Research Article

Tyrosine kinase titration for scabies and bacterial and viral infection of human's skin

Azhar Jabbar Khalaf Al-Subaihawi[1], Zinah Shakir Shallal[2]*, May Naji Al-Khanaq[3], Hadil Al-Hadi[4]

ABSTRACT

Background: Protein tyrosine kinase (PTK) is a key regulator for cellular proliferation and differentiation, growth, and death. PTK is a potent regulator for adaptive immune response. Thus, it could be an interesting antigen for serological to determine scabies and other bacterial infections that are diagnosed presumably on subclinical symptoms. **Objectives:** The objectives of the study were to compare the measurement of secretory PTK in the serum of scabies-infected group, bacteria-infected group, and virus-infected groups. **Materials and Methods:** PTK levels were tested in the sera of 50 patients with scabies infection and 14 patients with bacterial infection and 11 patients with viral infection using human enzyme-linked immunosorbent assay (ELISA). The control of this study included healthy individuals with no history of infections. **Results:** PTK levels in the sera of scabies-infected group were significantly higher compared to the healthy group (1.57 ± 0.34 vs. 0.99 ± 0.12, $P < 0.05$). PTK levels of virus-infected patients were found not significantly higher compared to the healthy group (1.61 ± 0.32 vs. 0.99 ± 0.12, $P > 0.05$). High levels of PTK detected in the sera of bacteria-infected group compared to the control but statistically the difference non-significant (1.5 ± 0.14 vs. 0.99 ± 0.12, $P > 0.05$). **Conclusion:** ELISA analysis showed that PTK levels were higher in the sera of scabies patients compared to the other infected patients. Titration of PTK levels using ELISA in the sera of patients with scabies, bacterial, and viral infections could provide sensitive and specific diagnostic approach for early detection.

KEY WORDS: Bacterial and viral infections, ELISA, PTK, Scabies

INTRODUCTION

Scabies is a contagious parasitic threatening disease infects human and animal health.[1] It caused by the mite called *Sarcoptes scabiei* that burrow forming a short, wavy, scaly, gray line on the surface of the skin. The main symptoms are skin inflammation, severe itching, and crusted thickness of skin lesions, leading to a number of health problems.[2] Scabies was classified by the World Health Organization as one of the top of neglected tropical diseases.[3] Globally, scabies infests 300 million individuals every year whom 50% are women and children, and more than 100 animal species resulting in serious health problems and economic losses.[4,5] Most scabies cases are accompanied by secondary bacterial infections, particularly with Group A streptococci and *Staphylococcus aureus* when the infested crusted skin lesions are not treated appropriately. The prevalence of scabies is high in poor and developing countries due to the poor and crowded environmental conditions.[6] Low awareness of health education, hygienic conditions, and treatment of scabies are obstacles to eradicate scabies in developing countries.[7]

Diagnosis of scabies is difficult. The parasitological investigations involve microscopic examination of eggs and mites existed in skin scrapping of the burrow. However, these techniques are insensitive as the symptoms of scabies are subclinical and cannot be distinguished from other dermatological diseases such as eczema, psoriasis, atopic dermatitis, diaper rash, and skin reaction to irritating agents such as lotions and soaps or lice, crab lice, and hairless tinea. Practically, patients are diagnosed with scabies based on the existed symptoms (e.g., severe itching and rash) which are not definitely confirmed scabies infection, this diagnostic approach is often generate a false-positive result. Therefore, it was suggested to implement a specific and sensitive blood test has been used to detect scabies

Access this article online	
Website: jprsolutions.info	ISSN: 0975-7619

[1]Department of Medicine, College of Medicine, Wasit University, Kut, Iraq, [2]Department of Pathological Analysis, College of Science, Wasit University, Kut, Iraq, [3]Department of Biology, Wasit University, Kut, Iraq, [4]School of Biomedical Sciences, Plymouth University, Drake Circus, Plymouth PL4 8AA, UK

***Corresponding author:** Zinah Shakir Shallal, Department of Pathological Analysis, College of Science, Wasit University, Kut, Iraq. E-mail: zalsoza@uowasit.edu.iq

Received on: 10-06-2019; Revised on: 16-07-2019; Accepted on: 17-08-2019

antibodies, this aiming to rapid and accurate diagnosis. For example, enzyme-linked immunosorbent assay (ELISA)[8-11] has been shown to detect secretory *Streptomyces scabies* proteins; however, the sensitivity and specificity are varied and not efficiently accurate during early stages of infestation. In addition, preliminary dot-ELISA and indirect ELISA have been used to detect recombinant proteins for diagnosis of scabies.[12] However, similar to conventional ELISA, these diagnostic approaches suffer from insufficient sensitivity and/or specificity for detection in the early stages of the disease due to the cross-antigenicity with dust mites, *Dermatophagoides farina, Dermatophagoides pteronyssinus,* and *Euroglyphus maynei* that are highly prevalent worldwide.[13,14]

Protein tyrosine kinases (PTKs) are a potential candidate which regulate the intercellular signaling cascades that prime cellular proliferation, differentiation apoptosis, and metabolic reactions.[15,16] PTK is the key regulatory factor of adaptive immune response. For instance, PTK is important during the signaling pathways that trigger T- and B-lymphocytes, during the phosphorylation signaling of tyrosines, PTKs associated with Syk or the related ZAP-70 tyrosine kinase, and orchestrate steps of recruitment and activation.[17] The latters are altered by PTK during the development of immune response when mice bite is occurred.[18] The present study aimed to titer and compare the tyrosine kinase of sera of three groups: Bacteria-infected group, virus-infected group, and scabies-infected group.

MATERIALS AND METHODS

Human Sera Preparation

Following ethical approval and informed consent, blood samples were collected from three groups of patients diagnosed with either scabies or bacterial or viral infection at Al-Israa Complex Medical Centre, Kut, Iraq. Serum samples were obtained from blood samples following a centrifugation at 1500–2000 rpm for 15 min at 2–8°C. The scabies-infected group was composed of 50 subjects (25 females + 21 males+ 4 children, 1–57 years of age) who their diagnosis confirmed by the recovery of live mites by skin scraping at the time of diagnosis. The clinical diagnosis of bacteria-infected group was confirmed by biochemical tests and swab culture of infected skin using mannitol salt agar and MacConkey agar to isolate the facultative pathogens-associated skin infections such as *S. aureus* and *Pseudomonas aeruginosa*. Bacteria-infected group was composed of 14 subjects (4 females +9 males, 4–45 years of age). Virus-infected group was composed of 11 subjects (8 males and 3 females, 18–42 years of age). Diagnosis was confirmed by doctors according to the clinical signs observed.

ELISAs

- Wash buffer: Wash buffer was diluted with 720 mL of deionized or distilled water to prepare 750 mL of wash buffer.
- Standard working solution: A standard was centrifuged at 10,000 × g for 1 min before the addition of 1.0 mL of reference standard and sample diluent. The solution was allowed to stand for 10 min and inverted it gently for several times. The dissolved solution was mixed thoroughly with a pipette to obtain a final concentration of 20 ng/mL. The final concentrations were used to make serial dilutions as follows: 20, 10, 5, 2.5, 1.25, 0.63, 0.31, and 0 ng/mL, as shown in Table 1.
- Biotinylated detection Ab working solution (100×): Biotinylated detection Ab working solution was diluted with 1 × working solution with biotinylated detection Ab diluent before use.
- Concentrated horseradish peroxidase (HRP) conjugate working solution (×100): Concentrated HRP conjugate working solution (×100) was diluted with 1 × working solution with concentrated HRP conjugate diluent to obtain sufficient amount of 100 µL/well before the experiment.
- ELISA.

Human PTK6 ELISA of the number of well plates (Elabscience) was applied for the quantitative measurement of PTK6 in the three groups with scabies, bacterial, and viral infections. Briefly, sera samples were added to the pre-coated wells with an antibody specific to human PTK6 wells and incubated for 90 min at 37°C. Following incubation, samples were removed and a volume of 100 µl of biotinylated detection antibody was added to each well. The plate was sealed and incubated at 37°C for 1 h; wells were washed 3 times with a wash buffer and HRP conjugate added appropriately to the wells and incubated for 30 min at dark at 37°C. Following washing process (5 times), a volume of 90 µl of substrate reagent was added to each well and incubated for 15 min at 37°C at dark. The plate was read immediately after adding 50 µl of stop solution to each well at 450 nm using a microplate reader (BioTek).

Statistical Analysis

Data were analyzed using GraphPad Prism software version 5.0. Results are presented as standard deviation

Table 1: Serial dilution standards of protein assay

Standards	ng/ml	Volume added to 1×diluent (µl)	Volume of 1×diluent (µl)
A	20	0	0
6	10	500 standard A	500
5	5	500 standard 6	500
4	2.5	500 standard 5	500
3	1.25	500 standard 4	500
2	0.63	500 standard 3	500
1	0.31	500 standard 2	500
0	0	0	500

(SD) of the mean. Statistical significance was determined using one-way ANOVA analysis followed by Bonferroni multiple *post hoc* analysis. Results were considered statistically significant when $P < 0.05$ against appropriate controls and marked with*.

RESULTS

The Results of ELISA Method

Before inducting ELISA experiment, standard curve was constructed according to the optical density obtained from the serial dilutions of the original standard (20 ng/ml). The results are shown in Table 2 and Figure 1.

For this study, the control represented PTK levels in sera obtained from healthy individuals with no history of infections. ELISA analysis of the serum of 50 scabies patients showed a significant increase in circulatory PTK levels compared to the healthy control (1.57 ± 0.34 vs. 0.99 ± 0.12, $P < 0.05$, Table 3 and Figure 2). Like scabies patients, serum of bacteria-infected patients showed increased levels of PTK, but statistically not significant compared to the control (1.5 ± 0.14 vs. 0.99 ± 0.12, $P > 0.05$, Table 3 and Figure 2). PTK levels of virus-infected patients were found slightly higher than circulatory PTK in bacteria-infected patients; however, the slight difference did not reach statistical significance (1.61 ± 0.32 vs. 0.99 ± 0.12, $P > 0.05$, Table 3 and Figure 2) and may have been due to analytical within run imprecision.

Scabies-infected patients showed a significant increase in PTK levels compared to the control ($P < 0.05$). No significant differences observed between PTK levels of bacteria- and virus-infected groups compared to the control ($P < 0.05$). The control represents the serum level of PTK in healthy individuals. Data are presented the mean ± SD of ELISA absorbance of scabies-infected patients ($n = 50$), bacteria-infected patients ($n = 14$), and virus-infected patients ($n = 11$). Percentages coefficient variation for control, scabies bacteria-, and virus-infected groups are 12.8%, 21.85%, 9.3%, and 18.15%, respectively.

DISCUSSION

In humans and animals, early diagnosis of scabies remains an obstacle as there are no specific commercial immunodiagnostic tools which are available to detect *S. scabies* infestation. The conventional protocols followed for scabies diagnosis depend on the observation of lesion and detection for any mites existed in skin scrapping to confirm the presence of infection. However, the traditional diagnosis methods are inconvenient, inaccurate, and unable to detect scabies at early stages of the manifestation. In addition, presumptive diagnosis depending on the clinical characteristics that are non-pathognomonic (rash and itchiness) could lead to potentially serious treatment due to the false-positive diagnosis. Therefore, such these diagnostic approaches require further confirmatory investigation to confirm scabies manifestation and other pathogenic infections associated scabies. In the current study, ELISA was used not only to detect the release of PTK in the serum of scabies patients but also in the serum of patients with bacterial and viral infection at early stages.

PTK is a key enzyme that modulates signaling transduction of immune cells.[19] Therefore, it could be a useful approach to detect secretory PTK in the serum of infected patients at early stages of manifestation since it has important role in modulating immune system such as differentiation, cytokine/chemokine

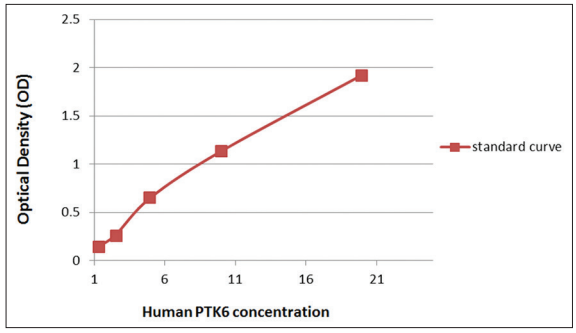

Figure 1: Standard curve for human protein tyrosine kinase 6 concentrations

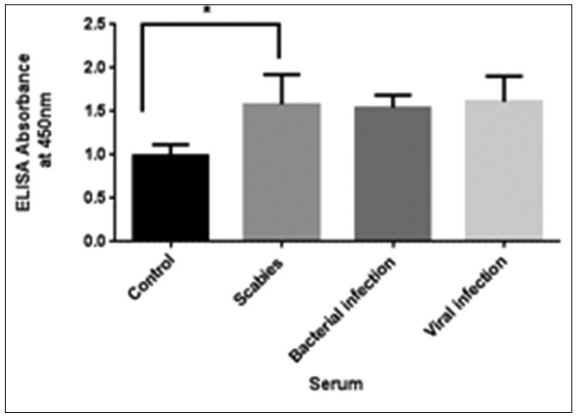

Figure 2: Protein tyrosine kinase levels in serum samples of three infected groups

Table 2: The results of the OD of the standards in ng/ml

Concentration (ng/ml)	20	10	5	2.5	1.25	0.63	0.31	0
OD	2.347	1.657	0.966	0.484	0.273	0.18	0.128	0.076
Corrected	2.361	1.581	0.89	0.408	0.197	0.104	0.052	-

OD: Optical density

Table 3: Protein tyrosine kinase concentrations (ng/ml) obtained from sera of healthy, scabies, bacterial, and viral infected people

Standards (ng/ml)	Control (ng/ml)	Scabies (ng/ml)	Bacterial infection (ng/ml)	Viral infection (ng/ml)
20	1.08	1.30	1.60	
10	0.90	1.50	1.40	1.79
5		1.40	1.70	1.80
2.5		1.50	1.50	1.70
1.25		1.60	1.60	1.19
0.63		1.40	1.80	1.20
0.31		1.50	1.40	1.70
0.13		0.34	1.39	1.90
		1.30	1.50	
		1.70		
		1.80		
		1.50		
		1.90		
		2.20		
		1.40		
		1.90		
		1.50		
		1.80		
		1.60		
		1.40		
		1.40		
		1.60		
		1.30		
		2.40		
		1.50		
		1.30		
		1.30		
		1.50		
		1.90		
		1.50		
		1.40		
		1.60		
		2.70		
		1.90		
		1.70		
		1.40		
		1.80		
		1.40		
		1.30		
		1.60		
		1.60		
		1.40		
		1.50		
		1.80		
		1.80		
		1.50		

release, and inflammatory process which are controlled by a variety of phosphorylated proteins and consequently by the corresponding protein kinases.[20] Following manifestation, anti-PTK levels are altered and thus can be detected by immune diagnostic tools like ELISA.

In this study, PTK release in the serum of scabies-, bacteria-, and virus-infected patients was determined and compared with the healthy individuals. The results clearly showed that all infected individuals exhibited high levels of PTK following the onset of infection compared to the control. Scabies patients had higher levels of PTK than bacteria- and virus-infected groups. These findings were similar to the previous study demonstrated that PTK levels in scabies patients detected by PTK-based ELISA increased at the early stages of manifestation with high rates of specificity and accuracy distinguishing mites infestation from parasitic infections in rabbits. The levels of PTK in serum samples of bacteria-infected patients and virus-infected patients were higher than the control. These findings are suggesting that PTK release is triggered by microbial infections stimulating signaling pathways and increasing phosphorylation of protein kinases. For instance, West Nile virus-infected microglial cells (human brain cells) are associated with elevated levels of protein kinases such as P38 MAPK, ERK, and JNK phosphorylation.[21] In the present study, samples were collected from patients infected with *S. aureus* and *Pseudomonas aeruginosa.* Cells infected with these pathogens have been shown to express focal adhesion-like complexes which their activities are cytoplasmic tyrosine kinases, focal adhesion kinase and SRC dependent. *P. aeruginosa* is involved in modulating Rho GTPases, cell shape, and mobility regulator[22]

Statistical analysis

Bonferroni's multiple comparisons test	**Mean diff.**	**95% CI of diff.**	**Significant?**	**Summary**	**Adjusted *P* value**	**A-?**	
Control versus scabies	−0.5891	−1.154–0.02411	Yes	*	0.0382	B	Scabies
Control versus bacterial infection	−0.5533	−1.165–0.05817	No	ns	0.0888	C	Bacterial infection
Control versus viral infection	−0.6214	−1.249–0.005757	No	ns	0.0529	D	Viral infection

Test details	Mean 1	Mean 2	Mean diff.	SE of diff.	*n*1	*n*2	*t*	DF
Control versus scabies	0.9900	1.579	−0.5891	0.2294	2	46	2.568	60
Control versus bacterial infection	0.9900	1.543	−0.5533	0.2483	2	9	2.229	60
Control versus viral infection	0.9900	1.611	−0.6214	0.2546	2	7	2.440	60

Descriptive statistics				
Number of values	2	46	9	7
Minimum	0.9000	0.3400	1.390	1.190
25% percentile	0.9000	1.400	1.400	1.200
Median	0.9900	1.500	1.500	1.700
75% percentile	1.080	1.800	1.650	1.800
Maximum	1.080	2.700	1.800	1.900
Mean	0.9900	1.579	1.543	1.611
Std. deviation	0.1273	0.3450	0.1437	0.2925
Std. error of mean	0.09000	0.05087	0.04790	0.1105
Lower 95% CI	−0.1536	1.477	1.433	1.341
Upper 95% CI	2.134	1.682	1.654	1.882

CI: Confidence interval

and the SCR and ABL families; nucleocytoplasmic tyrosine kinases.[23,24] This could indicate that tyrosine kinases are potential marker for early diagnosis not only in scabies manifestation but also for bacterial infections. Similar to PTK-dependent bacterial infection detection, secretory tyrosine kinases from patient serums could be one of the diagnostic mechanisms for early detection of viral infection. Upon viral infection, several signaling pathways are initiated and triggered by viral proteins. For instance, Epstein–Barr virus (EBV) latent membrane protein 1 (LMP1) stimulates phosphatidylinositol 3-kinase/Akt (PI3k) cascade. LMP1 induces EBV membrane protein to promote cell survival and induce cytoskeleton remodeling of EBV-infected cells.[25,26] In this study, secretory PTK levels in the serum of virus-infected group were higher compared to the healthy group and not remarkably different compared to scabies- and bacteria-infected groups.

CONCLUSION

The findings of this study clearly show that using ELISA to detect PTK in the serum of scabies-, bacteria-, and virus-infected groups could be sensitive and specific diagnostic approach for early diagnosis.

REFERENCES

1. Feldmeier H, Heukelbach J. Epidermal parasitic skin diseases: A neglected category of poverty-associated plagues. Bull World Health Organ 2009;87:152-9.
2. Alasaad S, Rossi L, Heukelbach J, Pérez JM, Hamarsheh O, Otiende M, *et al.* The neglected navigating web of the incomprehensibly emerging and re-emerging sarcoptes mite. Infect Genet Evol 2013;17:253-9.
3. World Health Organization. List of Neglected Tropical Diseases. Geneva: World Health Organization; 2019.
4. Walton SF, Holt DC, Currie BJ, Kemp DJ. Scabies: New future for a neglected disease. Adv Parasitol 2004;57:309-76.
5. Chosidow O. Scabies and pediculosis. Lancet 2000;355:819-26.
6. Walton SF, Currie BJ. Problems in diagnosing scabies, a global disease in human and animal populations. Clin Microbiol Rev 2007;20:268-79.
7. Hay RJ, Steer AC, Engelman D, Walton S. Scabies in the developing world – its prevalence, complications, and management. Clin Microbiol Infect 2012;18:313-23.
8. Kessler E, Matthes HF, Schein E, Wendt M. Detection of antibodies in sera of weaned pigs after contact infection with *Sarcoptes scabiei* var. Suis and after treatment with an antiparasitic agent by three different indirect ELISAs. Vet Parasitol 2003;114:63-73.
9. Löwenstein M, Kahlbacher H, Peschke R. On the substantial variation in serological responses in pigs to *Sarcoptes scabiei* var. Suis using different commercially available indirect enzyme-linked immunosorbent assays. Parasitol Res 2004;94:24-30.
10. van der Heijden HM, Rambags PG, Elbers AR, van Maanen C, Hunneman WA. Validation of ELISAs for the detection of antibodies to *Sarcoptes scabiei* in pigs. Vet Parasitol 2000;89:95-107.
11. Smets K, Vercruysse J. Evaluation of different methods for the diagnosis of scabies in swine. Vet Parasitol 2000;90:137-45.
12. Zhang R, Zheng W, Wu X, Jise Q, Ren Y, Nong X, *et al.* Characterisation and analysis of thioredoxin peroxidase as a potential antigen for the serodiagnosis of sarcoptic mange in rabbits by dot-ELISA. BMC Infect Dis 2013;13:336.
13. Falk ES, Dale S, Bolle R, Haneberg B. Antigens common to scabies and house dust mites. Allergy 1981;36:233-8.
14. Arlian LG, Vyszenski-Moher DL, Ahmed SG, Estes SA. Cross-antigenicity between the scabies mite, *Sarcoptes scabiei*, and the house dust mite, *Dermatophagoides pteronyssinus*. J Invest Dermatol 1991;96:349-54.
15. Arlian LG, Rapp CM, Morgan MS. Resistance and immune response in scabies-infested hosts immunized with dermatophagoides mites. Am J Trop Med Hyg 1995;52:539-45.
16. Hubbard SR, Till JH. Protein tyrosine kinase structure and function. Annu Rev Biochem 2000;69:373-98.
17. Fodor S, Jakus Z, Mócsai A. ITAM-based signaling beyond the adaptive immune response. Immunol Lett 2006;104:29-37.
18. Osman N, Lucas S, Cantrell D. The role of tyrosine

phosphorylation in the interaction of cellular tyrosine kinases with the T cell receptor zeta chain tyrosine-based activation motif. Eur J Immunol 1995;25:2863-9.
19. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev 2009;22:240-73.
20. Shen N, He R, Liang Y, Xu J, He M, Ren Y, *et al*. Expression and characterisation of a *Sarcoptes scabiei* protein tyrosine kinase as a potential antigen for scabies diagnosis. Sci Rep 2017;7:9639.
21. Keating JA, Striker R. Phosphorylation events during viral infections provide potential therapeutic targets. Rev Med Virol 2012;22:166-81.
22. Murali A, Rajalingam K. Small rho GTPases in the control of cell shape and mobility. Cell Mol Life Sci 2014;71:1703-21.
23. Pielage JF, Powell KR, Kalman D, Engel JN. RNAi screen reveals an abl kinase-dependent host cell pathway involved in *Pseudomonas aeruginosa* internalization. PLoS Pathog 2008;4:e1000031.
24. Swimm A, Bommarius B, Reeves P, Sherman M, Kalman D. Complex kinase requirements for EPEC pedestal formation. Nat Cell Biol 2004;6:795.
25. Herbein G, Gras G, Khan KA, Abbas W. Macrophage signaling in HIV-1 infection. Retrovirology 2010;7:34.
26. Brinkmann MM, Schulz TF. Regulation of intracellular signalling by the terminal membrane proteins of members of the gammaherpesvirinae. J Gen Virol 2006;87:1047-74.

Source of support: Nil; Conflict of interest: None Declared