

Total IgE, mosquito saliva specific IgE and CD4+ count in HIV-infected patients with and without pruritic papular eruptions

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Summary

Background: Pruritic Papular Eruption (PPE) is a skin disorders found in HIV infected patients. However, the exact etiology of PPE is not documented. It has been suggested that PPE might result from arthropod bites.

Objective: The aim of this study was to investigate those factors in the HIV patient contributing to the occurrence of PPE, including specific IgE against mosquito saliva allergens, total IgE, CD4 cell counts and their associations.

Methods: Specific IgE against saliva allergens of *Cx. quinquefasciatus* mosquito was measured in 25 HIV patients with PPE and in 60 HIV without PPE by a time-resolved fluorescence immunoassay (TRIFA). The total IgE levels and CD4 cell counts were also determined.

Results: Among the HIV patients with PPE, 84% (21/25) had CD4 cell counts less than 200 cells/ μ l in contrast to 30% (18/60) of the HIV without PPE patients. These differences were statistically significant ($p = 0.0005$, χ^2 test). The total IgE scores for the HIV patients with PPE were

significantly higher than for those without PPE. A comparison of the mean arbitrary scores of the specific IgE in HIV patients, with and without PPE, was non-significant ($p = 0.152$). However, 44% (11/25) of the HIV patients with PPE had an arbitrary score above the mean score of mosquito bite allergic subjects, as compared to only 3.3% (2/60) of HIV patients without PPE.

Conclusions: It may be concluded that the etiology of PPE in the HIV patient may be heterogeneous or multi-causal with allergic responses to the mosquito saliva allergen being only partially responsible. (*Asian Pac J Allergy Immunol* 2014;32:53-9)

Key words: pruritic papular eruptions, HIV, mosquito saliva allergens, specific IgE, total IgE

Introduction

A prominent dermatologic feature of HIV-infected individuals is Pruritic Papular Eruptions (PPE) which produce areas of dyspigmentation of the skin which in turn cause worsening self-esteem and social stigmatism.¹ This pathological condition is characterized by chronic/multiple sterile discrete pruritic papules (rarely pustules) with a symmetric distribution on the extensor surfaces of the arms, legs, face and trunk.² PPE may appear as an initial cutaneous manifestation of AIDS contributing to the progression to an advanced immunosuppressive stage.³ Currently, there is still a controversy concerning the clinical or histological criteria for the diagnosis, etiology or treatment of PPE.²

The prevalence of PPE in HIV patients varies according to the tropical countries within which it is found; for instance 11.7% in Brazil,⁴ 18% in Africa,⁵ 46% in Haiti,³ 26.6% and 51.2% in Thailand,⁶⁻⁷ respectively. In view of the observation that lesions are more commonly located on the trunk and exposed parts of the body, such as forearms, hands, shins and feet in tropical countries, it has been suggested that arthropod bites, may underlie the

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pathogenesis.⁸ Rosatelli et al have demonstrated, in the HIV subjects with PPE, high levels of eosinophilia, elevated IgE levels and a significant positive reaction to an insect body extract in the skin-prick test, probably due to a hypersensitive reaction to mosquito allergens.⁹ In addition, previous evidence has suggested that a dysfunction of the immune system concurrent with B-cell activation and a depletion of CD4+ helper T cells in both HIV-infected adults¹⁰ and children¹¹ can lead to elevated levels of serum IgE antibodies. It seems likely that high levels of IgE can be utilized in addition to low CD4+ cell counts as a prognostic indicator for disease severity in late-stage HIV disease.¹² Furthermore, several studies have indicated that HIV-infected patients have an increased incidence of asthma and atopic dermatitis.¹³⁻¹⁴

However, the etiology of PPE in HIV patients has remained elusive. It was hypothesized that an adverse reaction to the mosquito saliva allergen was the reason for the development of PPE in HIV immunocompromised patients. A better understanding of these relationships in these patients may ultimately explain the etiology of PPE in HIV patients.

In this study, we therefore sought to investigate the potential causative factors involved in the occurrence of PPE in HIV patients. The levels of specific IgE against *Culex quinquefasciatus* saliva allergens was determined. This mosquito was chosen in this study because it was determined that the protein profiles and potential allergens derived from mosquito saliva from four of common mosquitoes found in Thailand including *Cx. quinquefasciatus*, exhibited shared allergens that are immunologically identical with each other. Sensitization of allergic subjects by mosquito bites from one species can confer reactivity against another species and thus holds great promise for the diagnosis of patients' subject to exposure to a wide range of mosquito species.¹⁵ Total IgE and CD4⁺ cell counts were also measured. The associations among these factors with the presence of PPE were also determined.

Methods

Study samples

A total of 110 subjects were recruited, and included 25 HIV patients with PPE and 60 HIV patients without PPE. Twenty five mosquito bite allergic patients were also included as positive controls in this study.

All patients were evaluated by Dermatologists in the Skin Clinic at Siriraj Hospital, Bangkok, Thailand. At the time of the initial interview a complete history was taken and a physical examination was performed. Participant inclusion criteria consisted of: 1) HIV patients with PPE who had experienced pruritic skin eruptions > one month's duration, with evidence of multiple papular or nodular lesions, 2) HIV patients without PPE who had no evidence of PPE at the time of the dermatological examination (All the HIV patients in both groups had never received any antiretroviral therapy (ART)), and 3) Mosquito bite allergic patients who clinically manifested overt skin lesions, had a history of allergic reactions to mosquito bites, and who exhibited specific IgE antibodies against allergenic components in the saliva of *Cx. quinquefasciatus* (by an immunoblot assay), and all were HIV negative. The use of the mosquito saliva specific IgE from the mosquito allergic patients who served as positive control subject, provides a benchmark/standard by which to measure and compare the allergic responses of the HIV patients.

The study protocol was reviewed and approved by the Ethical Committee on Research Involving Human Subjects of the Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.

Determination of CD4 count

The CD4 cell counts of the sera of the HIV patients were performed using a Fluorescence Activated Cell Sorting (FACS) Instrument (Becton Dickinson, New Jersey, USA).

Quantitative determination of mosquito saliva specific IgE antibodies was carried out using a Time-resolved fluoroimmunoassay (TRFIA).

To determine the mosquito specific IgE in the study population, mosquito saliva was collected from living female *Culex quinquefasciatus* mosquitos, following the method of Boorman with some modifications.¹⁵ Then 100 µl of *Cx. quinquefasciatus* saliva protein (20 µg/ ml in carbonate-bicarbonate buffer, pH 9.6) was coated on each well of a 96 well microplate (Maxisorp; Nunc, Denmark). The microplate was incubated at 4°C overnight and washed with a washing buffer (DELFIA®, Perkin Elmer, Finland) five times. The wells were then blocked with 200 µl of a buffer consisting of 5% Skimmed milk in 0.01% PBST at 37°C for 1 hr, followed by washing as in the previous step. 100 µl of the study sample (dilution

1:2) was then added to the coated wells. Pooled positive and negative plasma samples were also included. One hundred microliters of the diluent, europium substrate, as well as BSA, were subsequently added to each well and used as a blank in the assay. Plates were incubated overnight. After washing, 100 μ l of anti-human IgE conjugated biotin (dilution 1: 1000) was added to each well. The plate was incubated for 1 hour at 37°C. Then, 100 μ l of Eu³⁺-labeled streptavidin (DELFI[®], Perkin Elmer, Finland) (50 ng/ml) was added followed by incubating at 37°C with continuous shaking for 60 minutes. After washing, as in the previous step, 100 μ l of the enhancer (DELFI[®], Perkin Elmer, Finland) was added to each well and agitated for 5 minutes (PerkinElmer[®] instrument). For the measurement of TRF counts, a Europium blank substrate was used. The noise value of the background absorbance of the blank was subtracted from the signal value of all test readings.

In TRFIA, the measured TRF counts were proportional to the concentrations of the specific IgE antibodies against the mosquito saliva allergens. Since no standard control (known unit) of the specific IgE against the mosquito saliva allergens was available, we set our arbitrary IgE units directly from the TRF counts. For example; sample with the TRF count of 345 counts was given 345 arbitrary IgE units.

Detection of total IgE antibodies

The total IgE levels in the sera of the subjects were measured using a commercial kit (VIDAS) according to the manufacturer's instructions (Biomérieux, F-69280 Marcy l'Etoile, France). The results were automatically calculated and the concentrations were reported in kIU/L. If the resultant values were < 150 kIU/L, the samples were classified as belonging to a non-atopic population and therefore considered to be normal.

Statistical analysis

Statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL).

Descriptive statistics were used to summarize the demographic and clinical data that were collected from the case records form (age, gender, CD4 cell count, the presence of PPE). Continuous variables were described as mean \pm standard deviation (SD). The Mann-Whitney U test was used to compare the total IgE scores, arbitrary scores of specific IgE against mosquito saliva allergen and CD4 counts of HIV-infected patients, with and without PPE. The

Chi-squared test was used to compare CD4 counts above and below the 200 $\times 10^6/L$ standard between the HIV with and without PPE groups. Spearman's correlations were used to measure the degree of association between the total IgE scores and the arbitrary scores of HIV-infected patients with and without PPE, and between the total IgE scores and the CD4 counts for the two groups of HIV patients.

Multivariate logistic regression analysis was used to determine the association between clinical data (CD4 cell counts, the total IgE scores, the arbitrary score of specific IgE) and the presence of PPE, after adjusting for gender and age. A value of $p < 0.05$ was considered as statistically significant.

Results

Demographic Data

The mean age and gender of the subjects in this study was as follows: of the 25 HIV patients with PPE, the mean age was 35.40 \pm 5.96 years (range 27-46 years) and 36% were female; in the HIV without PPE group, the mean age of the HIV subjects without PPE was 32.97 \pm 9.68 years (range 20-69 years) and 50% were female. The mean age of the 25 mosquito bite allergic subjects was 23.3 \pm 15.49 and 64% were female.

CD4 counts of HIV patients with and without PPE

Figure 1 presents a box plot of the CD4 data sets for the HIV patients, with and without PPE. In the HIV with PPE group the mean \pm SD of CD4 counts was 100.40 \pm 76.67 cells/ μ l (95%CI 68.75-132.05 cells/ μ l) while their median was 60.0 cells/ μ l (IQR 38-158.50 cells/ μ l). In the HIV without PPE group, the mean \pm SD was 313.83 \pm 178.38 cells/ μ L (95% CI 267.75-359.91 cells/ μ l) and their median was 287.50 cells/ μ l (IQR 175.50-465.75 cells/ μ l). These differences were statistically significant ($p = 0.00003$, Mann-Whitney Test). Among the HIV patients with PPE, 84% (21/25) had CD4 cell counts less than 200 cells/ μ l, whereas 30% (18/60) had CD4 cell counts below this value. These differences were also statistically significant ($p = 0.0005$, χ^2 test).

The arbitrary score of specific IgE against mosquito saliva allergen of HIV patients with and without PPE

Figure 2 depicts a box-whisker plot of the arbitrary specific IgE units of all subjects. The mean \pm SD of the arbitrary score of specific IgE against mosquito saliva allergen for the HIV with PPE was 2458.36 \pm 3887.86 (95%CI = 853.53 – 4063.19),



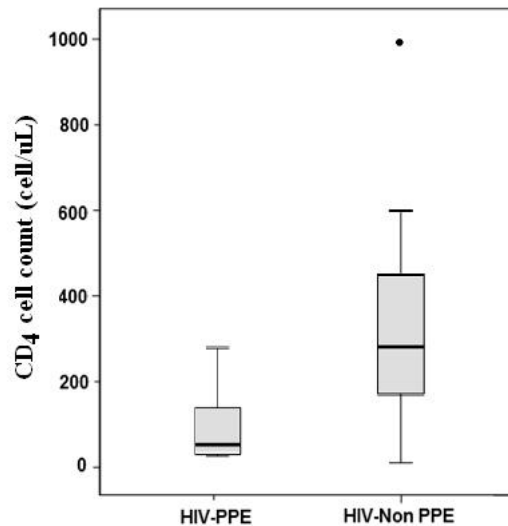


Figure 1. Box-Whisker Plots showing CD4 counts in the serum of HIV-infected patients. The line inside the box represents the median of each group. The length of the box is the interquartile range (IQR). Values more than 1.5 IQR's but less than 3 IQR's from the end of the box are labeled as outliers (●).

while its median was 660 with an IQR of 2976. The mean±SD of the non-PPE group was 280.22±415.46 (95%CI = 172.89 – 387.54). The median for the HIV patients without PPE was 197 and its IQR was over 10 times smaller than that for the HIV patients with PPE at 286.8. However, the Mann-Whitney test for the differences between these two groups was nonsignificant ($p = 0.152$). The mean ± SD of arbitrary score of specific IgE against mosquito saliva allergens for the mosquito bite allergic subjects was 1469.72±1432.91 (95%CI = 908.02 – 2031.42) while its median was 914. The only significant comparison involving the Mosquito Bite allergy subjects was that between the HIV-non PPE patients ($p < 0.0006$, Mann-Whitney U Test).

Eleven out of 25 (44%) HIV patients with PPE had an arbitrary specific IgE score above the mean arbitrary specific IgE score of the mosquito bite allergic subjects, as compared to only 2 out of 60 (3.3%) of the HIV patients without PPE. These differences were highly significant ($p < 0.0005$, χ^2 test). Of the remaining 14 HIV with PPE subjects, 10 had an allergen-specific IgE score of zero.

Multivariate logistic regression analysis showed that the significant predictors of the presence of PPE, after adjusting for gender and age, were CD4 cell counts and the arbitrary scores for IgE (adjusted odds ratio [OR] = 0.982, 95% CI 0.972-0.992, $p < 0.001$;

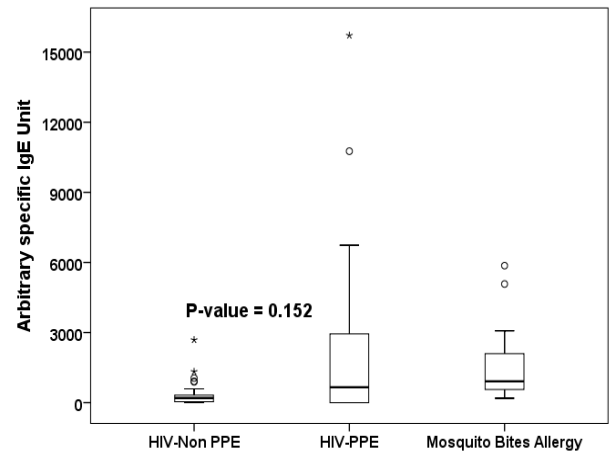


Figure 2. Box-whisker graph showing the arbitrary unit of specific IgE against mosquito saliva allergen in the serum of HIV infected patients and mosquito bite allergic patients. The median of each group is identified by the line inside the box. The length of the box is the interquartile range (IQR). P -value = 0.152 for the difference between HIV patients with and without PPE

adjusted OR = 1.001, 95% CI 1.000 – 1.002, $p = 0.013$, respectively).

The total IgE scores of HIV patients with and without PPE

Figure 3 presents a box plot of the total IgE scores for the two HIV groups and mosquito bite allergic patients. In the HIV with PPE group, the mean ± SD was 2903.13±3859.38 (95%CI 1310.06 – 4496.21). The median of this group was 866.56 with an IQR of 6592.25. In the non-PPE group, the mean ± SD was 590.66±1301.95 (95%CI 254.33 – 926.98). Its median was 240.08 and its IQR = 612.49. The total IgE scores in the HIV patients with PPE was significantly higher, as compared to those for the HIV patients without PPE ($p < 0.001$, Mann-Whitney U test). For the mosquito allergy patients, the mean ± SD was 613.87±353.23 with a 95% CI = 468.06 – 759.68. Its median was 716.19 and the IQR = 769.35. The Mann-Whitney U Test comparison between the Mosquito Allergy Patients and the HIV-Non PPE Ss was significant ($p = 0.004$) but not that between the HIV_PPE Ss.

Correlation of the arbitrary specific IgE scores and the CD4 counts with the total IgE scores

The arbitrary specific IgE score showed highly significant correlation with the total IgE scores in the HIV patients with PPE (Spearman's $r = 0.669$,

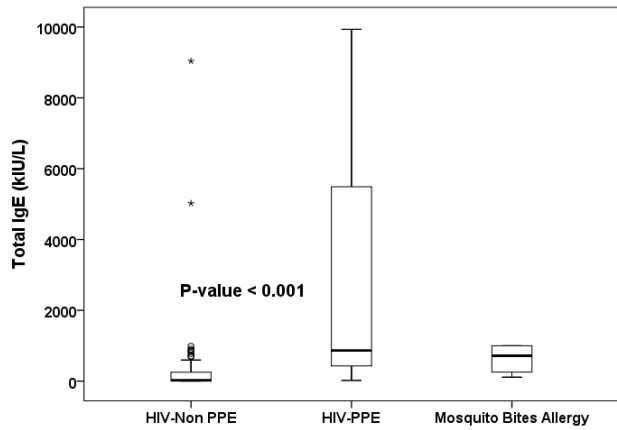


Figure 3. Box-Whisker Plots of the total IgE scores of HIV infected patients and mosquito bite allergic patients with the median of each group identified by the line inside the box, the IQR by the length of the box. P -value <0.001 for the difference between HIV patients with and without PPE.

$p < 0.001$). No correlation was found in the HIV patients without PPE ($r = 0.076$, $p = 0.562$). Spearman's r for the CD4 counts and the total IgE scores of the HIV with PPE group showed a significant negative correlation ($r = -0.526$, $p = 0.007$), but not for the HIV without PPE group ($p = 0.251$).

Discussion

Many studies have focused on the investigation of the etiology of PPE in HIV patients. It has been suggested that arthropod bites may underlie the pathogenesis. High titers of specific IgG antibodies against the salivary glands of paraffin embedded sections of the *Aedes taeniorhynchus* mosquito were detected in the sera of AIDS patients with PPE.¹⁶ Rosatelli et al. found that a skin prick test using various antigens (house dust, mites, fungi and insect body) showed significant differences between HIV patients with PPE and without PPE only from the insect body inoculum.⁹

The HIV infected patients with PPE demonstrated significantly greater total IgE levels compared to those without PPE. Corominas et al. showed that in the early stages of an HIV infection, 31% of these patients produced higher IgE levels as well as elevated percentages of CD23 expression on B cells, and that atopy was more frequent in HIV patients with high IgE values.¹⁷ Gingo et al. had performed a cross-sectional analysis of 223 HIV-infected subjects and found that HIV patients with

doctor diagnosed asthma manifested worse pulmonary symptoms and function than those not diagnosed by a doctor. Furthermore, parental history, obesity through changes in adipocytokine levels [leptin ↑, adiponectin ↓] and metabolic disturbances, such as lipodystrophy, chronic inflammation from the HIV infection, prior infection, absence of ART, and increased HIV-stimulated Th2 cytokines (IL-4) were possible mechanisms increasing the risk of HIV-associated asthma.¹³

Our study revealed that a significantly higher number of patients with PPE had the arbitrary scores of specific IgE against saliva allergens above the mean score of the mosquito bite allergic group, compared to HIV patients without PPE. This, however, left 56% (14/25) of the HIV with PPE subjects below this criterion with 10 of these patients showing no specific antibody against the mosquito allergen. These HIV patients who had the arbitrary scores for specific IgE against saliva allergens above the mean score of the mosquito bite allergic group may have had mosquito bite allergy as the underlying disease process.

Moreover, the specific IgE against mosquito saliva allergen in HIV patients with PPE was significantly associated with the total IgE scores, but no such correlation was found in the HIV patients without PPE. A similar observation has been made by Sturm et al. who found an association between total IgE levels and specific IgE levels generated by sting reactions to Hymenoptera venom, suggesting associations with inflammation and hypersensitivity reactions to specific allergen.¹⁸

One possible explanation for an allergic response to environmental allergens commensurate with a cell-mediated immune deficiency is the immunological changes due to switching from a Th1 to a Th2 immune response during HIV infection.¹⁹ An alternative reason explaining this phenomenon is that AIDS is characterized by a variety of disturbances in the regulation of cytokine expression, including a possible increase in Th2 cytokines and a decrease in the expression of Th1 cytokines.²⁰ The disorders prevalent in advanced HIV patients with an allergy may be attributed to an abnormal T cell regulation of increased Th2 cytokines, concomitant with an increased IgE synthesis by B cells and a decrement in Th1 cytokines. Sukvit et al.²¹ found that, in severely immunosuppressed Thai children born to HIV-1 infected mothers, a reduction in IL-2 concomitant

with an increase in IL-4 production, which supports the Clerici-Shearer switch model.²² Likewise, previous studies have suggested that arthropod bites, in particular by mosquitoes, underlie the pathogenesis of PPE.⁹

Nevertheless, our study revealed no significant differences between the mean arbitrary specific IgE scores in HIV with PPE and HIV without PPE. Although the differences in the number of subjects in PPE VS non-PPE might be one factor contributing to these insignificant differences, and increasing the number of assessments might improve our understanding of the situation, of greater importance, from a statistical point of view there are very large differences in the variability of the two groups (undoubtedly contributed by the outliers both in score values and ranks as well as the 10 HIV with PPE patients who didn't have specific antibody against the mosquito saliva antigen). In our opinion, the etiology of PPE in the HIV patient may be heterogeneous or multi-causal with allergic responses to the mosquito saliva allergen being only partially responsible.

In the present study, we also demonstrated significantly lower CD4 cell counts for the HIV patients with PPE as compared with HIV patients without PPE. In accordance with previous studies, the presence of PPE may be considered as a predictive marker for a progressive HIV infection.¹⁸

While this study is the first measuring specific IgE antibodies against mosquito saliva allergens in HIV infected subjects, with and without PPE, it may be concluded that the observed reactions in certain of the HIV patients with PPE patients to the mosquito bite allergy is only partially correct, it is not the definitive cause underlying all cases of PPE in HIV patients. The results did not show a clear link between the mosquito bite allergy and the presence of PPE, since the mean arbitrary scores of the specific IgE against mosquito saliva allergen in HIV with and without PPE revealed no significant differences. The lack of differences in specific IgE may due to the small sample size of the group with PPE, as compared to that without PPE. Nevertheless, it is possible that the absence of arbitrary specific IgE in ten of the HIV patients with PPE represents a subset of HIV patients in which PPE may have been due to other non-mosquito-bite associated antigens. This in turn suggests that the etiology of PPE in HIV patients may be heterogeneous or multi-causal with no singular environmental antigen being totally responsible for

all cases of PPE in the HIV patient. Further studies are necessary to investigate the precise mechanism(s) underlying this phenomenon.

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