Original Research Report

EXPRESSIONS OF INTERLEUKIN-4 AND INTERLEUKIN-5 IN NODULAR PRURIGO AND PRURITIC PAPULAR LESIONS

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ABSTRACT

Pruritic papular eruption is a dermatosis characterized by pruritic symptoms in patients with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS). Similarly, nodular prurigo is an itchy dermatosis with lesions and histopathological features that resemble those of pruritic papular eruption. Both conditions share a common etiopathogenesis, which involves the cytokines produced by T helper 2 (Th2) cells. Nodular prurigo and pruritic papular eruption are chronic and frequently recalcitrant, thus posing challenges in treatment. The use of biological agents represents a treatment development for chronic and recalcitrant dermatoses. This study aimed to determine the difference in the mean percentage of interleukin-4 (IL-4) and interleukin-5 (IL-5) expressions between nodular prurigo and pruritic papular eruption lesions, which may establish a basis for further biological agent therapy. A cross-sectional study was conducted using paraffin block preparations of the skin lesions of patients diagnosed with nodular prurigo (n=16) and pruritic papular eruption (n=16). Each paraffin block preparation involved immunohistochemical staining using IL-4 and IL-5 monoclonal antibodies. The expressions of IL-4 and IL-5 were assessed through ImageJ for Windows, version 1.53 (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, USA) by pathologists. The data were analyzed using an unpaired t-test with a significance level of $p<0.05$. The analytical results indicated that data on the average age of the two groups, disease duration, and storage sample duration followed a normal distribution ($p>0.05$). The mean percentage of IL-4 expression was significantly different between the nodular prurigo and pruritic papular eruption groups ($p=0.000$). However, the mean percentage of IL-5 expression was not significantly different between the two groups ($p=0.060$). In conclusion, the expression of IL-4 was higher in the nodular prurigo group in comparison to the pruritic papular eruption group. Nonetheless, the expression of IL-5 was comparably high in both the nodular prurigo and pruritic papular eruption groups.

Keywords: Human immunodeficiency virus (HIV); interleukin-4; interleukin-5; immunohistochemistry

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Highlights:
1. An examination of IL-4 and IL-5 expressions in nodular prurigo and pruritic papular eruption is crucial for enhancing the effectiveness of biological agent therapy, specifically for HIV/AIDS patients.
2. The findings offer evidence suggesting the possibility of IL-4 as a treatment target for individuals diagnosed with nodular prurigo, as well as IL-4 and IL-5 for those diagnosed with pruritic papular eruption.

INTRODUCTION

Pruritic papular eruption is a chronic manifestation of a skin disorder that commonly occurs in patients living with the human immunodeficiency virus (HIV). Several factors are considered to play a role in the etiology and pathogenesis of pruritic papular eruption. These include hypersensitivity to insect bites in patients with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), host cellular immune responses to abnormal infectious processes, drug reactions, autoimmune reactions in the skin, and the direct effects of viruses on immune regulation and skin tissue (Chua et al. 2014, Garg & Sanke 2017, Vijayan et al. 2017).

In the advanced stage of HIV infection, the T helper 1 (Th1) cells are suppressed, resulting in the dominance of the T helper 2 (Th2) cells. This dominance of Th2 cells plays a role in the pathogenesis of pruritic papular eruption. As a result, it causes an aberration of immune responses characterized by a decrease in clusters of differentiation 4 (CD4) T lymphocytes and an increase in clusters of differentiation 8 (CD8) T lymphocytes. This aberration is accompanied by hyperactivation of polyclonal B cells, which act in mast cell degranulation, and an increase in interleukin-5 (IL-5). IL-5 also contributes to the transfer of immunoglobulin G (IgG) isotypes to immunoglobulin E (IgE) isotypes. The presence of IgE both in the local area and in the bloodstream, followed by the differentiation of eosinophils, enables an increase in the sensation of itching on the skin. The increase in serum IL-5 levels has been found to be proportional to the severity of the pruritic papular eruption. The mean serum IL-5 levels in HIV patients with a pruritic papular eruption have been observed to be higher compared to HIV patients without an eruption (Vijayan et al. 2017, Mohammed et al. 2019).

Nodular prurigo is a type of chronic inflammatory dermatosis that shares similarities with a pruritic papular eruption. It is characterized by the clinical features of excoriations in various forms, including papules and nodules. Histologically, it is characterized by a combination of inflammatory infiltrates such as lymphocytes, eosinophils, and neutrophils (Wong & Yen 2022). The pathophysiology of nodular prurigo is not fully known. However, dysregulations of the immune and nervous systems are involved in the pathogenesis of nodular prurigo. Th-1 and Th-2 cytokines play a role in the pathogenesis of nodular prurigo through signal transducer and activator of transcription (STAT) 1, 3, and 6. Phosphorylated STAT (pSTAT) 1 and 6 function as the distinguishing characteristics of Th1 and Th2, respectively (Williams et al. 2020, Shao et al. 2023). A previous research by Agrawal et al. (2022) showed a high detection rate of pSTAT6. In the study, most cases exhibited that the entire epidermis was stained with anti-pSTAT6, which serves as a marker for Th2 cytokines including IL-4, IL-5, and IL-13. The immunohistochemical findings suggest that Th2 cytokines have a major impact on the pathogenesis of nodular prurigo.

Cytokines can be detected in both serum and tissue. However, the measurement of cytokines in serum can be influenced by circadian rhythms, food intake, and comorbidities (Amsen et al. 2009). Immunohistochemistry is a cytokine examination method that can overcome these deficiencies. Immunohistochemical methods are performed to analyze tissue biopsy samples, allowing the examiner to visualize the presence, location, and distribution of the target protein. In addition, immunohistochemistry is more accessible and affordable than other methods such as quantitative polymerase chain reaction (qPCR) and enzyme-linked immunosorbent assay (ELISA) (Maity et al. 2013).

A previous study by Yao et al. (2018) reported the distribution of race-specific allelic variants in cytokine genes. Analysis of cytokine gene polymorphisms showed that African Americans had increased levels of IL-5, tumor necrosis factor (TNF-) and interferon alpha (IFN-α). Concurrently, European Americans exhibited elevated levels of IL-4, interleukin-10 (IL-10), and interferon gamma (IFN-γ). Variations in cytokine levels that are associated with race can indirectly affect disease severity (Ness 2004). The presence of race-specific allelic variants in cytokine genes and the clinical picture of chronic inflammatory dermatoses, which are predominantly influenced by Th2 lymphocytes, raise the question of whether there is a difference in the expressions of IL-4 and IL-5. This study examined the expressions of IL-4 and IL-5 in nodular prurigo and pruritic papular eruption lesions with the objective of identifying any difference in the mean percentage of these interleukins.

MATERIALS AND METHODS

This study used a cross-sectional design to analyze paraffin block preparations of nodular prurigo and pruritic papular eruption. The data collection started by examining the medical records of patients who visited Dr. Sardjito Central General Hospital in Sleman, Indonesia. Subsequently, an investigation was conducted after obtaining paraffin blocks from the Department of Anatomical Pathology of the hospital. Sample tracing was carried out over a period of time spanning from January 2015 to December 2020. There were 16 subjects in each of the prurigo nodular and pruritic papular eruption groups. The paraffin blocks were recut and incubated with monoclonal antibodies targeting IL-4 and IL-5 (Grillo et al. 2017).

The expression of cytokines was measured by anatomical pathologists from the Faculty of Medicine, Public Health, and Nursing at Universitas Gadjah Mada, Sleman, Indonesia. An immunohistochemical method was selected for staining using IL-4 and IL-5 monoclonal antibodies and chromogen 3,3'-diaminobenzene (DAB). ImageJ for Windows, version 1.53 (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, USA) was utilized to observe the expressions of IL-4 and IL-5 in the cytoplasm of inflammatory cells. The

obtained data were subjected to the Shapiro-Wilk test to assess the normal distribution. The statistical analysis employed an independent t-test with a 95% confidence level. A significance level of $p<0.05$ was applied to declare statistical significance (Kim 2019). The analysis was performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA).

This research obtained ethical clearance from the Medical and Health Research Ethics Committee (MHREC) of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada-Dr. Sardjito Central General Hospital, Sleman, Indonesia, with reference No. KE/FK/0666/EC/2020 on 15/6/2020.

RESULTS

This study used 16 paraffin block preparations each from patients who underwent skin lesion biopsies for nodular prurigo and those who had skin lesion biopsies for pruritic papular eruption. The nodular prurigo group had a mean age of 42.63±14.25 years. This average was higher than the mean age of the pruritic papular eruption group (34.50±7.87 years). The mean disease duration in the nodular prurigo group was 18.31±8.58 months, which was similar to the mean disease duration in the pruritic papular eruption group (18.75±3.34 months). The storage duration of preparations in the nodular prurigo group was 35.00±18.01 months. This duration was slightly shorter than the average storage duration of preparations in the pruritic papular eruption group (37.06±7.28 months).

The immunohistochemistry, utilizing IL-4 and IL-5 monoclonal antibodies and chromogen 3,3'-diaminobenzene (DAB), demonstrated the production of a strong and permanent brown stain. The analysis using ImageJ for Windows, version 1.53 (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, USA) revealed positive IL-4 and IL-5 expression readings, as indicated by the appearance of brown stains on the cytoplasm of inflammatory cells. However, it was observed that not all inflammatory cells expressed IL-4 or IL-5. The Shapiro-Wilk test indicated that the data pertaining to the characteristics of the research sample conformed to a normal distribution. The detailed characteristics of the research sample are shown in Table 1.

Table 1. Normal distribution of data pertaining to the characteristics of the research sample.

Characteristics	NP	PPE	p
Age (years)			
Lowest	13	21	
Highest	63	47	
Mean	42.63±14.25	34.50±7.87	0.059
Disease duration (months)			
Lowest	6	12	
Highest	39	25	
Mean	18.31±8.58	18.75±3.34	0.205
Storage duration (months)			
Lowest	8	23	
Highest	60	48	
Mean	35.00±18.01	37.06±7.28	0.473

Legends: NP=nodular prurigo; PPE=pruritic papular eruption.

Yellow circles in Figure 1 represent positive IL-4 expressions in the nodular prurigo and pruritic papular eruption groups. The count of IL-4-expressing inflammatory cells was shown on the counter feature in ImageJ for Windows, version 1.53 (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, USA). However, among these preparations, it was found that not all inflammatory cells exhibited the expression of IL-4. This included plasma cells (indicated by red arrows), lymphocytes (indicated by yellow arrows), mast cells (indicated by red circle marks), and eosinophils (indicated by green circle marks).

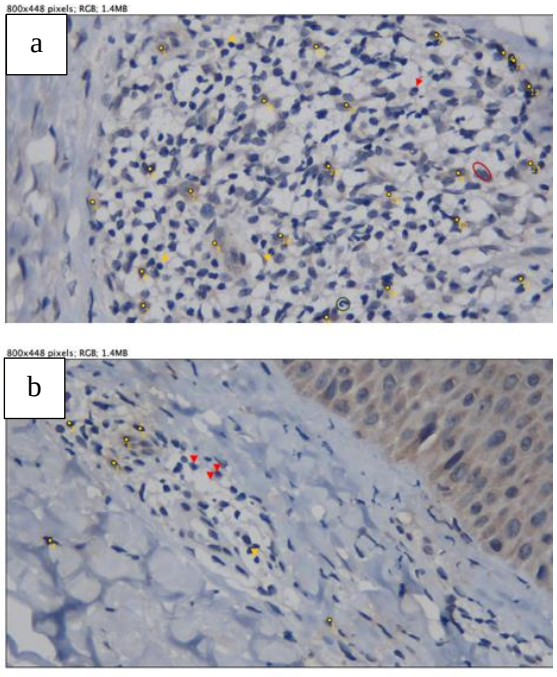

Figure 1. The expressions of IL-4 in (a) nodular prurigo lesions and (b) pruritic papular eruption lesions.

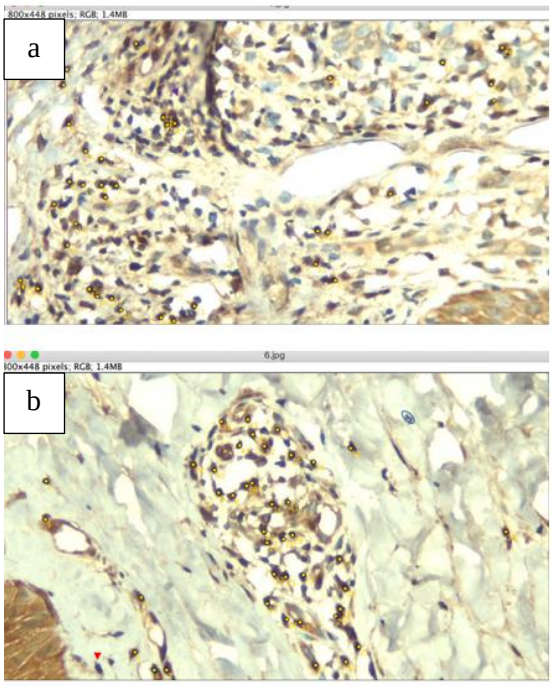

Figure 2. The expressions of IL-5 in (a) nodular prurigo lesions and (b) pruritic papular eruption lesions.

Similar to the IL-4 expressions shown in the previous figure, yellow circles in Figure 2 marked the positive expressions of IL-5 in the nodular prurigo and pruritic papular eruption groups. The count of inflammatory cells that exhibited IL-5 expressions was observable from the counter feature of ImageJ for Windows, version 1.53 (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, USA). However, several inflammatory cells in these preparations did not exhibit any IL-5 expression. Plasma cells (indicated by red arrows), lymphocytes (indicated by purple arrows), and eosinophils (indicated by green circles) were among these inflammatory cells.

Table 2. Differences in the average percentage of IL-4 and IL-5 cytokine expressions between the nodular prurigo and pruritic papular eruption groups.

Characteristics	NP	PPE	p
IL-4 cytokine expressions (%)			
Lowest	7.00	3.40	
Highest	40.00	9.90	
Mean	15.97±8.52	5.76±1.85	0.000
IL-5 cytokine expressions (%)			
Lowest	12.40	10.90	
Highest	63.70	29.90	
Mean	29.97±15.22	20.59±6.48	0.060

Legends: NP=nodular prurigo; PPE=pruritic papular eruption.

An analysis was carried out to determine the differences in the average percentage of IL-4 and IL-5 cytokine expressions between the nodular prurigo and pruritic papular eruption groups. The difference test analysis was performed following the normality test. Table 2 presents the differences in the mean percentage of IL-4 and IL-5 cytokine expressions between the nodular prurigo and pruritic papular eruption groups, as determined by an unpaired t-test.

DISCUSSION

A prior research conducted by Wang et al. (2022) revealed that age has an impact on the expression of cytokines. In the elderly, there is a decline in the activation of macrophages, which causes a decrease in the immunosurveillance of memory T lymphocytes as well as chronic and non-specific inflammatory cytokine responses. As per the Statistics Indonesia (2020), the elderly are defined as those who are over 60 years of age. The mean age of the subjects in the nodular prurigo and pruritic papular eruption groups in this study did not fall within the advanced age category. The data on the average age of the two groups were normally distributed ($p>0.05$), indicating that the average age did not affect the results of this study.

Nodular prurigo and pruritic papular eruptions are chronic pruritic dermatoses. The sample for this study was obtained from chronic lesions, as indicated by the similar mean disease duration of 18 months in both the nodular prurigo group and the pruritic papular eruption group. Nodular prurigo is characterized by chronic itching that lasts for a duration of at least six weeks (Ekpe 2019). Meanwhile, more than half of the subjects in the pruritic papular eruption group experienced skin manifestations that had appeared several months before the diagnosis was established.

Various factors that affect the measurement of cytokines in tissue biopsies include the methods used for collecting and handling tissue preparations, the duration of storage, and the frequency of using the preparations. The sample preparations in this study were not used repeatedly and were derived from paraffin blocks, which were utilized as stable storage media for further deep cutting and immunohistochemical staining (Keustermans et al. 2013, Grillo et al. 2015). Grillo et al. (2017) demonstrated that antigens located on the cell membrane and nucleus exhibited a decrease in the intensity of staining when stored in paraffin blocks for more than ten years. In the nodular prurigo group, the mean storage duration of paraffin block preparations was 35.00±18.01 months. Similarly, the mean storage duration in the pruritic papular eruption group was 37.06±7.28 months. Thus, the

intensity of the antigen in the staining was preserved.

The statistical analysis conducted in this study found a significant difference (p=0.000) in the percentage of IL-4 cytokine expressions between the nodular prurigo and pruritic papular eruption groups. The results additionally indicated that IL-4 cytokines were mainly expressed in the nodular prurigo group. Nodular prurigo is a chronic inflammatory condition characterized by secondary skin changes resulting from constant scratching due to severe itching. It is considered a hypersensitivity reaction affecting the dermis. The pathogenesis of nodular prurigo involves dysregulation of the immune system, specifically an increase in the levels of Th2 lymphocyte cell cytokines, as indicated by the detection of high pSTAT6. In addition, neural dysregulation is implicated in the pathogenesis of nodular prurigo (Zeidler et al. 2019, Agrawal et al. 2022). Th2 lymphocyte cells require exogenous IL-4 induction to initiate further differentiation and synthesis of IL-4. This suggests that the IL-4 cytokine appears in both the acute and chronic phases. IL-4 signaling is further required to regulate immunoglobulin class switching in B cells responsible for the induction of chronic itch (strong pruritogens) in nodular prurigo (Kaminuma et al. 2018, Heeb et al. 2020).

Previous immunohistochemical studies by Agrawal et al. (2022) and Kaminuma et al. (2018) demonstrated a high level of IL-4 messenger ribonucleic acid (mRNA) expression, which infiltrated the skin lesions of nodular prurigo. The findings additionally showed that IL-5, a cytokine produced by Th2 lymphocyte cells, predominantly appeared during the chronic phase. Its primary role is to promote the terminal differentiation of eosinophil precursors and enhance their activation and chemotaxis/migration. Eosinophils play a role in the induction of eosinophilic inflammation. The inhibition of the IL-4 signaling cascade can have a broader interference effect on the inflammatory response of Th2 lymphocytes compared to the proliferation and activity of more selective eosinophils through the inhibition of the IL-5 pathway (Kaminuma et al. 2018, Wu et al. 2019). The results of this study corroborate previous studies indicating that IL-4 cytokines were highly expressed, especially in the nodular prurigo group.

When individuals with advanced HIV infection experience a viral infection, the normal Th1 lymphocyte response switches to a Th2 lymphocyte response. The measurement of serum cytokine levels in HIV-infected patients revealed an increase in Th2 cytokines (Chimbetete et al. 2023). During the early stages of HIV infection, the presence of IL-4 triggers differentiation, leading to the development of cytokine dominance in Th2 lymphocytes as the disease progresses to an advanced stage. Afterwards, Th2 lymphocyte cells promote the proliferation of B cells. This is followed by an increased secretion of IL-5, which results in elevated IgE levels in both the local area and the bloodstream. Consequently, it enables the intensification of the itchy sensation on the skin (Chua et al. 2014, Vijayan et al. 2017). In a study conducted by Mohammed et al. (2019), it was found that most of the subjects exhibited IL-5 expression (40% of the subjects) as opposed to IL-4 expression (10% of the subjects). The majority of the subjects also had elevated levels of eosinophils in both their blood and tissues. The results of this study further support previous studies, which have shown that IL-5 cytokines were highly expressed in the pruritic papular eruption group.

A previous study carried out by Jung et al. (2013) revealed that the expressions of IL-4 and IL-5 cytokines in the epithelium of inflammatory lesions were 45.46±15.35% and 50.02±4.32%, respectively. In the nodular prurigo group of this study, the expressions of IL-4 and IL-5 cytokines were found to be 15.97±8.52% and 29.97±15.22%, respectively. In the pruritic papular eruption group, the expressions of IL-4 and IL-5 cytokines were 5.76±1.85% and 20.59±6.48%, respectively. Race differences can influence differences in the expression of these cytokines. In a previous research conducted by Yao et al. (2018), the distribution of allelic variants specific to different races in cytokine genes was documented. The analysis of cytokine gene polymorphisms showed that individuals of African American descent had high levels of IL-5, TNF- and IFN-α, whereas individuals of European American descent displayed elevated levels of IL-4, IL-10, and IFN-γ. The results of this study demonstrated that IL-5 cytokines were highly expressed in both the nodular prurigo and pruritic papular eruption groups. Therefore, IL-5 cytokines could potentially be used in the development of biologic agents for treating nodular prurigo and pruritic papular eruption in local races. The suggestions in this study propose conducting research to measure all cytokines involved in nodular prurigo and pruritic papular eruption. The purpose was to determine a specific cytokine that may be used as an alternative to developing biological agents or identifying atopic risk factors in the study sample.

Strength and limitations

This study examined the expressions of IL-4 and IL-5 in HIV patients who were diagnosed with nodular prurigo and pruritic papular eruption. The findings of this study suggested that IL-4 and IL-5 can be considered as potential treatment targets for the

treatment of nodular prurigo and pruritic papular eruption. However, this study had limitations that should be taken into consideration for further research. One of the limitations was the absence of other cytokines involved in nodular prurigo and pruritic papular eruption from the examination. A measurement of all cytokines associated with the two disorders is, in fact, important to identify a particular cytokine in the development of an alternative biological agent for treatment purposes. Another limitation was the absence of fresh skin preparations and the exclusion of atopic risk factors from the study sample.

CONCLUSION

The expression of interleukin-4 (IL-4) is higher in nodular prurigo compared to that in pruritic papular eruption. The difference in IL-4 expressions between the nodular prurigo and pruritic papular eruption groups is significant. However, there is no significant difference in IL-5 expressions between the nodular prurigo and pruritic papular eruption groups. The expressions of IL-5 in both groups are comparably high.

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Conflict of interest

None.

Ethical consideration

The Medical and Health Research Ethics Committee (MHREC) of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada-Dr. Sardjito Central General Hospital, Sleman, Indonesia, issued the ethical clearance for this study (No. KE/FK/0666/EC/2020 dated 15/6/2020).

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Author contribution

AKP drafted the article, provided the study materials, provided administrative, technical, and logistic support, as well as collected and assembled the data. Both DRAW and SRP contributed to the critical revision of the article for important intellectual content, gave final approval of the article, and obtained funding.

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