



Cross-sectional Study

Effect of breastfeeding on children's health and its relationship to NRAMP1 expression: A cross-sectional study



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ABSTRACT

Toddlers with exclusive breastfeeding can increase immunity in preventing infectious diseases such as Upper Respiratory Tract Infections (ARI). The body's resistance to disease is controlled by Natural Resistance-Associated Macrophage Protein 1 (NRAMP1). NRAMP1 contributes to the pathophysiology of several intercellular infections, including ARI. The purpose of this study was to determine the effect of breastfeeding on children's health and its relationship to NRAMP1 expression. A cross-sectional study was conducted on 124 toddlers in October 2020–June 2021. Toddlers were selected purposively from three Community Health Centers in Central Jakarta. The first group (n = 62) was exclusive breastfeeding and the second group (n = 62) was not exclusive breastfeeding. The characteristics of mothers and toddlers were collected using a structured questionnaire supported by medical record data. Meanwhile, NRAMP1 expression and NRAMP1 protein levels were obtained from the examination of blood samples. Examination of NRAMP1 gene mRNA expression by real-time PCR method and serum NRAMP1 protein levels by ELISA method. Data were analyzed using t-test, ANOVA, and multiple linear regression. The results obtained that the average mRNA expression of NRAMP1 gene and protein levels of NRAMP1 in infants who were not exclusive breastfeeding were 6.88 fold change (FC) and 315.02 pg/ml compared to those who received exclusive breastfeeding of 11.36 FC and 1087.74 pg./ml. Parity, immunization history, exclusive breastfeeding, and frequency of ARI were significantly associated with NRAMP1 gene mRNA expression and NRAMP1 protein levels (P < 0.05). Maternal and under-five age, gender, and nutritional status were not significantly related (P > 0.05). Exclusive breastfeeding was the dominant factor influencing NRAMP1 gene mRNA expression (OR: 4268) and NRAMP1 protein content (OR: 737,362). Antibodies obtained from exclusive breastfeeding in synergy with the NRAMP1 gene form the body's immunity in infants suffering from ARI.

1. Introduction

According to WHO & UNICEF in all countries, the coverage of exclusive breastfeeding only reaches 60% [1]. Meanwhile in Indonesia, based on the 2018 Basic Health Research data, the coverage of exclusive breastfeeding has decreased significantly, namely 54.3% in 2013 to 37.3% in 2018. The highest coverage is in Bangka Belitung Province (56.7%) and the lowest was in West Nusa Tenggara (20.3%).

Meanwhile, the coverage of exclusive breastfeeding in DKI Jakarta Province is slightly higher than the national figure of almost 48% [2].

WHO recommends that after exclusive breastfeeding is given, breastfeeding is continued until the baby is 2 (two) years old with additional complementary foods [3]. Success in breastfeeding is determined since the mother's pregnancy [4–6]. Exclusive breastfeeding is a protective factor against the incidence of ARI, especially in the early phase of a baby's life, because breast milk contains various substances

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that increase the baby's immunity [7]. Various studies have shown that the incidence of ARI can be prevented by exclusive breastfeeding. Oktaria et al., in 2017 reported the results of an observational study that infants who are not exclusive breastfeeding are at risk for respiratory tract infections compared to infants who are exclusive breastfeeding [8]. Furthermore, infectious diseases in toddlers have a direct impact on the occurrence of malnutrition, especially the occurrence of stunting [9]. Scientific evidence has shown that nutritional factors are the biological basis that supports the process of transcription and translation of genes that play a role in breast milk production to work optimally [10].

One of the genes that play a role in controlling the body's resistance to disease is Natural Resistance-Associated Macrophage Protein 1 (NRAMP1). The NRAMP1 gene contributes to the pathophysiology of several intercellular infections [11–13]. A case-control study conducted on residents of Andhra Pradesh in India showed that patients with the gene controlled by NRAMP1 were 3 times more likely to develop tuberculosis than patients not controlled for the NRAMP1 gene [14]. The value of NRAMP1 gene expression in the healthy group was 4.61 times higher than inactive TB patients [11]. Based on this research, this study was conducted to know the effect of breastfeeding on children's health and its relationship to NRAMP1 expression.

2. Methods

2.1. Study area and period

This research was conducted at the Integrated Management of Childhood Illness (IMCI) polyclinic of Community Health Centers in Central Jakarta Regional, Indonesia during October 2020–June 2021. Central Jakarta is one of the administrative cities located in the State capital as the center of government, service, and trade, with development activities, carried out by the government high enough. The estimated population of this municipality is 924,686, the lowest compared to the other 4 (four) municipalities where most of the residents are highly educated. Central Jakarta consists of 8 (eight) sub-districts with lowland morphology [15].

2.2. Design and samples

A cross-sectional study with an independent test on children under five suffering from ARI. The population in this study were children under five who treated ARI at three Community Health Centers in Central Jakarta. Toddlers were selected from three Community Health Centers which became the research population. All eligible toddlers were included in the study, and respondents were selected using the purposive sampling technique.

2.3. Inclusion and exclusion criteria

Toddlers who were involved in this study were toddlers who were treated for ARI at the Community Health Centers and aged 7–48 months, who were breastfeeding, had a birth weight of more than 2500 g. Mothers are willing to have their toddlers participate in the research program by signing informed consent. Exclusion criteria were infants who were not breastfeeding and mothers who had tuberculosis.

2.4. Sample size determination

The sample size was calculated using a two-sample proportion test formula with a two-tailed alternative hypothesis, using the following assumptions: 95% confidence level, 30.8 infants with ARI who received exclusive breastfeeding (P1), and infants with ARI who did not receive exclusive breastfeeding (P2) in the previous study, 90% power, 10% contingency for loss to follow up. Therefore, using the following formula, the calculated sample size was 124 (62 per group) children under five suffering from ARI were selected for this study, which was

calculated based on the proportion of the population in the previous study using the purposive sampling technique [16]:

2.5. Data collection and measurements

The instrument used is a structured questionnaire supported by medical record data to see the characteristics of mothers and toddlers including age, gender, parity, immunization history, frequency of ARI in the last 6 months, nutritional status (Weight/Age), and history of exclusive breastfeeding. Measurements of NRAMP1 gene mRNA expression and NRAMP1 protein levels were obtained from the examination of blood samples taken as much as 100 l, then centrifuged at 3000 rpm for 10 min to separate blood cells and serum. Blood serum was taken and put into an Eppendorf tube and stored in a freezer/refrigerator at -80°C until all samples were filled. After the sample is fulfilled, it is then taken to the Molecular Biology and Immunology Laboratory, Faculty of Medicine, Hasanuddin University in a container that meets the requirements at the time of delivery for further examination. Examination of NRAMP1 gene mRNA expression by real-time PCR method according to the protocol of the previous study [17–25]. Serum NRAMP1 protein levels using the Enzym linked Immunosorbent assay (ELISA) method according to the protocol of the previous study [26–31].

2.6. Data processing and analysis

All data in the questionnaire were examined including the characteristics of mothers and children under five. Furthermore, the data was coded and input using SPSS version 22.0. Variables with continuous data, NRAMP1 gene mRNA expression and NRAMP1 protein levels were analyzed for normality using the Kolmogorov-Smirnov test. Based on the Kolmogorov test, all variables including NRAMP1 gene mRNA expression and NRAMP1 protein levels showed normal distribution. Descriptive statistics consisting of mean, standard deviation, percentage, and categorical were analyzed by univariate analysis. 95% confidence levels and $P < 0.05$ were used to assess statistical significance. Independent t-test and ANOVA were used to see significant differences between groups, the dominant factors affecting the independent variables were analyzed by multivariate multiple linear regression analysis.

2.7. Ethical consideration

This research has passed the ethical test based on the research ethics notification letter from the Faculty of Medicine, Hasanuddin University Number 438/UN.4.6.4.5.31/PP36/2020. Data collection was carried out by the main researcher and assisted by enumerators (midwifery students). The mother filled out the questionnaire herself after receiving information about the study and signing the informed consent. In this study, the authors confirmed that all methods were carried out under the relevant guidelines and regulations (Helsinki Declaration) under the number researchregistry7118. This manuscript have been reported in line with the STROCSS criteria [32].

3. Result

Based on the results of interviews with 124 respondents, the characteristics of mothers aged 20–35 years were 55.6%. Judging from maternal parity, multiparas were more than primiparas and grandes, namely 57.3%. The characteristics of the children under five who were studied, aged 13–24 months were slightly more, namely 33.1%, gender was almost comparable between 49.2% men and 50.8% women. Most of the children under five have received 75.0% complete immunization. Community Health Centers is the choice of place for delivery of the mother with the most 66.1%. 81.5% of children under five were born spontaneously. The frequency of ARI in children under five was more than 2 times in the last six months 45.2% and the frequency of ARI less than two time was 54.8%. Toddlers who received 50.0% exclusive

breastfeeding and 50.0% did not get exclusive breastfeeding. Toddlers with good nutritional status were 82.3% and as many as 16.1% were undernourished.

In Table 1, it can also be seen that the average mRNA expression of the NRAMP1 gene was 9.120 FC \pm 2.591 SD 5.039–13.785. The average protein levels of NRAMP1 was 701.377 pg/ml \pm 442.570 SD 14.576–1414.052.

The results of this study indicate that the average NRAMP1 gene mRNA expression at the age of the mother <20 years is higher. In toddlers aged 7–12 months, the highest mRNA expression of the NRAMP1 gene decreased with the increasing age of toddlers. The sexes of men and women are not much different. Likewise, the nutritional status is not much difference between good and poor nutritional status in toddlers. This shows that there is no difference in the average NRAMP1 gene mRNA expression based on maternal age, age of a toddler, gender, and nutritional status (p-value >0.05) (Table 2).

The findings of this research in Table 2 show that the average mRNA expression of the NRAMP1 gene was higher in the multiparous group, under-fives with a history of complete immunization, exclusive breastfeeding, and the frequency of ARI once in the last six months. Also in this study shows that immunization history, breastfeeding status, and the frequency of ARI were related to the NRAMP1 gene mRNA expression (p-value <0.05). Multiparous mothers had a higher average expression of NRAMP1 gene mRNA than primiparous and grande mothers. In addition, this research shows a positive relationship between complete immunization and exclusive breastfeeding, where completed immunization and exclusive breastfeeding can increase the average NRAMP1 gene mRNA expression. The average NRAMP1 gene mRNA expression was higher in toddlers who were fully immunized than in toddlers who

Table 1
Univariate analysis.

Variables	N	%
Mother's Age		
<20 years	7	5.6
20–35 years	69	55.6
>35 years	48	38.7
Parity		
Primipara	47	37.9
Multipara	71	57.3
Grande	6	4.8
Toddler Gender		
Boy	61	49.2
Girl	63	50.8
Toddler Age		
7–12 months	30	24.2
13–24 months	41	33.1
25–36 months	29	23.4
37–48 months	24	19.4
Immunization history		
Incomplete	31	25.0
Complete	93	75.0
Exclusive breastfeeding		
No	62	50.0
Yes	62	50.0
ARI frequency		
≥ 2 times	56	45.2
<2 time	68	54.8
Nutritional Status (Weight/Age)		
Malnutrition	1	0.8
Poor nutrition	20	16.1
Good nutrition	102	82.3
Overnutrition	1	0.8
NRAMP1 gene mRNA expression	9.12 FC	
Mean	2.59	
SD	5.04–13.78	
Min-Max		
NRAMP1 protein levels	701.38 pg/ml	
Mean	442.57	
SD	14.58–1414.05	
Min-Max		

Table 2
Factors that affect NRAMP1 Gene mRNA Expression.

Characteristics	Mean	SD	95% CI	p-value
Mother's Age	9.70 FC	2.44	7.44–11.96	0.294
<20 years	9.35 FC	2.77	8.70–10.03	
20–35 years	8.67 FC	2.31	8.00–9.34	
>35 years				
Toddler Age	9.55 FC	2.46	8.63–10.47	0.524
7–12 months	9.25 FC	2.61	8.43–10.08	
13–24 months	8.97 FC	2.56	7.99–9.95	
25–36 months	8.53 FC	2.78	7.36–9.70	
37–48 months				
Parity	8.50 FC	2.62	7.74–9.28	0.018*
Primipara	9.66 FC	2.45	9.07–10.24	
Multipara	7.53 FC	2.65	4.74–10.32	
Grande				
Toddler Gender	8.88 FC	2.54		0.308
Boy	9.35 FC	2.63		
Girl				
Immunization history	7.79 FC	2.20		0.001*
Incomplete	9.56 FC	2.57		
Complete				
Exclusive breastfeeding	6.88 FC	1.20		0.000*
No	11.36 FC	1.38		
Yes				
ARI frequency	7.69 FC	2.14		0.000*
≥ 2 times	10.30 FC	2.33		
<2 times				
Nutritional Status				
Poor nutrition	8.83 FC	2.21		0.581
Good nutrition	9.18 FC	2.67		

were not fully immunized. Toddlers who had exclusively breastfed also had a higher average expression of NRAMP1 gene mRNA than toddlers who had not breastfed exclusively. However, there is a reverse relationship between the NRAMP1 and the ARI frequencies where the more often toddlers suffer from ARI, the lower the average NRAMP1 gene mRNA expression. The average NRAMP1 gene mRNA expression was higher in toddlers who had ARI less than two times compared with toddlers who suffered from ARI less than two times within the last six months.

Table 3 shows that the average protein levels of NRAMP1 in mothers aged <20 years is higher. In toddlers aged 7–12 months and 25–36 months the highest. The sexes of men and women are not much different. Likewise, the nutritional status is not much difference between good and poor nutritional status in toddlers. This shows that there is no difference in the average NRAMP1 protein levels based on maternal age, age of toddlers, gender, and nutritional status (p-value > 0.05).

The average protein levels of NRAMP1 was higher in the multiparous group, children under five with a history of complete immunization, receiving exclusive breastfeeding, and the frequency of ARI once in the last 6 months. From the results of the study, it was found that a history of complete immunization, exclusive breastfeeding, and the frequency of ARI was associated with NRAMP1 protein levels (p-value <0.05).

Differences in gene mRNA expression and protein levels of NRAMP1 between infants who were not exclusive breastfeeding and those who were exclusive breastfeeding are presented in Fig. 1 mRNA gene expression and protein levels of NRAMP1 in infants who were not exclusive breastfeeding were lower than those who were exclusively breastfeeding.

Multiple linear regression analysis was used to determine the most influential factors on gene mRNA expression and NRAMP1 protein content. Table 4 shows that for every child who is exclusive breastfeeding, the mRNA gene expression and protein levels of NRAMP1 will increase by 4268 FC and 737,362 pg/ml. For each child with an ARI frequency once every 6 months, the expression of gene mRNA and protein levels of NRAMP1 will increase by 2195 FC and 365,390 pg/ml. The factor with the most dominant influence on gene mRNA expression and NRAMP1 protein content was exclusive breastfeeding.

Table 3
Factors that affect NRAMP1 protein levels.

Characteristics	Mean	SD	95% CI	p-value
Mother's Age	830.16 pg/ml	339.10	516.55–1143.78	0.181
<20 years	750.77 pg/ml	480.77	635.28–866.27	
20–35 years	611.60 pg/ml	386.28	499.42–723.74	
>35 years				
Toddler Age	714.29 pg/ml	410.65	560.95–867.63	0.513
7–12 months	752.62 pg/ml	409.76	623.29–881.96	
13–24 months	713.72 pg/ml	483.53	529.79–897.64	
25–36 months	582.77 pg/ml	488.31	376.58–788.96	
37–48 months				
Parity	591.69 pg/ml	420.30	468.27–715.08	0.050*
Primipara	784.94 pg/ml	439.60	680.90–889.00	
Multipara	571.83 pg/ml	505.34	41.50–1102.15	
Grande				
Toddler Gender	671.31 pg/ml	432.30		0.459
Boy	730.49 pg/ml	453.84		
Girl				
Immunization history	486.40 pg/ml	416.20		0.002*
Incomplete	773.03 pg/ml	429.67		
Complete				
Exclusive breastfeeding	315.02 pg/ml	196.28		0.000*
No	1087.74 pg/ml	230.17		
Yes				
ARI frequency	461.50 pg/ml	365.55		0.000*
≥2 times	898.92 pg/ml	402.72		
<2 times				
Nutritional Status				
Poor nutrition	693.19 pg/ml	410.50		0.926
Good nutrition	703.05 pg/ml	450.70		

4. Discussion

In this study, the results showed that there was no difference in the average NRAMP1 gene mRNA expression based on maternal age, toddler age, gender, and nutritional status, so there was no relationship with the NRAMP1 gene mRNA expression. Recent studies have found that there is a relationship between polymorphisms in the NRAMP1 gene with susceptibility to various diseases, including tuberculosis, ARI, or other bacterial and viral infections. This is associated with the ability of the innate immune system in a person's body to deal with the infection. Research related to this gene in several age groups and sexes has been widely published. Ben-Selma et al. conducted a study by looking at the relationship of tuberculosis patients with polymorphisms in the NRAMP1 gene. This study identifies the distribution of two forms of polymorphism. The first is a non-conservative substitution on a base at codon 543 in exon 5 which converts aspartic acid to asparagine, this polymorphism is called D543 N. The second form is the deletion of TGTTG in the untranslated region 3 at nucleotide number 55, the last codon of

exon 15, this type of polymorphism is called 3'-UTR. Ben-Selma et al. found that the D543 N polymorphism was significantly more found in the tuberculosis group than in the control group. It turns out that this type of polymorphism is more common in women under the age of 30 years [33]. However, this is different from the results of other studies where susceptibility to the NRAMP1 gene occurs with age. For example, the AA+874 A/T genotype variant can affect the performance of IFN-cytokines thereby increasing the risk of worsening respiratory tract infections in men aged 30–49 years [34].

In addition to age and gender, the environment is also a factor in gene susceptibility in infection control mechanisms. Malik et al. stated that the susceptibility of the NRAMP gene to respiratory diseases, especially tuberculosis, was detected more often in families who were frequently exposed to M. tuberculosis. When exposed to frequent exposure, the performance of the NRAMP1 protein becomes weak and ultimately fails the optimal immune response [35].

The early period of life is very vulnerable to ARI. The neonate's immune system is still compartmentally deficient, so the innate immune response tends to be weak. Antigen presentation is also weak in stimulating the immune response, resulting in the weak activity of memory immune cells. In neonates, the innate immune response is more important than the adaptive immune response, because in the early period of life there is continuous exposure to new and diverse antigens. Thus, the ability to distinguish between an antigen and a harmless substance is essential. In addition, the ability to fight at an early stage is also very important. It is clear from this that the susceptibility of infants to infectious diseases is closely related to the ability of the compartments of cells that play a role in the innate immune response. The NRAMP1 gene is known to be abundant in macrophages, monocytes, and neutrophils, so the polymorphism that occurs in this gene may cause susceptibility to infectious diseases [36].

The NRAMP1 gene does not only work on macrophages or monocytes against the causative agent of ARI. Recent studies have found that this gene also plays a role in the upregulation of TNF-, interleukin (IL)-1, MHC class II expression, nitric oxide release, and apoptotic processes.

Table 4
Factors that affect NRAMP1 Gene mRNA Expression and NRAMP1 Protein Levels in Multivariate Analysis.

	NRAMP1 Gene mRNA Expression			NRAMP1 Protein Levels		
	OR	p-value	R Square	OR	p-value	R Square
exclusive breastfeeding	4268	0.000	0.931	737,362	0.000	0.937
ARI frequency	2195	0.000		365,390	0.000	

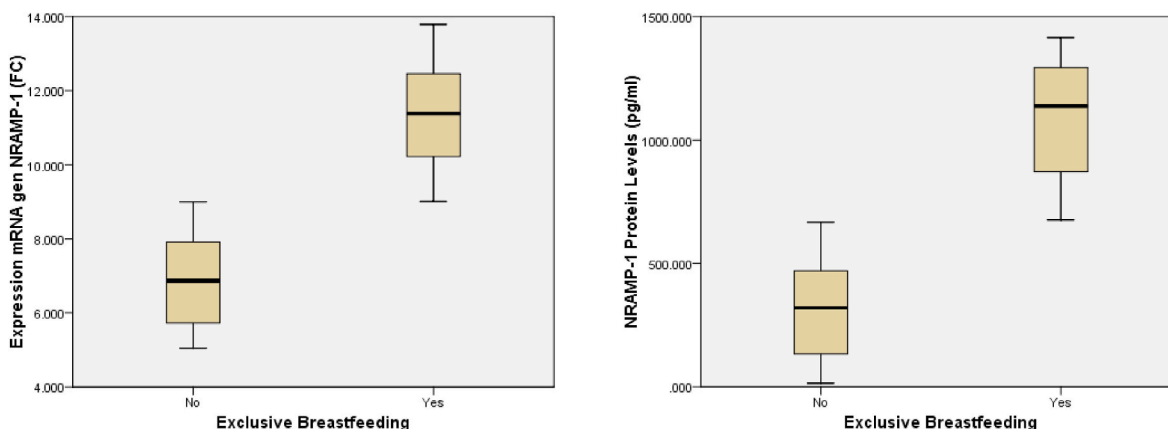


Fig. 1. Boxplot Expression mRNA gen NRAMP1 and NRAMP1 Protein Levels.

This finding, therefore, supports the theory that the NRAMP1 protein directly not only plays a role in intracellular bactericidal processes but also plays a role in macrophage differentiation and activation [36].

Based on the nutritional status of the study, which is in line with the results of this study, a case-control study conducted on TB patients in a TB endemic area in Jakarta found that the NRAMP1 gene polymorphism was not significantly associated with iron deficiency anemia [36]. The different NRAMP1 gene mRNA expression between complete and incomplete immunization history showed a significant relationship. Another study found that the NRAMP1 gene could influence the effectiveness of the BCG vaccine, suggesting that NRAMP1 plays an important immune regulatory function related to pathogen response [37].

The NRAMP1 gene and NRAMP1 Protein Levels for children under five who were not exclusive breastfeeding were lower than those who were exclusive breastfeeding. This study indicates that exclusive breastfeeding is a dominant factor associated with the NRAMP1 gene mRNA expression and NRAMP1 Protein Levels. Oktaria et al. reported the results of an observational study that infants who are not exclusive breastfeeding are at risk for respiratory tract infections compared to infants who are exclusively breastfeeding [8]. The nutritional content and nutrients, and bioactive factors in breastmilk have benefits in the formation of body immunity, achieving optimal growth and development, especially in the first six months of a baby's life. Lactoferrin in breastmilk plays a role in the body's defense of infants through the mechanism of binding iron, binding to bacterial membranes, inhibition of tumor necrosis factor-alpha (TNF- α), and interleukin-1 β (IL-1 β), and other roles in increasing body immunity in early life period [8,38].

NRAMP1 is known to increase immunity against the causative agents of ARI. There are many hypotheses about how the performance of the NRAMP1 protein in killing bacteria or viruses. Barton et al. stated that the phosphorylation of the SH3 domain on the NRAMP protein was able to mediate the transport of L-arginine, which is a substrate for the synthesis of Nitric Oxide (NO). This theory is interesting because NO is a potent antimicrobial agent produced by macrophages. A study was conducted on mice, mice that produce more NO are called BCG gene (Bcgr) while mice that produce less NO are called Bcgs animals. Then the mice were stimulated with interferon- γ (IFN- γ) and lipopolysaccharide or BCG. Bcgr mice were able to inhibit *M. tuberculosis* by producing more NO. Many functional differences between the two macrophages are produced from these different mice. Among them are increased expression of genes that produce IL-1 and TNF-, higher MHC expression, which makes macrophages from Bcgr mice stronger in the inflammatory process and antigen presentation [12,39].

This study indicates that NRAMP1 and the ARI frequency have a reverse relationship where the more often children under five suffer from ARI, the lower the average NRAMP1 gene mRNA expression. In other words, children who have a higher NRAMP1 gene will suffer fewer ARI frequencies. NRAMP1 gene contributes to the immune response and has a direct effect on pathogens that persist in macrophages. It is related to the role of the NRAMP1 gene in controlling pathogens inside the macrophage. Also, the NRAMP1 gene has the potential to keep the balance of Th1 and Th2 in the adaptive immune response mechanism towards an infection [12].

Acute Respiratory Infection (ARI) is one of the diseases that causes a high mortality rate in children under 5 years. This disease is mostly caused by rhinovirus, respiratory syncytial virus (RSV), influenza, parainfluenza, and coronavirus. In addition to viruses, bacteria also play a role in the pathogenesis of ARI. In the upper respiratory tract, there are commensal bacteria that have the potential to turn into pathogens. These bacteria include *Streptococcus pneumoniae* (pneumococcus), *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus*. This infection is usually limited to the upper respiratory tract but can progress to the lower respiratory tract and cause other, more severe infections, such as bronchiolitis and bronchopneumonia. Several polymorphisms related to the NRAMP1 gene are known to be associated with the pathogenesis of respiratory tract diseases, namely INT4, D543 N, and

3-UTR which are associated with susceptibility to tuberculosis in African and Asian races [40,41].

Research conducted by Handayani stated that the mRNA expression of the NRAMP1 gene was lower in patients with active pulmonary TB than latent TB, however, there was no significant difference between the groups of active pulmonary TB and latent TB [11]. Research conducted on pulmonary tuberculosis patients and nurses in Surabaya found that the 3'untranslated region (3'UTR) polymorphism of the NRAMP1 gene increased the risk of pulmonary tuberculosis [42].

The main component of innate and adaptive immunity against *Mycobacterium tuberculosis* is NRAMP1, the relationship between susceptibility to tuberculosis and polymorphisms in the NRAMP gene has been studied in various geographies. Studies conducted in Venezuela confirmed the association of the NRAMP1 3'UTR polymorphism with *Mycobacterium tuberculosis* infection and disease progression [43]. Polymorphisms in the NRAMP1 gene were statistically associated with susceptibility to pulmonary tuberculosis in the Han Chinese population [44]. A meta-analysis has shown that polymorphisms in the SLC11A1 gene contribute to TB (both pulmonary and extrapulmonary tuberculosis), particularly in Asia [45].

Different results were found by Hatta et al. who stated that no significant association was found in the three NRAMP1/SLC11A1 gene variants with tuberculosis in the population in South Sulawesi [46].

In this study, it was found that maternal age, under-five age, gender, and nutritional status did not have a significant relationship with NRAMP1 protein levels.

Infants infected with microbes in their respiratory tract have differences in their genetic and immunological control. Recent studies have found that the NRAMP protein is associated with susceptibility to respiratory infections. The NRAMP protein is an integrated membrane protein with a transport function. This protein can regulate the antimicrobial activity of macrophages against intracellular pathogens in the early phase of infection. Therefore, it is called a protein related to natural immune function. The distinctive structure of this protein is the transmembrane domain, which consists of an N-linked glycosylation cluster and a consensus form of transport. Currently, two genes related to the NRAMP protein, namely NRAMP1 and NRAMP2, have been characterized and identified in mammals such as mice and humans. NRAMP1 is highly expressed on the endosome and lysosomal membranes of macrophages, dendritic cells, neutrophils, and myeloid pathway immune cells, and is also found in neuronal cells. The NRAMP1 gene encodes an ion transporter located on the lysosomal membrane during phagocytosis of mycobacterium or other pathogens. This is possible due to the ability to regulate divalent cations [47].

The regulation of Fe²⁺ ions at the phagosome levels is very important in the process of microbial phagocytosis. Because the mineral iron is essential for the biological system of bacteria or viruses. Humans as hosts compete with bacteria and viruses for the availability of iron as a support system. If the iron mineral is sufficient, the host cells can supply the devices to defend themselves from pathogens such as ROS and nitrogen [35,36].

In the multiparous group and complete immunization history, the mean NRAMP1 protein levels was higher, this indicates parity and immunization history was associated with NRAMP1 protein level.

The average protein levels of NRAMP1 for infants who were not exclusive breastfeeding was lower than those who were exclusive breastfeeding, indicating the relationship between exclusive breastfeeding and NRAMP1 protein levels and was the most dominant factor. NRAMP1 (SLC11A1) transports ferrous iron transmembrane (importer) for iron in phagocytic vesicles. NRAMP1 plays an important role in the body's defense against various intracellular infections mediated by macrophages, including Leishmania, *Mycobacterium*, and *Salmonella*. *Mycobacterium tuberculosis* requires iron as a cofactor for the formation of superoxide dismutase and catalase, which are enzymes that neutralize antimicrobial action in phagolysosomes so that bacteria can survive in macrophages. NRAMP1 pumps iron ions out of the phagosome, bacteria

will be deprived of this metal nutrient so that their defense against macrophage bactericidal activity will be weakened. Several NRAMPT polymorphisms are known to influence TB susceptibility and progression [12,13,46].

This study showed that the frequency of ARI was associated with protein levels of NRAMPT. Research conducted by Handayani stated that NRAMPT protein levels were lower in patients with active pulmonary TB than latent TB, but there was no significant difference between the groups of active pulmonary TB and latent TB [11]. In contrast to the examination of the NRAMPT genotype of tuberculosis patients and healthy controls in Timor Tengah Selatan, East Nusa Tenggara showed an association between NRAMPT polymorphisms and susceptibility to tuberculosis [48].

5. Conclusion

Exclusive breastfeeding and history of ARI frequency in children under five were significantly associated with NRAMPT gene mRNA expression, and NRAMPT protein levels showed a different pattern of association. Exclusive breastfeeding has a positive relationship to NRAMPT gene mRNA expression and NRAMPT protein levels, where children under five who had breastfed exclusively had a higher NRAMPT gene mRNA expression than children under five who had not breastfed exclusively. There is a reverse relationship between the NRAMPT and the ARI frequency, where the less often children under five suffer from ARI, the higher the NRAMPT gene mRNA expression and NRAMPT protein levels.

Of the two main factors, exclusive breastfeeding is the most dominant factor affecting NRAMPT gene mRNA expression and NRAMPT protein levels. Therefore, it is necessary to make strategic efforts to promote exclusive breastfeeding since the baby is born until the first six months of life, both in all health services and in the community. It is needed to evaluate the Exclusive Breastfeeding policy to assess the achievement of the target and overcome the obstacles in the practice of that policy.

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Declaration of competing interest

The authors declare that there are no competing interests associated with the manuscript.

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Appendix A. Supplementary data

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References

- [1] WHO and UNICEF, Global Breastfeeding Scorecard, 2017: Tracking progress for breastfeeding policies and programmes, 2017.
- [2] R.I. Kemenkes, Laporan Nasional Riset Kesehatan Dasar 2018, "Badan Penelitian dan Pengembangan Kesehatan, Jakarta, 2019.
- [3] WHO, Indicators for Assessing Infant and Young Child Feeding Practices Part I, 2008.
- [4] T.A.E. Permatasari, et al., Exclusive breastfeeding intention among pregnant women, *Kesmas* 12 (3) (2018) 134–141, <https://doi.org/10.21109/kesmas.v12i3.1446>.
- [5] T.A.E. Permatasari, N.W. Sudiartini, Do health workers play a role in exclusive breastfeeding among working mothers in industrial area? *J. Nutr. Sci. Vitaminol. (Tokyo)*. 66 (Supplement) (2020) S94–S98, <https://doi.org/10.3177/jnsv.66.S94>.
- [6] T.A.E. Permatasari, F. Rizqiya, W. Kusumaningati, I.I. Suryaalamah, Z. Hermiwahyoeni, The effect of nutrition and reproductive health education of pregnant women in Indonesia using quasi experimental study, *BMC Pregnancy Childbirth* 21 (1) (2021) 180, <https://doi.org/10.1186/s12884-021-03676-x>.
- [7] J. Khan, L. Vesel, R. Bahl, J.C. Martines, Timing of breastfeeding initiation and exclusivity of breastfeeding during the first month of life: effects on neonatal mortality and morbidity—a systematic review and meta-analysis, *Matern. Child Health J.* 19 (3) (2015) 468–479, <https://doi.org/10.1007/s10995-014-1526-8>.
- [8] V. Oktaria, et al., Nutritional status, exclusive breastfeeding and management of acute respiratory illness and diarrhea in the first 6 months of life in infants from two regions of Indonesia, *BMC Pediatr.* 17 (1) (Dec. 2017) 211, <https://doi.org/10.1186/s12887-017-0966-x>.
- [9] L. Nurbaiti, N. Taslim, M. Hatta, A. Bukhari, Evaluation of feeding practices for infants and children (PMBA) for stunting children in Lombok, *Ann. Rom. Soc. Cell Biol.* 25 (1) (2021) 2019–2027.
- [10] I. Iskandar, et al., Gene prolactine receptor (PRLR) and signal transducer and activator of transcription 5 (STAT5) on milk production, *Med. Clínica Práctica* 4 (2021) 100223, <https://doi.org/10.1016/j.mcpsp.2021.100223>.
- [11] I. Handayani, et al., Contribution of NRAMPT gene expression and protein level in pulmonary and latent TB infection in Indonesia, *J. Appl. Pharmaceut. Sci.* 11 (8) (2021) 17–21, <https://doi.org/10.7324/JAPS.2021.110803>.
- [12] T.D. Wahyuni, M. Hatta, A. Bukhari, A. Santoso, M.N. Massi, Increasing Natural Resistance Associated Macrophage Protein 1 serum level after Miana treatment in BALB/c induced Klebsiella pneumoniae experimental research, *Ann. Med. Surg.* 65 (2021) 102262, <https://doi.org/10.1016/j.amsu.2021.102262>.
- [13] R. Dwiyantri et al., "Association of typhoid fever severity with polymorphisms NOD2, VDR and NRAMPT genes in endemic area, Indonesia," *J. Med. Sci.*, vol. 17, no. 3, pp. 133–139, Mar. 2017, doi: 10.3923/jms.2017.133.139.
- [14] R.V. Medapati, S. Suvvari, S. Godi, P. Gangiseti, NRAMPT and VDR gene polymorphisms in susceptibility to pulmonary tuberculosis among Andhra Pradesh population in India: a case-control study, *BMC Pulm. Med.* 17 (1) (2017) 89, <https://doi.org/10.1186/s12890-017-0431-5>.
- [15] Dinkes DKI Jakarta, DKI Jakarta Province Health Profile 2018, *Profil Kesehatan Provinsi DKI Jakarta Tahun 2018*, 2018.
- [16] S. Lemeshow, D.W. Hosmer, J. Klar, S.K. Lwanga, *Adequacy of Sample Size in Health Studies*, World Health Organization, 1990.
- [17] P.R.S. Banjarnahor, S.P. Rahardjo, E. Savitri, M. Hatta, F.X.B. Suhadi, A. Bukhari, The correlation between tmn and YY1 and P53 mRNA expression in nasopharyngeal cancer, *Biomed. Pharmacol. J.* 14 (1) (2021) 105–111, <https://doi.org/10.13005/bpj/2104>.
- [18] I. Farid, M. Hatta, S. Rauf, and N. Abdullah, "Relationship between age, parity and history of chronic cough as a risk factor for uterine prolapse based on expression of mRNA and soluble protein estrogen receptor alpha (ER α) and estrogen receptor beta (ER β)," *Gazz. Med. Ital. Arch. Sci. Med.*, vol. 180, 2021, doi: 10.23736/S0393-3660.19.04193-7.
- [19] M.F. Farid, N. Abdullah, M. Tahir, M. Hatta, S. Rauf, Budu, Correlation between mRNA tissue expression and serum concentration of Cyr61 in adenomyosis, *Gazz. Med. Ital. Arch. Sci. Med.* 180 (1–2) (2021) 48–53, <https://doi.org/10.23736/S0393-3660.19.04234-7>.
- [20] Purnamanita, et al., The effectiveness of triamcinolone injection on risk of postoperative operations with the conjunctiva autograft technique and its association with change of VEGF mRNA Expression, *Biomed. Pharmacol. J.* 13 (2) (2020) 543–549, <https://doi.org/10.13005/bpj/1916>.
- [21] Farsida, et al., Relationship between expression mRNA gene Treg, Treg, CD4+, and CD8+ protein levels with TST in tuberculosis children: a nested case-control, *Ann. Med. Surg.* 61 (2020) 44–47, <https://doi.org/10.1016/j.amsu.2020.12.011>.
- [22] Farsida, et al., The correlation of Foxp3 + gene and regulatory T cells with scar BCG formation among children with Tuberculosis, *J. Clin. Tuberc. other Mycobact. Dis.* 21 (2020) 100202, <https://doi.org/10.1016/j.jctube.2020.100202>.
- [23] U.K. Amsyah, M. Hatta, H. Tahir, G. Alam, A. Asmawati, Expression of IL-10 in A. actinomycetemcomitans induced rat treated by Purple Miana Leaves, *Biomed. Pharmacol. J.* 12 (4) (2019) 2099–2104, <https://doi.org/10.13005/bpj/1845>.
- [24] M.H. Oley, et al., Effects of hyperbaric oxygen therapy on vascular endothelial growth factor protein and mRNA in crush injury patients: a randomized controlled trial study, *Int. J. Surg. Open* 29 (2021) 33–39, <https://doi.org/10.1016/j.ijso.2021.01.003>.
- [25] R. Sitepu K, et al., Relation of mRNA expression of hMLH1 and p53 gene in colorectal carcinoma patients' faecal, *Int. J. Pharmacol. Res. Supplement* (1) (2020) 898–904, <https://doi.org/10.31838/ijpr/2020.SP1.138>.
- [26] T. Tommy, et al., Effect of folic acid on serum homocysteine, TNF α , IL-10, and HMGB1 gene expression in head injury model, *Ann. Med. Surg.* 65 (May 2021) 102273, <https://doi.org/10.1016/j.amsu.2021.102273>.
- [27] M.C. Oley, et al., Hyperbaric oxygen therapy ameliorates the symptoms of post-concussion syndrome by inhibiting MMP-9 activity: a randomized controlled trial in Indonesia, *F1000Research* 10 (2021) 501, <https://doi.org/10.12688/f1000research.53289.1>.
- [28] R. Karnina, et al., Systemic lidocaine administration influences NF- κ B gene expression, NF- κ B and TNF- α protein levels on BALB/c mice with musculoskeletal

- injury, *Ann. Med. Surg.* 69 (Sep. 2021) 102660, <https://doi.org/10.1016/j.amsu.2021.102660>.
- [29] H. Agustín, et al., Analysis of CD4 and CD8 expression in multidrug-resistant tuberculosis infection with diabetes mellitus: an experimental study in mice, *Ann. Med. Surg.* 68 (Aug. 2021) 102596, <https://doi.org/10.1016/j.amsu.2021.102596>.
- [30] R. Marlina, M. Hatta, E. Sridiana, I. Djaharuddin, I. Patellongi, F. Murtiani, The effect of miana (*coleus scutellarioides* [L]) on vascular endothelial growth factor expression in Balb/C mice infected with mycobacterium tuberculosis, *Biomed. Pharmacol. J.* 14 (2) (2021) 525–532, <https://doi.org/10.13005/bpj/2154>.
- [31] I.S. Wardani, et al., Serum vitamin D receptor and High Mobility Group Box-1 (HMGB1) levels in HIV-infected patients with different immunodeficiency status: a cross-sectional study, *Ann. Med. Surg.* 63 (Mar. 2021) 102174, <https://doi.org/10.1016/j.amsu.2021.02.020>.
- [32] R. Agha, A. Abdall-Razak, E. Crossley, N. Dowlut, C. Iosifidis, G. Mathew, STROCSS 2019 Guideline: strengthening the reporting of cohort studies in surgery, *Int. J. Surg.* 72 (Dec. 2019) 156–165, <https://doi.org/10.1016/j.ijsu.2019.11.002>.
- [33] W. Ben-Selma, H. Harizi, M. Letaief, J. Boukadida, Age- and gender-specific effects on NRAMP1 gene polymorphisms and risk of the development of active tuberculosis in Tunisian populations, *Int. J. Infect. Dis.* 16 (7) (2012) e543–e550, <https://doi.org/10.1016/j.ijid.2011.11.016>.
- [34] T.H.M. Ottenhoff, F.A.W. Verreck, E.G.R. Lichtenauer-Kaligis, M.A. Hoeve, O. Sanal, J.T. van Dissel, Genetics, cytokines and human infectious disease: lessons from weakly pathogenic mycobacteria and salmonellae, *Nat. Genet.* 32 (1) (Sep. 2002) 97–105, <https://doi.org/10.1038/ng0902-97>.
- [35] S. Malik, et al., Alleles of the NRAMP1 gene are risk factors for pediatric tuberculosis disease, *Proc. Natl. Acad. Sci. U. S. A.* 102 (34) (Aug. 2005) 12183–12188, <https://doi.org/10.1073/pnas.0503368102>.
- [36] E. Sahiratmadja, et al., Iron deficiency and NRAMP1 polymorphisms (INT4, D543N and 3'UTR) do not contribute to severity of anaemia in tuberculosis in the Indonesian population, *Br. J. Nutr.* 98 (4) (2007) 684–690, <https://doi.org/10.1017/S0007114507742691>.
- [37] A.A. Badawy, R.S. Yahya, S.I. Awad, G.A. Al-Sawah, N.A. Kizilbash, Relationship between NRAMP1 gene polymorphism and efficacy of BCG vaccine in a helminth-infected population, *Genet. Mol. Res.* 12 (3) (Jan. 2013) 3048–3056, <https://doi.org/10.4238/2013.January.30.4>.
- [38] N.T. Cacho, R.M. Lawrence, Innate immunity and breast milk, *Front. Immunol.* 8 (2017), <https://doi.org/10.3389/fimmu.2017.00584>. May.
- [39] D. Radzioch, I. Kramnik, E. Skamene, Molecular mechanisms of natural resistance to mycobacterial infections, *Circ. Shock* 44 (3) (Nov. 1994) 115–120.
- [40] M.J. Blaser, S. Falkow, What are the consequences of the disappearing human microbiota? *Nat. Rev. Microbiol.* 7 (12) (Dec. 2009) 887–894, <https://doi.org/10.1038/nrmicro2245>. England.
- [41] T.F. Murphy, L.O. Bakaletz, P.R. Smeesters, Microbial interactions in the respiratory tract, *Pediatr. Infect. Dis. J.* 28 (10 Suppl) (Oct. 2009) S121–S126, <https://doi.org/10.1097/INF.0b013e3181b6d7ec>.
- [42] R. Angraini, 3'UTR polymorphism of NRAMP1 gene and susceptibility to lung tuberculosis among patients and nurses in Surabaya, Indonesia, *Indones. J. Trop. Infect. Dis.* 1 (1) (2010) 17, <https://doi.org/10.20473/ijtid.v1i1.3717>.
- [43] M. Fernández-Mestre, Á. Villasmil, H. Takiff, Z. Fuentes Alcalá, NRAMP1 and VDR gene polymorphisms in susceptibility to tuberculosis in Venezuelan population, *Dis. Markers* 2015 (2015), <https://doi.org/10.1155/2015/860628>.
- [44] W. Liu, et al., VDR and NRAMP1 gene polymorphisms in susceptibility to pulmonary tuberculosis among the Chinese Han population: a case-control study, *Int. J. Tuberc. Lung Dis. Off. J. Int. Union against Tuberc. Lung Dis.* 8 (4) (Apr. 2004) 428–434.
- [45] Q. Meilang et al., "Polymorphisms in the SLC11A1 gene and tuberculosis risk: a meta-analysis update.," *Int. J. Tuberc. Lung Dis. Off. J. Int. Union against Tuberc. Lung Dis.*, vol. 16, no. 4, pp. 437–446, Apr. 2012, doi: 10.5588/ijtld.10.0743.
- [46] M. Hatta, Ratnawati, M. Tanaka, J. Ito, T. Shirakawa, and M. Kawabata, "NRAMP1/SLC11A1 gene polymorphisms and host susceptibility to Mycobacterium tuberculosis and M. leprae in South Sulawesi, Indonesia," *Southeast Asian J. Trop. Med. Publ. Health*, vol. 41, no. 2, pp. 386–394, Mar. 2010.
- [47] S. Gruenheid, E. Pinner, M. Desjardins, P. Gros, Natural resistance to infection with intracellular pathogens: the Nramp1 protein is recruited to the membrane of the phagosome, *J. Exp. Med.* 185 (4) (1997) 717–730, <https://doi.org/10.1084/jem.185.4.717>.
- [48] M.G. Ramadhan, Pengaruh polimorfisme NRAMP1 dengan tuberkulosis paru di Kab Timor Tengah Selatan Nusa Tenggara Timur Indonesia, " Universitas Indonesia, 2014.