Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 51, Number 1, 2019; 54-62
http://dx.doi.org/10.19106/JMedSci005101201907

The role of nickel contact allergy in nummular dermatitis in Indonesia

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Submited: 2019-04-19
Accepted : 2019-04-24

ABSTRACT

In the recurrence of nummular dermatitis (ND) as a problem for patients, it is necessary to identify interferon-γ (IFN- γ), interleukin-4 (IL-4), interleukin-17 (IL-17), and stimulation of lymphocytes against nickel. This study aimed to investigate the role of nickel contact allergy in ND. Forty-two patients with ND were studied and 42 healthy subjects who were equal in age, sex and atopy history as control. All subjects underwent nickel skin patch test, detection of IFN-γ, IL-4, IL-17 in blood, and lymphocyte stimulation assays. To determine cut off point of the variables, receiver operator characteristic (ROC) curves were calculated. Bivariate and multivariate analyses were performed to measure the strength of association using odds ratio (ORs) and 95% confidence intervals (95% CI). Statistical analysis was performed using McNemar X^2-square test and multiple conditional logistic regression. Nickel contact allergy was shown by nickel patch test (OR= 3.5; 95% CI = 1.09–14.60), stimulation index/SI (OR= 29; 95% CI = 4.81-1184.43), IFN-γ (OR= 4.25; 95% CI = 1.39–17.36). These results were supported after multivariate analysis with conditional logistic regression which showed nickel patch test (OR= 9.63; 95% CI= 1.02–109.38; p= 0.04), SI (OR= 42.19; 95% CI = 2.32–766.03; p= 0.01), IFN-γ (OR= 11.51; 95% CI = 1.08–122.63; p= 0.04). Nickel contact allergy is an important risk factor for ND. Patients with ND are recommended to be tested for nickel contact allergy.

ABSTRAK

Dermatitis numularis (DN) merupakan kelainan kulit yang bersifat kambuhan. Perlu diidentifikasi interferon-γ (IFN-γ), interleukin-4 (IL-4), interleukin-17 (IL-17), dan stimulasi limfosit terhadap nikel untuk mengetahui peran alergi kontak nikel pada DN. Sebanyak 42 orang pasien dengan DN dan 42 subyek sehat yang setara secara usia, jenis kelamin dan riwayat atopi diikutkan dalam penelitian. Semua subjek menjalani uji tempel kulit alergen nikel, deteksi *IFN-γ,* IL-4, IL-17 dalam darah perifer, dan uji stimulasi limfosit. Untuk menentukan titik potong variabel, digunakan kurva *receiver operator characteristic* (ROC). Analisis bivariat dan multivariat dilakukan untuk mengukur kekuatan hubungan menggunakan *odds ratio* (*ORs*) dan *confidence intervals* 95% (95% *CI*). Dilakukan analisis statistik dengan menggunakan uji McNemar *X2-square* dan *multiple conditional logistic regression*. Hasil penelitian menunjukkan bahwa alergi kontak nikel ditunjukkan dengan uji tempel nikel (OR = 3,5; 95% CI = 1,09-14,60), indeks stimulasi/IS (OR = 29; 95% CI = 4,81-1184.43), IFN-γ (OR = 4,25; 95% CI = 1.39–17.36). Hasil ini didukung setelah dilakukan analisis multivariat dengan multiple conditional logistic regression yang menunjukkan uji tempel nikel (OR = 9,63; 95% CI = 1,02-109,38; p = 0,04), indeks stimulasi (OR = 42,19; 95% CI = 2,32-766,03; p = 0,01), IFN-γ (OR = 11,51; 95% CI = 1,08-122,63; p = 0,04). Dapat disimpulkan bahwa alergi kontak nikel merupakan faktor risiko penting pada DN. Pasien dengan DN direkomendasikan untuk dilakukan uji terhadap alergi kontak nikel.

Keywords:
interferron-γ
interleukin
lymphocyte
nickel
nummular dermatitis

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INTRODUCTION

Nummular dermatitis (ND) is a recurrent inflammatory skin disease characterized by skin lesions that form an oval or coin-shaped plaque with an exudative surface. In general, patient suffer from itching lesion on the low extremities.[1-3] It is also known as nummular eczema or discoid eczema, frequently occurs in the population (between 2% – 12%).

The pathogenesis of ND remains unclear.[4] Many factors contribute to the etiology of ND including endogenous and exogenous factors. Dry skin and emotional stress are endogenous factors that have been implicated in the onset of ND, and appear alone or together.[5-7] Exogenous factors that play a role in ND pathogenesis are topical allergens (sensitizers), infections or colonization or of bacteria, chemical trauma, local physical trauma, irritants, low humidity, hot weather, warm water, and winter season.[5,7] To date, previous studies described nickel exposure found to be the most frequent allergen in ND.

Patients with nickel contact dermatitis are mostly having a tendency to also suffer from ND (3.36% and 6.2%, respectively).[8,9] Since nickel is widely known as the most common metal substance in contact sensitizer characteristic, it has been known that 50% ND patients are associated by nickel contact allergy.[10] A similar result showed that nickel was the most prevalent allergen of ND (63%: 31 out of 49 cases).[11]

In allergic contact dermatitis (ACD), T helper1 cells (T_H1 cells) seem to be specifically act as an effector of T cells, who produce IL-2 and IFN-γ.[12] An *in vitro* study showed that the activation of nickel specific-T cells is followed by the proliferation and induction of cytokines of T_H1 (IL-2, IFN-γ), T_H2 (IL-3, IL-4, IL-13), and IL-10 by the regulatory T cells.[13] In any other study, patients with a positive patch test results indicated that the sensitization to nickel and other kind of metals involves an immune response similar to the cytokine profile from both T_H1 and T_H2 cells.[14] Exposure to nickel has been reported to induce IL-23 secretion through keratinocytes, a response mediated by T_H17. Interleukin-17-secreted T cells in peripheral blood samples have been found in the patients with nickel contact allergy. Interleukin-17 may contribute to the development of the contact allergy reactions by the capacity of T lymphocytes which are involved in the tissue damage.[15] By addressing the two theories above about ND and nickel contact dermatitis, we therefore determined the role of nickel lymphocyte stimulation, IFN-γ, IL-4 and IL-17 on the recurrence of nummular dermatitis. Thus, the insights lies within two following observations: first, the most common recurrence of ND is due to the exposure of nickel as allergen material; second, lymphocyte stimulation of nickel allergen, IFN-γ, IL-4, and IL-17 are the mediation parties that assist the encouragement of nickel ACD.

This study aimed to investigate the role of nickel contact allergy in the ND, focusing on the role of lymphocyte stimulation of nickel allergen, IFN-γ, IL-4, and IL-17. The study is necessary to our knowledge, as there has been no study, specifically *in vitro* study conducted in Indonesia.

MATERIALS AND METHODS

Design and participants

The study was performed using a matched-case control design to investigate the risk factors of ND (nickel contact allergy). Patch tests were performed, moreover, lymphocyte stimulation, IFN-γ, IL-4 and IL-17 stimulation were measured to demonstrate the nickel contact allergy. Patiens with ND were recruited from Dermatology and Venereology Clinic at Dr. Sardjito General

Hospital, Yogyakarta from the period of January 2014 to December 2015. Non ND indivisuals which matched for age, sex, and atopy history were considered as control group. In total 84 participants were included consisted of nummular dermatitis group (n=42; 18-55 years old) and control group (n=42; 18-55 years old).

Protocol of study

Peripheral blood samples (12 mL) were collected from each participants in both groups. Peripheral blood mononuclear cells (PBMC) were isolated for lymphocyte stimulation assay.[18] Briefly, a suspension of 10^5 PMBC in Roswell Park Memorial Institute (RPMI) then stimulated using nickel sulphate as an antigen (Sigma®). Phytohemagglutinin as medium was cultured using flat-bottom 96 wells microplate (Iwaki®). Medium control were stimulated without nickel. On a serial testing of our preliminary study, we tested several concentration of nickel sulfat (0.025; 0.05; 0.1, and 1.0 µg/mL). The best concentration of this antigen was found to be 0.025 µg/mL. The level of lymphocyte proliferation was measured with microculture tetrazolium assay (MTT assay), and stimulation index (SI) was calculated between antigen stimulated wells and the control wells. Supernatants of these cultures were tested for IFN-γ, IL-4, and IL-17 using enzyme-linked immunoabsorbent assay (ELISA).

Before the assay, patch tests with nickel sulfate as the allergen were done and interpreted in accordance with the International Contact Dermatitis Research Group (ICDRG) criteria.

Statistical analysis

Statistical analysis was done by bivariate analysis using McNemar test and multivariable using Multiple Conditional Logistic Regression. An Odds Ratio (OR) and 95% Confidence Interval (95% CI) were calculated to determine the significancy of these factors.

RESULTS

Characteristics of subject

From each study group, nine male and 33 female participants were included in the study (21.43%); mean age was 27.48 and 27.6 years old respectively in ND and control group. 82 out of 42 participants are diagnosed with positive atopic family history (66.66%). It means that the prevalence of atopic history among participants is relatively high (TABLE 1).

TABLE 1. Characteristics of subjects

Characteristics	ND (n=42)	Control (n=42)
Sex [n (%)]		
• Male	9 (21.4)	9 (21.4%)
• Female	33 (78.6)	33 (78.6%)
Age (mean ± SD year)	27.48 ± 12.32	27.6 ± 12.35
Atopic history [n (%)]		
• Positive	28 (66.7)	28 (66.7)
• Negative	14 (33.3)	14 (33.3)

ND : nummular dermatitis

Receiver operator characteristics (ROC) analysis

To determine the cut-off values of positivity of variables i.e. SI, IFN-γ, IL-4, and IL-17 levels, ROC analysis were applied to put the values of the cytokine. Area under the curve (AUC) of SI showed to be in a medium degree (FIGURE 1) and for IFN-γ, the degree was high (FIGURE 2)

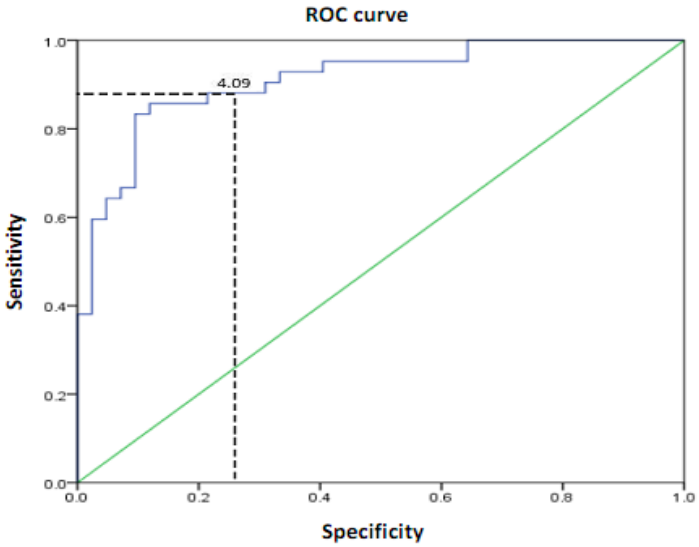

FIGURE 1. ROC curve of SI variable

Low degree AUC was found on IL-4 and IL-17. Cut off points for SI in our study was 4.1 (88.1% sensitivity; 78.6% specificity), whereas cut off points for IFN-γ level was 31.05 (69.05% sensitivity; 61.90% specificity). The references for the cut off points of ROC were used on McNemar tests on IL-4, IL-17. However, McNemar tests on SI, IFN-γ are analyzed by using the new cut off points. These analyses are summarized on TABLE 2.

TABLE 2. ROC analysis of all variables

Variables (cut off points)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	AUC
SI (>4.095)	88.1 (78 - 98)	78.6 (66 – 91)	0.91
IFN-γ (>31.05)	69 (55 – 83)	61.9 (47 – 77)	0.73
IL-4(>0.63)	4.8	97.6	0.51
IL-17(>8.5)	62	31	0.48

SI : stimulation index; AUC: area under the curve

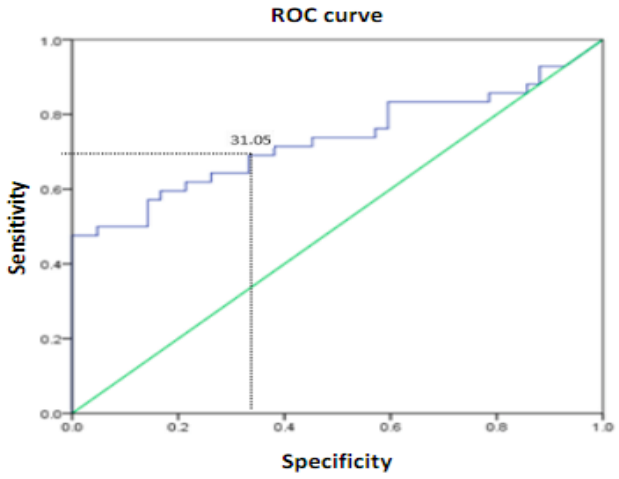

FIGURE 2. ROC curve of IFN-γ variable

Bivariate analysis results

Nickel patch tests, SI, IFN-γ were significantly different between groups ($p < 0.05$) on the TABLE 3. On the other hand, no significant differences were identified between groups for variables IL-4, IL-17 ($p > 0.05$); McNemar test results in the variables as follows: IL-4 (p= 1.00), IL-17 (OR=1; 95% CI = 0.19- 5.37; p= 1.00). Significant p value, OR, and 95% CI were found as follow: patch test (OR=3.5; 95% CI = 1.09-14.60; p= 0.03), SI (OR= 29; 95% CI= 4.81-1184.44; p= 0.00), IFN-γ (OR= 4.25; 95% CI = 1.39-17.36; p= 0.0072).

TABLE 3. Association between variables and ND using bivariate analysis

Variables	OR	95% CI	p
PT	3.5	1.09-14.6	0.03
SI >4.095	29	4.81-1184.44	0.00
IFN-γ >31.05	4.25	1.39-17.36	0.01
IL-4 >10	-	-	1.00
IL-17 >7.5	1.00	0.19-5.37	1.00

PT : Patch test; SI: stimulation index; IFN-γ: interferon-γ; IL-4: interleukin 4; IL-17: interleukin-17; OR: Odds Ratio; CI: confidence interval

Multivariate logistic regression analysis

After being tested by multivariate conditional logistic regression, we found the significant variables for patch tests (p= 0.048; OR= 9.63; 95% CI = 1.02–109.38), SI (p= 0.011; OR= 42.20; 95% CI = 2.32–766.03), IFN-γ (p= 0.043; OR= 11.51; 95% CI= 1.08–122.63) (TABLE 4).

TABLE 4. Results of association between variables and ND after multivariate conditional logistic regression analysis

Variables	OR	95 % CI	p
SI	42.19	2.32 – 766.03	0.01
IFN-γ	11.51	1.08 – 122.63	0.04
PT	9.63	1.02 – 109.38	0.04

SI: stimulation index; IFN-γ : interferon-γ; PT : patch test; OR: odds ratio; CI: confidence interval

DISCUSSION

The current study showed that nickel contact allergy was significantly different between ND group and control group (p= 0.03; OR= 3.5; 95% CI = 1.19 – 8.52) as demonstrated by patch test for nickel. This indicated that a person who had a positive nickel patch test would have a 3.5 times risk higher than a person with a negative nickel patch test. In line with our study, nickel sulfate patch test was showed to give a positive result on 46% ND patients.[10] Moreover, nickel sulfate also appears to be the most potential risk factor in the onset of ND by assessing the positive patch test results on 50% of 48 ND patients.[9] Another study using nickel patch tests found a positive results in 63% of 100 ND patients in Thailand; followed by other allergen such as rubber, fragrance, gold, formaldehyde, neomycin, and chromium.[11] In addition, 56% of 50 ND patients also gave a positive nickel patch test results. This later finding found that the relative contact sensitivity occurs markedly on patients with persistent and recurrent ND.[16] Different mechanisms may be influence the exacerbation of ND such as metal ingestion or a direct contact with the skin.[17]

Metal allergen identification with a patch test is necessary to perform on ND patients who are not respon to therapy.[17] In our study, nickel allergy was found by the positive patch test results, SI, and IFN-γ. Conversely, the diagnosis was less likely supported by IL-4 and IL-17 level.

In current study, we found a new cut off points of nickel SI (4.1) in ND. For this reason, individuals with an nickel SI >4.1 would have 29 times higher risks to develop ND than the one who has an nickel SI <4.1. Study by Cederbrant *et al.*[19] showed a promising result using PBMCs with nickel as the allergen of contact dermatitis only (positive results with a classification of SI >2).[19] Therefore, the current study is a novel study that measure SI with PBMC-stimulated nickel sulfate in contact dermatitis patients suffering from ND by using Lymphocyte Stimulation Test as one of the tools to confirm ND.

We used 0.025% (50 μM) of nickel sulfate, which was the same as the concentration of nickel sulfate in previous studies.[14,18-21] In disagreement with our cut off points, a previous study used 1.8 (63% sensitivity; 75% specificity) for the SI.[20] This cut off points were obtained by assessing 43 ACD patients; by using the PBMC culture treated with nickel sulfate 50 μM (0.029%).[22] In order to improve the cut off points of the SI, extra cytokine supplementation was specifically needed when culturing to better set the diagnose of *in vitro* nickel contact allergy.[20] However, we did not add cytokine supplementation in our lymphocyte stimulation tests.

In this study, the significantly difference between IFN-γ on ND group and control group was observed by using bivariate analysis with McNemar test; this

value was obtained from the new cut off points of ROC IFN-γ. Therefore, it means that a person who has a IFN-γ >31.05, he/she would have a 4.25 risk to develop ND than a person who has IFN-γ <31.05. Different from IFN-γ result, we failed to find a significant difference between IL-4 on ND group and control group (p= 1.00). We suggested one explanation for the non-significant result of IL-4 –stimulated nickel sulfate on both groups. The IL-4 is a cytokine that subsequently produced by T_H2 cells. Whereas in our study, the mechanism of hypersensitivity of nickel sulfate allergen was mediated by IFN-γ, which is produced by T_H1 cells. There was no study measure IL-4 concentration with PBMC-stimulated nickel sulfate on ND patients before.

Several studies demonstrated the role of cytokine as the sensitive indicator or biomarker of allergic contact dermatitis.[13] Such a pathway possibly happens in the allergic contact dermatitis patients.[12] With intention to this theory, several studies have been performed. One *in vitro* study found the activation of T cells-specific nickel, proliferation and induction of T_H1 cells (IL-2, IFN-γ), proliferation and induction T_H2 cells (IL-3, IL-4, IL-13), and proliferation and induction of T cells regulator (IL-10) have been suggested as the relevant response to nickel.[13] Nickel sulfate and other metal sensitization involves in the immune response which is similar to the cytokine profile of T_H1 together with T_H2.[14]

In the same way as IL-4 result, we also did not find the significant difference between IL-17 on ND group and control group. In fact there was no study measuring IL-17 with PBMC-stimulated nickel sulfate on ND patients before. Interleukin-17 level, as the rather new pro-inflammatory cytokine, were occasionally elevated on ACD and psoriasis patients.[23] Interleukin-17 has been known to not only regulate the molecule expression but also regulate the keratinocytes chemokine.[23]

In our study, we observed the significant IFN-γ level on NP group; T_H2 cells (IL-4) secretion and IL-17 were not increased. Note that the cut off points to analyze the IFN-γ refer to the ROC analysis, meanwhile, to analyze IL-4 and IL-7 refer to the previous study.[24,25] Some similarities have been found between the results from bivariate analysis and results from multivariate analysis conditional logistic regression. Nevertheless, OR from multivariate analysis conditional logistic regression on PT nickel sulfate, SI, and IFN-γ were found higher than their bivariate analysis.

Oral nickel intake (hematogen) gives rise to the sensitivity value and stimulated-lymphocyte response. Clinical responses will frequently emerge on the majority of allergic contact dermatitis patients when oral nickel intake is higher or equal to 5mg per day.[26] Nickel delayed type hypersensitivity response is known to be a remarkable pattern. This response will eventually change the peptide bonds on MHC II, therefore, stimulating cell T activity.[27]

CONCLUSION

In conclusion, nickel contact allergy is the most important factor of ND. These are demonstrated by the nickel patch test, SI, and IFN-γ level. Therefore, we recommend to avoid nickel contact to prevent the recurrence of ND. As for health practitioners, examination on hypersensitivity factors by using patch test is becoming an urge in order to treat a persistent and recurrent ND. It is important to note that the current study is a novel study that measure IFN-γ level and lymphocyte stimulation with PBMC-stimulated nickel sulfate in nickel contact dermatitis patients suffering from ND.

ACKNOWLEDGEMENTS

We would like to thank Mrs. Dewi from Clinical Epidemiology & Biostatistics

Unit (CE&BU), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta for valuable assistance in statistical analysis.

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