ 4 ชั่วโมงหลังทดสอบ ได้ผลลบทางคลินิกในผู้ป่วยทุกราย ซึ่งสอดคล้องกับผลการตรวจทางอิมมูโนพยาธิวิทยา ที่ไม่พบอิมมูโนโกลบูลินชนิด IgG, IgM, IgA และ C_3 ตรงตำแหน่งที่ทดสอบ สำหรับผลการตรวจทางจุลพยาธิวิทยาพบว่าได้ผลบวก 4 ใน 5 ราย โดย 3 ราย ได้ผลบวกทางคลินิกด้วย อีก 1 ราย ได้ผลบวกเฉพาะทางจุลพยาธิวิทยา แต่ได้ผลลบทางคลินิก ผู้ป่วยที่เหลืออีก 1 ราย ผลการทดสอบได้ผลลบทุกวิธี

คณะผู้วิจัยสรุปว่าการทดสอบ pathergy โดยวิธี intradermal prick ใช้เข็มฉีดยาใหม่ขนาด 20G และทำความสะอาดผิวหนังด้วย 70%แอลกอฮอล์ ได้ผลไวพอสมควรในการวินิจฉัย Behçet's disease ควรอ่านผลเมื่อ 48 ชั่วโมงภายหลังการทดสอบ โดยการตรวจดูอาการทางคลินิกและตัดชิ้นเนื้อตรวจทางจุลพยาธิวิทยา

INTRODUCTION

Behçet's disease, first described by a Turkish dermatologist, is a complex multisystem disease.[1] It is characterized by oral ulceration, genital ulceration and ocular symptoms.[2] It can also involve other systems such as gastrointestinal tract, cardiovascular system, central nervous system, lungs and joints. Because of the overlap of its clinical signs with other systemic diseases and the lack of a pathognomonic laboratory test[1], diagnostic criteria are needed for the diagnosis of Behçet's disease,[3,4] and the pathergy test is one of these criteria.

There are many methods of performing the pathergy test such as intradermal normal saline injection, intradermal histamine injection and the needle prick test. The International Study Group for Behçet's disease (ISG) recommended the needle prick[5] test because this is a convenient method to perform. The prevalence of positive results varies between countries[6,7], for example, more than 60% in Turkey, Iran, and Tunisia and less than 20% in the United Kingdom and USA. In the present study, we performed the intradermal prick pathergy test in Thai patients with Behçet's disease to evaluate the results in terms of clinical, histopathological and immunopathological findings.

MATERIALS AND METHODS

The skin pathergy tests were performed, after obtaining informed consent, on Thai patients with active Behçet's disease, who attended the Skin Clinic of the Department of Dermatology, Siriraj Hospital, Bangkok, from January 1998 to August 1998. The diagnosis was made by using ISG criteria (table 1) or O' Duffy criteria. The intradermal needle prick method was chosen, using a 20 gauge needle and 70% alcohol for cleaning the skin. The needle prick tests were performed at 2 avascular and hairless areas of both forearm symmetrically. The needle was introduced into the skin without rotation making a 45° angle with the skin surface to a depth of 3 mm (just above the bevel). The results were evaluated clinically at 4 hours and 48 hours after the needle prick. A positive result was defined as papules or a pustule at the prick site. If there was only erythema or a trace of the prick was seen, the result was interpreted as negative. Skin biopsies were performed, after clinical evaluation at a test site on the right forearm for direct immunofluorescence study at 4 hours after the needle prick, and at a test site on the left forearm for histopathological study at 48 hours after the prick.

Table 1. International Study Group criteria for Behçet's disease

Recurrent oral ulceration	Minor aphthous, major aphthous, or herpetiform ulceration observed by physician or patient that recurred at least 3 times in one 12-month period
plus 2 of the following criteria	
Recurrent genital ulceration	Aphthous ulceration or scarring observed by physician or patient
Eye lesions	Anterior uveitis, posterior uveitits, or cells in vitreous on slit lamp examination; or retinal vasculitis observed by ophthalmologist
Skin lesions	Erythema nodosum observed by physician or patient, pseudofolliculitis or papulopustular lesions; or acneiform nodules observed by physician in postadolescent patients not receiving corticosteroid treatment
Positive pathergy test	

Biopsy specimens for routine light microscopic examination were fixed in formalin embedded in paraffin and stained with hematoxylin and eosin. The test was considered positive when the section showed leukocytoclastic vasculitis, Sweet's like pattern or perivascular infiltration of polymorphonuclear cells or lymphocytes.

The biopsy specimens for direct immunofluorescence study were snapped frozen in precooled isopentane. They were then sectioned and incubated with fluorescein-conjugated monospecific antiserum to human IgG, IgM, IgA, and C_3. Positive and negative controls were also provided to verify the specificity of each antiserum.

RESULTS

There was 1 male and 4 females (table 2), the mean age was 25.8 years (20-34 years). At 4 hours after the test (table 3), there was no clinically positive result in any of the patients and direct immunofluorescence findings (DIF) were negative. At 48 hours after the test (table 4), 3 patients had a papular lesion at the test site. All 3 patients also had positive histopathological findings with mixed inflammatory cell infiltrate composed of polymorphs, lymphocytes and histiocytes around a superficial vascular plexus. Evidence of vascular injury was found in one patient with vessel wall edema and red blood cell extravasation. No fibrinoid degeneration of vessel wall was

Table 2. Clinical findings in 5 Behçet's disease patients

Pt.	Sex	Age	Oral aphthous	Genital aphthous	Eye	Skin	Joint
1	M	20	+	-	-	+	+
2	F	24	+	+	+	-	-
3	F	34	+	+	-	+	-
4	F	24	+	+	-	+	-
5	F	27	+	+	-	-	-

Table 3. Clinicopathological findings after pathergy test

Pt.	Clinical at 4 hrs.	DIF	Clinical at 48 hrs.	H&E
1	-ve	-ve	+ve	Superficial perivascular PMN, lymphocytes, histiocytes
2	-ve	-ve	+ve	Superficial perivascular PMN, vessel wall edema, RBC extravasate
3	-ve	-ve	+ve	Superficial perivascular PMN, lymphocytes, dust
4	-ve	-ve	-ve	Superficial and deep perivascular and interstitial PMN, lymphocytes
5	-ve	-ve	-ve	Normal

found. A clinically negative result was found in 2 patients, but one of these had positive histopathological findings with neutrophilic infiltration around a superficial and a deep vascular plexus and in the interstitium, where as the other showed no pathological changes.

DISCUSSION

The applicability of the pathergy test to Behçet's disease has been the subject of ongoing debate. The results of the pathergy test can be influenced by the size of needle,[8] sharpness of the needle (used-resterilized vs disposable needle),[3,7] method and site of the needle prick,[3] and the type of disinfectant used for skin cleaning before the test. Previous studies indicated that a positive result was more frequently obtained when using a blunt (used-resterilized) and large (size 0.9 mm, 20G) needle[3,7,8] and using 70% alcohol for skin cleaning.[9] We used 70% alcohol for cleaning and pricked with a disposable 20G needle. Although a sharp disposable needle may reduce the number of positive results, it is more efficacious in the prevention of HIV infection.

There is no standardized method for conducting the pathergy test. Intradermal injection of normal saline or histamine; or needle prick intradermally, intravenously, or subcutaneously we are all used. Haim, et al[10] did not find any difference between the needle prick method and saline intracutaneous injection. Subsequently, Gilhar, et al[11] found an equal response between the test performed by histamine and saline intracutaneous injection. We used the needle prick method as recommended by the International Study Group for Behçet's disease (ISG) because it is more convenient to perform.

Our study confirms the moderately high sensitivity of the pathergy test and supports that it is a useful tool for the diagnosis of Behçet's disease in Thai patients. The test results were positive clinically in 3 out of 5 patients and histopathologically in 4 of 5 patients. This study also revealed clinicopathological non-correlation as one patient had a negative clinical test result but a positive histopathological test result. Jorizzo, et al showed a similar finding in a previous study[1] that histopathological responses were similar no matter whether clinical responses were strong, minimal or negative. Therefore the pathergy test should be evaluated both clinically and histopathologically in the diagnosis of Behçet's disease.

The timing of evaluation of the pathergy test is important in selection of its positivity.[4] Most investigators evaluated the results clinically and histopathologically at 48 hours after test.[3,5,6,8,9,12] ISG recommends either 24 or 48 hours after test as the appropriate time to evaluate the result;but Dilsen, et al[7] stated that false-positive results could be found at 24 hrs after the test in healthy controls and in patients without Behçet's disease but none had a positive reaction at 48 hrs.

It has been noticed that pathergy positivity decreases with long standing disease.[8] The results may fluctuate over time, i.e., negative results are frequently found during a remission period or after treatment.[1,3] In this study, we performed the pathergy test during active disease and the occurrence of a positive result was in line with the rates reported from Japan (30-77%).[12]

The mechanism of pathergy is still uncertain. Detection of immunoreactants in dermal blood vessels at 4 hours after the test followed by histopathological changes as leucocytoclastic vasculitis at 24 hours after test suggested circulating immune complex-mediated mechanism in one study[1] but this could not be reproduced in a subsequent study[11] as well as in our study. The negative DIF study found in our study does not support circulating immune complex as a mechanism of the pathergy test.

Different histopathological results related to the pathergy test have been reported in various studies. Perivascular infiltration of lymphohistiocyte,[3,10] periadnexal infiltration[10] and leucocytoclastic vasculitis[1] have been reported. Evidence of vascular damage has been reported as blood vessel thickening and erythrocyte extravasation,[3] but thrombosis or necrosis of vessel wall has not been reported. Our histopathological results were similar to previous reports but polymorphs were found around blood vessels in every cases, endothelial swelling and red blood cell extravasation were found in one case, but no fibrinoid necrosis of the vessel wall was seen.

CONCLUSION

Though the number of our patients is very small, the result of our study shows that the pathergy test is a useful tool for the diagnosis of active Behçet's disease in Thai patients. The recommended method is an intradermal prick test with a 20G disposable needle after cleaning the skin with 70% alcohol because it can be done conveniently and yields a high number of positive result. Histopathological study at 48 hours after test should be done in conjunction with clinical evaluation because a positive result (mixed inflammatory cell infiltration around blood vessels and evidence of vascular injury) can be found while the clinical result is negative.

REFERENCES

1. Jorizzo JL, Solomon AR, Cavallo T. Behçet's syndrome: immunopathologic and histopathologic assessment of pathergy lesions is useful in diagnosis and follow-up. Arch Pathol Lab Med 1985;**109:**747-51.
2. Arbesfeld SJ, Kurban AK. Behçet's disease: new perspectives on an enigmatic syndrome. J Am Acad Dermatol 1988;**19:** 767-79.
3. Akmaz O, Erel A, Gurer MA. Comparison of histopathologic and clinical evaluations of pathergy test in Behçet's disease. Int J Dermatol 2000; **39:**121-25.
4. International Study Group for Behçet's disease. Criteria for diagnosis of Behçet's disease. Lancet 1990; **335:** 1078-80.
5. Magro CM, Crowson AN. Cutaneous manifestations of Behçet's disease. Int J Dermatol 1995; **34:**159-65.
6. Tuzun Y, Yazici H, Pazarli H, Yalcin B, Yurdakul S, Muftuoglu A. The usefulness of the nonspecific skin hyperreactivity (the pathergy test) in Behçet's disease in Turkey. Acta Derm Venereol 1979; **59:** 77-79.

7. Dilsen N, Konice M, Aral O, Ocal L, Inanc M, Gul A. Comparative study of the skin pathergy test with blunt and sharp needles in Behçet's disease: confirmed specificity but decreased sensitivity with sharp needles. Ann Rheum Dis 1993; **52:** 823-25.
8. Ozarmagan G, Saylan T, Azizlerli G, Övul C, Aksungur VL. Re-evaluation of the Pathergy Test in Behçet's disease. Acta Derm Venereol 1991; **71:** 75-76.
9. Fresko I, Yazici H, Bayrannicli M, Yurdakul S, Mat C. Effect of surgical cleaning of the skin on the pathergy phenomenon in Behçet's syndrome. Ann Rheum Dis 1993; **52:** 619-20.
10. Haim S, Sobel JD, Friedman-Birnbaum R, Lichtig C. Histological and direct immunofluorescence study of cutaneous hyperreactivity in Behçet's disease. Br J Dermatol 1976; **95:** 631-36.
11. Gilhar A, Winterstein G, Turani H, Landau J, Etzioni A. Skin hyperreactivity response (pathergy) in Behçet's disease. J Am Acad Dermatol 1989; **21:** 547-45.
12. Krause I, Molad Y, Mitrani M, Weinbeyer A. Pathergy reaction in Behçet's disease: lack of correlation with mucocutaneous manifestations and systemic disease expression. Clin Exp Rheum 2000; **18:** 71-74.