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# Determination Factors Associated with Immune Responses Towards First Dose Edmonston-Zagreb (EZ) Measles Vaccine in Indonesian Infants

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## ABSTRACT

**Background:** Measles outbreaks under high coverage of measles vaccination in Indonesia is gaining a necessary evaluation of factors associated with measles-vaccine immune responses. **Objective:** The purposes of recent study were to evaluate the association of sociodemographic and anthropometric parameters and specific immune responses towards first dose Edmonston-Zagreb (EZ) measles vaccine in Indonesian infants. **Methods:** A total of 35 infants were enrolled in this cohort study. Measles immune responses were followed up at one and six months after vaccination then analyzed for its association with sociodemographic, anthropometric, and nutritional parameters. The plaque-reduction microneutralization assay was conducted to measure the titer of measles specific IgG antibody. The level of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells that exhibiting gamma interferon (IFN- $\gamma$ ) secretion were analyzed by flow cytometry. The association between variable was analyzed by linear regression. The difference immune response among variable were analyzed with Mann-Whitney test. **Results:** Vitamin A supplementation and breastfeeding were predicted as associated factor for humoral and cellular immune response after one month and six months measles vaccination among Indonesia Infants. **Conclusion:** Nutritional factor is associated with measles vaccination immune response in Indonesian infants.

**Keywords:** Age, Breastfeeding, Vitamin A, Immune, Measles.

## 1. BACKGROUND

Measles is an extremely contagious acute viral infection, particularly in children. Despite the global accelerated reduction was achieved in the last decades, the resurgence occurrence in the past years has been arising global concern. Worldwide reported cases of measles escalated by 167% with 142.300 deaths in 2018 (1). The complication and sequelae symptoms of measles such as pneumonia, permanent hearing loss, ocular complication and neurological disturbance are considering as common cause of morbidity and mortality of measles (2).

Fortunately, measles vaccination program is successful in preventing measles outbreak and decrease mortality rates (3). Indonesia has measles vaccination coverage by 80% in the past five years. However, a higher incidence of measles outbreak in Indonesia was reported in 2017 with the total cases was 15.104 and incidence rates was 5.77 case/100.000 population. An awareness consideration is arising due to 45% children measles cases in Indonesia were observed among they who had received first-dose of measles vaccination (4).

The effectiveness of vaccine is taken part by the contribution of B-cells and T-cells. The differentiation of B cells to plasma cell that secreting antibody is involved as protection effects of vaccine. Meanwhile, the B- memory cells is featured as second defense against infection. The cellular adaptive immunity is appeared as vaccine specific memory cells such as CD8<sup>+</sup> and CD4<sup>+</sup> T cells (5). Previous study was suggested the secretion of gamma interferon (IFN- $\gamma$ ) by memory T cells as specific memory for measles virus on vaccinated subjects. Therefore, the release of IFN- $\gamma$  can be adopted as identification the generation of measles specific memory on cellular immunity (6).

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Seroconversion is major vaccine effectiveness factors. Previous research was found different seroconversion response among children in different age (7). Heterogeneity immune responses towards vaccines should be put on consideration (8). Previous study was recorded the influence of human leucocyte antigen (HLA) gen, non-HLA gene and multigenic on measles vaccine immune response (9).

Literature reviews were reported that induction of protective immunity against measles by vaccination may be influenced by several factors such as including the maternal passive antibodies, sex, ethnicity, age at vaccination, concurrent infections, environmental exposures to antigens, number of doses and type of vaccine (10-12). There were conflicting results about the role of these factors on vaccine immune response. It has been suggested that immune responses to measles vaccine was different among gender and race (13). Other different study reported that genetical ancestry of African American was associated with higher titer of neutralizing antibody and higher secretion of IFN- $\gamma$  than Caucasia lineage (14).

Conversely, several past decades studies unable to show the relationship between anthropometric and nutritional measles antibody seroconversion rates (15, 16). Latest further investigations were necessary to determine the associated factors of immune response to measles vaccination especially in Indonesian infants. This study would provide better understanding about the contributing factors driving measles vaccine immune responses to evaluate the vaccine efficacy, increase the protective effect of measles vaccine and provide policy brief to the stakeholders of vaccination program.

## 2. OBJECTIVE

The aim of this study were evaluated the sociodemographic, anthropometric and nutrition parameters that associated to specific immune responses against first dose Edmonston-Zagreb (EZ) measles vaccine in Indonesian infants.

## 3. MATERIAL AND METHODS

### Study participants

Research was carried out in Primary Health Center, Plumbon, Cirebon, West Java, Indonesia. The population of this study are infants between 9-12 months without history of measles infection and has not been receive measles vaccination. We were excluded the subject who were assessed with nutritional or medical condition problems by the general practitioner. A total of thirty-five participants which are meet the inclusion requirement were enrolled in this study.

### Research design

Baseline peripheral blood sampling was conducted before vaccine application. Afterwards, EZ strain of measles vaccine combined with MR vaccine (Serum Institute of India) was applicated to the participants. Subsequent follow up blood sampling was scheduled at 1 month and 6 months after baseline.

### Demographic and anthropometric profiles

At the baseline visit, the sociodemographic data of participants was retrieved for sex, age, and ethnicity. Participants were assigned for anthropometric measurement and nutritional status. The body weight, length and body mass index (BMI) was measured as previously described (17, 18). Nutritional status was interpreted as normal and undernourished as mention before (19).

### Peripheral blood mononuclear cells treatment

EDTA-treated blood was isolated for PBMC according to previous protocol with minor modification (20). Briefly, PBMC extracted using ficoll-hipaque method. The fractionated PBMC cells then suspended in a serum-free medium with 5% CO<sub>2</sub> at 37°C for 24 hours. Stimulation of PMBC was conducted using H61 and H1 peptides (Genscript, Singapore) as described in our previous protocol (21)

### Flow cytometry

The cells suspension was labelled with FITC anti-human antibody (Biolegend:317408) and PE/Cy5 anti-human CD8a antibody (Biolegend:300910). Intracellular staining using PE Anti-human IFN- $\gamma$  antibody (Biolegend:502509) was conducted to identified the expression of IFN- $\gamma$  The following procedures were referred to our previous research by flow cytometry (BD FACSCalibur, USA) (21).

### Measles-specific neutralizing antibody assay

Recombinant of measles virus strain was utilized for neutralizing antibody assay using previous gold standard method (22). Dilution of 1:8 and 1:1024 of sample were arrange in cell culture media. A total of 50  $\mu$ l of 100 tissue culture infectious dose 50 (TCID<sub>50</sub>) virus was added to each serum dilution as well as the plates controls. Furthermore, plates were incubated for 1 h at 36°C. After the addition of Vero cells, the microtiter plates were incubated at 36°C and observed for the presence of CPE at fifth days. The titer of protective neutralizing antibody was  $\geq$ 1:8 than converted into mIU/ml unit (21).

### Ethic

This observational cohort study was approved by Health Research Ethic Committee of Faculty of Medicine, Universitas Brawijaya, Indonesia (No:250/EC/KEPK-S3/09/2019). Informed consent of study enrollment was given by the parents of participants.

### Statistical analysis

Descriptive statistic, t-test or Mann-Whitney and Linear regression analysis were used in this study. Software of SPSS Statistics, version 25.0 (IBM Corporation, New

Variable	n (%)	
Sex	Male	19 (54.3%)
	Female	16 (45.7%)
Age at vaccination	9 months	17 (48.5%)
	> 9 months	18 (51.5%)
Ethnicity	Javanese	13 (37.1%)
	Sundanese	22 (62.9%)
Nutritional status	Normal (-2 to +2 SD)	32 (91.4%)
	Undernourished (< -2 SD)	3 (8.6%)

Table 1. Sociodemographic and nutrition status of participants

Variable	IgG Antibody Level (mIU/ml)	P- value <sup>a</sup>	CD4 <sup>+</sup> T-cells producing IFN-γ (%)	P- value <sup>b</sup>	CD8 <sup>+</sup> T-cells producing IFN-γ (%)	P- value <sup>b</sup>
<b>Gender</b>						
Male	453.57±386.10	0.140	7.81±3.84	0.445	5.19±3.53	0.990
Female	259.42±160.30		6.83±3.62		5.20±3.43	
<b>Age at vaccination</b>						
9 months	369.12±363.05	0.493	7.86±3.17	0.423	6.02±3.05	0.143
>9 months	371.44±272.72		6.89±4.27		4.30±3.21	
<b>Ethnicity</b>						
Javanese	460.17±394.50	0.226	7.20±3.26	0.842	4.28±2.35	0.232
Sundanese	317.11±257.83		7.46 ±4.04		5.73±3.88	
<b>Nutritional Status</b>						
Normal	383.73±329.24	0.811	7.30±3.77	0.750	5.29±3.50	0.602
Undernourished	240.95±88.10		8.04±3.70		4.18±2.88	

**Table 2. The differences response of antibody production and cellular immunity according to participant characteristic distribution following one month after first dose of measles vaccine. aMann-Whitney test, bIndependent T-test, significant value was p < 0.05**

York City, USA) was used for analysis. Significant level was set at p value <0.05.

#### 4. RESULTS

##### Characteristic of participants

The proportion of male (54.3%) and female (45.7%) participants was similar in this study with the average of age was 9.83 ± 0.86 months old at the baseline. The participants predominantly Sundanese (62.9%) as major domestic ethnic in West Java. Anthropometric measurements were revealed the average of body weight by 8.26 ± 1.05 kg, body length 68.67 ± 3.00 cm, and BMI was 17.52 ± 1.69 kg/m<sup>2</sup> (Table 1).

##### Factor associated with measles-specific immune response.

Measles-specific antibody levels were not differing in both sex (p = 0.140), age (p = 0.493) and ethnic variations (P = 0.226). From anthropometric measurement, no significant differences in measles-specific antibody levels were found between normal and undernourished group (P = 0,811) (Table 2). We did not find significant association between measles-specific T cells producing IFN-γ with sociodemographic and anthropometry parameters (Table 3). The analysis of univariate was demonstrated that vitamin A supplementation is a potential factor for immune response at 1 moth after vaccination, meanwhile after 6-month vaccination breastfeeding is an important factor to immune response initiation (Table 4).

#### 5. DISCUSSION

Our previous research was reported the increase of measles-specific IgG antibody after one month vaccine application. The level was higher than baseline as well as at six month of follow up measurement (21). Recently, we evaluated the factors that related to the humoral and cellular immune response after measles vaccination specially among Indonesian infant population.

Variable	Measles-Specific Antibody Level (p-value)	CD4+ T cells producing IFN-γ (p-value)	CD8+ T cells producing IFN-γ (p-value)
Gender	0.057	0.445	0.990
Age	0.491	0.617	0.601
Ethnicity	0.202	0.842	0.232
Body weight	0.051	0.766	0.320
Body length	0.427	0.190	0.900
BMI	0.053	0.976	0.787

**Table 3. The association between sociodemographic, anthropometric, and specific immune response towards measles Linear regression test, significant value was p < 0.05**

Previous report was revealed factor of sex, age at vaccination, ethnicity, genetic factors, maternal antibody, nutrition, and environmental exposure were influenced

Covariates	Univariate		Multivariate	
	p-value	OR	p-value	OR
<b>After 1-month vaccine:</b>				
Age	0.153	0.103	0.095	0.098
Gender	0.864	0.777		
Ethnicity	0.595	0.385		
Height for Age	0.999	0.000		
Weight for Age	0.999	1.722		
Breastfeeding status	0.201	7.933	0.347	3.737
Vitamin A Supplementation	0.140	16.621	0.029	18.146
<b>After 6-month vaccine:</b>				
Age	0.630	0.322		
Gender	0.305	0.117		
Ethnicity	0.221	0.108	0.256	0.222
Height for Age	0.611	3.346		
Weight for Age	0.999	1.723		
Breastfeeding status	0.240	19.196	0.256	4.500
Vitamin A Supplementation	0.626	0.375		

**Table 4 Univariate and multivariate analysis between socio-demographic and nutritional factors with sero-conversion rate of specific immune response following one- and six-month measles vaccination**

the response to vaccination (11). Our recent finding was demonstrated the opposite outcome. We found the factor of sex, age, and ethnicity were not associated with measles-specific immune response. Similarly, Voigt, et al (14) suggest no associations between sex and production of measles antibody as well as cellular immune response after measles vaccination. The genetic lineage of race was reported as one of associated factors. A study performed in 6-month-old Papua New Guinean infants reported that age, anthropomorphic characteristics and maternal factors were not significantly associated with antibody response (23).

However, our results were showed that anthropometric parameters not associated with measles-specific immune response. Dao, et al (16) were reported the similar outcome that seroconversion of measles-specific antibody was not significantly related to age and anthropometric indices (16). Study conducted in undernourished Gambian children conclude that there were no consistent associations between age, gender, nutritional status measurements and the development of seroconversion in measles vaccination (24).

The variability of vaccine response strongly influenced by the genetic variations in human. Host genetic factors are resulting interindividual variation in measles vaccine immune response (9). Previous report was revealed the elaboration of genetic variability towards response to measles vaccine, such as variation allele of human leukocyte antigen and polymorphisms in several function genes i.e., cytokine related genes, cytokine receptor genes, genes associated the innate immunity, vitamin A receptor gene, etc. (25).

Interestingly, we found that vitamin A supplementation and breastfeeding were increase the probability of immune response after vaccination. Vitamin A play a role for enhance both innate and cellular immunity (26). Preclinical study was demonstrated that supplementation vitamin A as retinyl palmitate improve the level of IgM and IgG of vaccinated mice (27). Another factor, breastfeeding was previously proposed to be support according to the significant influence for several vaccine during infancy (28). Humoral response to measles was positively correlated with longer breastfeeding period (29).

## 6. CONCLUSION

Nutritional factor such as vitamin A supplementation and breastfeeding are associated to measles vaccine immune response in Indonesian infants. Since our study is a single-center-based study and has methodological limitations including small sample size, the results cannot be generalized. Further large scale of research is necessary to evaluate broad factor associated with immune response to measles vaccination.

- **Patient consent form:** Informed consent of study enrollment was given by the parents of participants.
- **Author contributions:** TNHR, WB, IWA, HMSCK: concept and research design; TNHR, NSB: data acquisition; TNHR, WB,

TLS: drafting article. All authors revising it critically for intellectual content.

- **Conflict of interest:** No conflict of interest
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