https://doi.org/10.48047/AFJBS.6.Si4.2024.660-668

African Journal of Biological Sciences

The sensitivity and specificity of self-saliva skin prick test versus conventional pathergy skin test with severity and activity of Behçet's disease

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Article History

Volume 6, Issue Si4, 2024
Received: 25 May 2024
Accepted: 15 June 2024
doi:
10.48047/AFJBS.6.Si4.2024.660-668

Abstract

Background: A multi-system vasculitis illness called Bechet's disease (BD) is characterized by recurring mouth ulcers, genital ulcers, and eye problems.

Objectives: to determine the sensitivity and specificity of self-saliva skin prick test versus conventional pathergy skin test with severity and activity of Bechet's disease.

Patients & methods: This case controls research was performed on 90 cases divided into: 30 Behçet's caseshave confirmed diagnosis concerning ICBD for BD criteria updated in 2013, 30 recurrent aphthous stomatitis (RAS) cases & thirty matched healthy controls. patients were selected from the clinics of internal medicine department, especially rheumatology and immunology clinic, Beni-Suef university hospital.

Results: Skin prick test with saliva revealed significantly higher sensitivity (96.7) and specificity (93.3) in diagnosis of Behçet's disease compared to normal healthy control. Skin prick test with filtered saliva showed significantly greater sensitivity (83.3) more than skin prick test with saliva & pathergy skin test in detecting RAS patients. While Skin prick test with saliva revealed significantly greater specificity (93.3) more than pathergy skin test & filtered saliva in excluding RAS disease.

Conclusion: skin prick test with neat self-saliva is reliable and cost-effective tool for diagnosing of BD, as it is accurate and inexpensive. However, it doesn't correlate with uveitis activity, so it's not recommended to use SPT NSS to predict BD uveitis.

Key words: Bechet's disease, self-saliva skin prick test, pathergy skin test

Introduction

BD is multisystem vasculitis condition distinguished by recurrent oral ulcers, genital ulcers, & ocular complications. The geographical distribution of this phenomenon follows the silk road countries, with recent nationwide survey in Egypt revealing a male predominance of 2.6:1 over females [1].

Recurrent aphthous stomatitis beginning in childhood or early adolescence is the clinical manifestation of BD, with genital ulcers afflicting more than fifty percent of cases [2].

By stimulating the T Helper 1 response and releasing cytokines, systemic inflammation is induced. Histopathology reveals the presence of neutrophils, lymphocytes, & plasma cells infiltrating a non-specific vasculitis [3].

A potential correlation among the formation of BD & HLA-B51, HLA-B 27, could result in either an induced or spontaneous Th1 immune response [4].

It is also hypothesised that infections, such as those caused by bacteria and viruses, may induce BD via the synthesis of HSP. HSP are distinct proteins that are capable of eliciting Th1 and B cell responses & are produced by cells in response to stressful stimuli. One of these HSPs is HSP60, that induces a Th1 response and systemic inflammation when recognised by TLR [5].

TLR triggers release of pro-inflammatory cytokines, involving IL-6, IL-12, IL-15, & TNF-a, which leads to development of T-cell clones that are specifically reactive to human HSP60. [6].

The previously hypothesised association among S. sanguinis & BD development was confirmed, & significantly higher rate of positive pathergy test results was seen when employing either dead bacteria or streptococcal antigens compared to the standard approach. Furthermore, the DNA of S. sanguinis was detected in cytoplasm of inflammatory cells obtained from BD mucocutaneous biopsies [7].

The aim of present research was to detect the sensitivity & specificity of self-saliva skin prick test versus conventional pathergy skin test with severity and activity of Bechet's disease.

Patients & methods

This case controls study was conducted on 90 patients divided into: 30 Behçet's caseswith confirmed diagnosis concerning ICBD for BD criteria updated in 2013, 30 RAS patients & 30 matched healthy controls. The patients were selected from the clinics of internal medicine department, especially rheumatology and immunology clinic, Beni-Suef university hospital.

Ethical considerations: The research obtained ethical approval from Human Research Ethics Committee at Beni-Suef University Hospitals. Obtained informed permission from all participants after clearance from the ethics committee at Beni-Suef - Faculty of Medicine.

Inclusion criteria: 30 BD patients. thirty cases who had RAS that had not been diagnosed, as well as another thirty healthy controls.

Exclusion criteria: cases diagnosed with rheumatic diseases and either inflammatory bowel disease or cancer.

Methods:

Pathergy test: Circumstract the region in which the needle was inserted. Subsequently, a section of the epidermis was examined 48 hours after the test. The interpretation of the results was as follows: Simple erythema or a needle mark (0); a pustule (1); or a pustule (2) [8].

Skin prick test with self-saliva: The experiment was conducted triplicate for each subject by a clinical immunologist utilising saline as a control, filtered self-saliva, and clean self-saliva. A freshly obtained sample of saliva was diluted with two cm of sterile water prior to mingling.

Self-saliva was sterilised using filter paper with porous dimensions of 0.2 mm and a diameter of 47 mm. The participants' forearm epidermis was incised utilising Prick Lancetter. skin needle test utilising purified self-saliva was replicated subsequent to the alcohol sterilisation of the forearm. The diameter (mm) of papular and erythematous cutaneous reactions will be assessed 48 hours later. A positive test result was obtained when erythema measured greater than ten mm in cases with BD, five mm in those with RAS, or the control group, and pustules measured greater than 2 mm in BD cases after twenty four hours [9].

Statistical analysis:

The data was analysed utilising IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY) and JMP Version 13.2.1 (SAS Institute Inc., Cary, NC). The continuous numerical variables were expressed as the mean & standard deviation and were contrasted utilizing t-test. The data, which was not evenly distributed, was represented using median & interquartile range. The Mann-Whitney U test was utilized to compare 2 groups. The categorical data was shown as numerical values & percentages, & then compared utilizing Fisher's exact test. Diagnostic value of the tests was evaluated using a Receiver-operating characteristic (ROC) curve. Values with p-value below 0.05 were deemed statistically significant.

Results

a statistically significant variances was noted among the examined groups as regard age and gender (Table 1).

Table (1): Age & sex distribution of examined participants

		Behçet (No=30)		**RAS (No=30)**		**Controls (No=30)**		***P value***
		No.	**(%)**	**No.**	**(%)**	**No.**	**(%)**	
Age (Mean±SD)		31.87±10.6		29.43±9.4		36.07±10.4		0.042*
Sex	**Male**	20	66.7	15	50.0	10	33.3	0.036*
	Female	10	33.3	15	50.0	20	66.7	

Statistical analysis was done using Analysis of variance test & Qui square test

Among 30 Behçet's patients, 11 (36.7%) reacted positive to saline in pathergy test. The number of positive reacting cases increased to 16/30 (53.3%) in filtered self-saliva prick test and to 23/30 (76.7%) in neat self-saliva prick test. And there were statistically significant variances between them (P value above 0.001) (Table 2).

Table (2): Findings of different skin tests among the studied groups

		Behçet's disease		**RAS**		**Controls**		***P value***
		Count	**%**	**Count**	**%**	**Count**	**%**	
Pathergy skin test with Saline	Negative	19	63.3	22	73.3	29	96.7	0.006*
	Positive	11	36.7	8	26.7	1	3.3	
Skin prick test with neat self-saliva	Negative	7	23.3	7	23.3	28	93.3	0.001*
	Positive	23	76.7	23	76.7	2	6.7	
Skin prick test with filtered self-saliva	Negative	14	46.7	12	40.0	28	93.3	0.001*
	Positive	16	53.3	20	60.0	2	6.7	

Statistics was carried out using Qui-square test.

Table (3) demonstrate that skin prick test with saliva revealed significantly higher sensitivity (96.7) and specificity (93.3) in diagnosis of BD compared to normal healthy control.

Table (3): ROC curve for determination of Behçet's disease

Tests	**Skin prick test with saliva**	**Skin prick test with filtered saliva**	**Pathergy skin test with saline**
AUC**	0.972	0.953	0.947
Cutoff	≥ 4.5 mm	≥ 2.5 mm	≥ 0.5 mm
Sensitivity	96.7	90.0	96.7
Specificity	93.3	86.7	86.7
P value	0.001*	0.001*	0.001*
95% CI	0.937-1.000	0.906-1.000	0.881-1.000

Table (4) demonstrate that skin prick test with filtered saliva revealed significantly greater sensitivity (83.3) greater than skin prick test with saliva and pathergy skin test in detecting RAS patients. While Skin prick test with saliva revealed significantly greater specificity (93.3) greater than pathergy skin test & filtered saliva in excluding RAS disease.

Table (4): ROC curve for determination of RAS cases

Test (s)	**Skin prick test with saliva**	**Skin prick test with filtered saliva**	**Pathergy skin test with saline**
AUC*	0.907	0.887	0.778
Cutoff	≥ 4.5 mm	≥ 2.5 mm	≥ 0.5 mm
Sensitivity	76.7	83.3	66.7
Specificity	93.3	86.7	86.7
P value	0.001*	0.001*	0.001*
95% CI	0.833-0.980	0.801-0.972	0.655-0.902

*AUC/ Area under the curve

Table (5) demonstrate that the three studied tests are considered poor diagnostic tests in detecting patients with uveitis.

Table (5):ROC curve for determination of Behcet patients with uveitis

Test (s)	**Skin prick test with saliva**	**Skin prick test with filtered saliva**	**Pathergy skin test with saline**
AUC*	0.459	0.552	0.423
Cutoff	≥ 3.5 mm	≥ 8.5 mm	≥ 0.5 mm
Sensitivity	100.0	84.6	100.0
Specificity	5.9	47.1	5.9
P value	0.706	0.630	0.477
95% CI	0.250-0.669	0.342-0.762	0.216-0.630

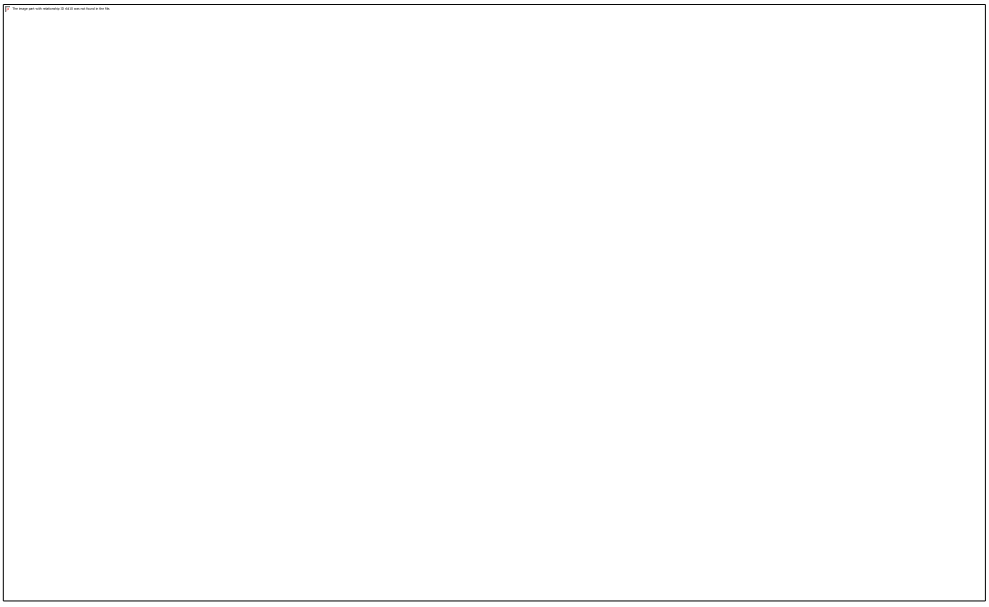

Figure (1): ROC curve for determination of Behcet patients with uveitis

Table (6) revealed that significant moderate positive relationship was noted among the ESR & CRP levels of the studied participants and the diameter of the skin reaction to saline (P value <0.05).

Table (6): Correlation between the laboratory findings (ESR and CRP) and the pathergy skin test with saline

		ESR	**CRP**
Saline (mm)	**Pearson Correlation (r)**	0.44	0.30
	P value	0.001*	.004*

Figure (2): Correlation between CRP level of the studied participants and their pathergy skin test diameter.

Discussion

There was a statistically significant variances among the examined groups regarding age & gender.

Among 30 Behçet's patients, 11 (36.7%) reacted positive to saline in pathergy test. The number of positive reacting cases increased to 16/30 (53.3%) in filtered self-saliva prick test and to 23/30 (76.7%) in neat self-saliva prick test. And there were statistically significant variances between them (P valueabove 0.001).

Skin prick test with saliva revealed significantly higher sensitivity (96.7) and specificity (93.3) in diagnosis of BD compared to normal healthy control.

Skin prick test with filtered saliva showed significantly higher sensitivity (83.3) more than skin prick test with saliva and pathergy skin test in detecting RAS patients. While Skin prick test with saliva revealed significantly greater specificity (93.3) greater than pathergy skin test & filtered saliva in excluding RAS disease.

The three studied tests are considered poor diagnostic tests in detecting patients with uveitis.

Significant moderate positive relationship was noted between the ESR & CRP levels of the studied participants and the diameter of the skin reaction to saline (P value <0.05).

The present results corroborated the outcomes of **Togashi et al,** [10], who conducted a study on ten patients with BD. Out of these ten patients, nine showed positive skin prick tests unique to their own oral microorganism(s). Additionally, three out of five patients with recurring oral ulcers had weak positive test results, whereas healthy controls had negative results [11].

Moreover, **Kaneko et al.** [11] reported that over 90% of cases with BD exhibited a favourable response to the salivary prick test.

Togashi et al. [10] evaluated the skin prick test utilizing self-saliva and found that majority of cases with BD (9/10, 90%) exhibited positive skin puncture that was specific to their intra-oral microorganism. The same pattern was observed in RA cases' skin pricks containing saliva (3/5, 60%).None of the other controls, including 3 cases with herpetic aphthous ulceration, 2 cases with BD-unrelated EN, & 6 healthy participants, had positive results when tested with self-saliva skin prick. This indicates that skin prick test is extremely specific for detecting BD & RA. Streptococcal species often exist in oral micro-flora, particularly Streptococcus (S) sanguinis & S. mitis, which showed a large increase in saliva from individuals with BD[12]. By considering the data that shows a heightened sensitivity to oral streptococci in patients with RA & the discovery of streptococcal colonies in saliva cultures from representative cases with BD, it can be inferred that positive skin prick reaction might be result of a localised hypersensitivity to streptococcal antigen(s). The histological resemblance among oral aphthous lesions in BD & skin pathergy reactions is reinforced by the presence of perivascular lympho-histiocytic infiltrate in dermis [14]. Immunohistologically, the mononuclear cells that invaded the pricked skin areas were primarily CD4+T cells & CD68+ monocyte/macrophages. This composition indicates DTH reaction. Furthermore, the clinicopathology of these skin pathergy reactions was suppressed by a modest dosage of systemic corticosteroid medication administered before to skin prick test using self-saliva. However, the reactions reappeared when the skin prick test was repeated following discontinuation of corticosteroid treatment (data not displayed). extrapolation based only on sequence of these pieces of data suggests that unintentional small injuries and/or repetitive irritation, such as toothbrushing, in oral mucous membrane may enable specific species of streptococcal bacteria to enter the underlying oral tissue. streptococcal antigen(s) can be processed to be correctly recognised by intra-epidermal immunocompetent cells, as Langerhans cells. This is due to the tip of "Prick-Lancetter" employed in their investigation never reached the

dermis, but instead consistently remained on skin surface. Subsequently, antigen-responsive helper T cells that have undergone processing may be stimulated, allowing them to reach the primary immunised oral mucosa, ultimately leading to the development of aphthous ulceration in BD & RA [15]. It is currently uncertain whether genetic predisposition, as the association with HLA-B51, is linked to streptococcal hypersensitivity in clinicopathology of BD. However, it is noteworthy that BD patients without HLA-B51 exhibited a stronger production of IL-12 from PBMCs when stimulated by streptococcal antigen, compared to those with HLA-B51 [16]. IL-12 has the capacity to trigger DTH reaction & has several biological consequences as early inflammatory accelerator in BD [17]. The prevalence of the rare KTH-1 form of S. sanguinis is considerably higher in oral bacterial population of cases with BD contrasted to healthy individuals [18]. Furthermore, BD patients have a significant presence of detectable IgG antibodies against S. sanguinis in their blood, & these antibodies exhibit cross-reactivity with synthetic oligopeptides of HSP65, which is extremely similar to streptococcal HSP-60. Therefore, it is possible that individuals with BD develop an acquired immune response to the identical sequences seen in both streptococcal & human HSP molecules. From a species-specific perspective, an investigation using a mouse model has shown that several clinical signs of BD may be reproduced by injecting S. sanguinis from the oral cavity of BD cases [19]. This indicates that administering S. sanguinis locally through the oral mucous membrane can induce symptoms similar to those of BD in specific species. cases with BD & RA who naturally harbour streptococci in their mouth may exhibit comparable immunological responses to the streptococci residing in their oral cavity. There is a definite connection among BD and streptococcal hypersensitivity. This is because the Bes-1 DNA, which contains certain parts of the S. sanguinis genomic sequences, can be found in the mononuclear cells that infiltrate the blood vessels in oral/genital ulcerations & EN-like lesions of BD cases [20].

Conclusion

The skin prick test with neat self-saliva is reliable and cost-effective tool for diagnosing of BD, as it is accurate and inexpensive. However, it doesn't correlate with uveitis activity, so it's not recommended to use SPT NSS to predict BD uveitis. The test can be used to assess ESR and CRP levels and the diameter of the skin reaction to saline, suggesting it may be a useful tool for BD activity and severity.

Study limitations:

It didn’t specify the effect of BD patients’ current medications especially steroids on SPT positivity.

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