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Effect of micronutrient deficiency on protozoal infection in stunting toddler

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ABSTRAK

Latar Belakang: *Stunting merupakan gangguan tumbuh kembang yang terjadi akibat kurangnya asupan gizi pada balita dalam jangka waktu lama, sehingga mengakibatkan kondisi kurang gizi, diantaranya zat besi dan seng. Hal ini akan mempengaruhi kekebalan balita dan membuatnya lebih rentan terhadap penyakit infeksi, seperti protozoa usus. Saluran pencernaan yang terinfeksi akan mengganggu penyerapan gizi dan mengurangi nafsu makan sehingga menyebabkan infeksi tidak kunjung sembuh dan berlangsung kronis. Infeksi kronis tersebut akan memicu terbentuknya sitokin pro-inflamasi yang mempengaruhi pertumbuhan tulang sehingga menghambat pertumbuhan balita dan menyebabkan stunting.*
Tujuan: *Menganalisis korelasi asupan mikronutrien zat besi dan seng dengan infeksi protozoa usus pada balita stunting di Kabupaten Jember.*
Metode: *Jenis penelitian analitik observasional dengan studi desain cross sectional. Subjek penelitian sebanyak 568 balita stunting berusia 0-59 bulan di Kabupaten Jember. Asupan zat besi dan seng dikumpulkan melalui wawancara food-recall 2x24 jam dan diolah menggunakan aplikasi Nutrisurvey. Metode direct smear dan modifikasi Ziehl Neelsen digunakan untuk mengidentifikasi status infeksi protozoa pada sampel feses balita. Uji korelasi Spearman dipilih sebagai metode analisis data.*
Hasil: *Asupan mikronutrien pada balita stunting di Kabupaten Jember sebagian besar tergolong kurang, dengan rerata asupan zat besi sebesar 2,56±3,06 mg dan rerata asupan seng sebesar 1,8±1,47 mg. Hasil menunjukkan bahwa prevalensi infeksi protozoa usus pada balita stunting di penelitian ini sebesar 15,7% dengan spesies yang teridentifikasi meliputi Entamoba histolytica (3,3%), Giardia lamblia (2,6%), Cryptosporidium parvum (3,2%), Blastocystis hominis (5,3%), dan infeksi campuran (1,2%). Hasil analisis statistik menunjukkan tidak ada korelasi yang signifikan antara asupan mikronutrien zat besi (p=0,91; r=0,005) dan seng (p=0,76; r=0,013) dengan infeksi protozoa usus pada balita stunting di Kabupaten Jember.*
Kesimpulan: *Berdasarkan hasil penelitian, infeksi protozoa bisa diakibatkan oleh berbagai faktor seperti pola asuh dan sosial ekonomi keluarga. Namun, pemahaman tentang gizi harus tetap ditingkatkan serta perlunya pemberian suplementasi mikronutrien tambahan untuk mengurangi prevalensi gizi kurang pada balita*

KATA KUNCI*: defisiensi; protozoa usus; seng; stunting; zat besi*

ABSTRACT

Background: *Stunting is a developmental disorder caused by chronic inadequate nutritional intake in toddlers, leading to deficiencies in various nutrients, including iron and zinc. This can weaken toddlers' immunity, making them more vulnerable to infectious diseases, such as intestinal protozoa. An infected digestive tract can interfere with absorbing nutrients and reduce appetite, causing the infection to persist and become chronic. Chronic infection triggers the formation of pro-inflammatory cytokines that affect bone growth, hindering growth and causing stunting.*

Objectives: *This study investigates the correlation between iron and zinc micronutrient intake and intestinal protozoan infection in stunting toddlers in Jember District.*

Methods: *The research is observational and cross-sectional, with 568 stunting toddlers aged 0-59 months in Jember District, East Java as study subjects. Iron and zinc intake were collected through 2x24-hour food-recall questionnaire interview and processed using the Nutrisurvey software. Protozoan infection status in toddler faeces samples was identified using direct smear and modified Ziehl-Neelsen methods. The data analysis method chosen was the Spearman correlation test.*

Results: *The study found that stunted toddlers in Jember District had mostly deficient micronutrient intake. The average iron intake is 2.56±3.06 mg and the average zinc intake is 1.8±1.47 mg. Protozoan infection was found in this study has a prevalence of 15.7%, with identified species including Entamoeba histolytica (3.3%), Giardia lamblia (2.6%), Cryptosporidium parvum (3.2%), Blastocystis hominis (5.3%), and mixed infections (1.2%). The statistical analysis confirms that there is no significant correlation between the intake of micronutrients iron (p=0.91;r=0.005) and zinc (p=0.76;r=0.013) and intestinal protozoan infection in stunting toddlers in Jember District.*

Conclusions: *Based on the data, protozoan infection may be caused by multiple factors, such as parenting practices and family socioeconomics. However, improving nutrition by education and giving additional supplementation are crucial to reduce the prevalence of malnutrition in toddlers.*

KEYWORD*:* *deficiency; iron; intestinal protozoa; stunting; zinc*

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INTRODUCTION

Stunting is a chronic nutritional issue caused by prolonged inadequate nutritional intake due to feeding practices that fail to meet children's nutritional needs (1). The 2018 Riskesdas data shows that in Indonesia, 30.8% of children suffer from stunting, with 19.3% being short and 11.5% being very short (33). According to the Study of Indonesian Nutritional Status 2022, the stunting rate in East Java Province was reported to be 19.2%, with Jember District having the highest prevalence at 34.9% (34).

The high rate of stunting is caused by complex and overlapping factors, including low socioeconomic status, poor personal and environmental sanitation, lack of knowledge about breastfeeding and balanced nutrition for children under five (2). Furthermore, inadequate food intake can lead to toddlers experiencing deficiencies in various nutrients. Macronutrients, including carbohydrates, proteins, and fats, as well as micronutrients, such as vitamins and minerals, are essential for the growth and development of toddlers (35). Research by Zogara et al. (2020) has shown that stunting children have a lower intake of iron and zinc compared to non-stunting children (3). Kundarwati et al. (2022) found a significant association between iron intake (p value 0.005) and zinc intake (p value 0.001) and the incidence of developmental delay in children aged 1-3 years (4).

Iron and zinc deficiencies are linked to weakened immune systems in children, making them more vulnerable to infectious diseases, including intestinal parasitic infections due to their underdeveloped immune systems (5). This statement is supported by the well-established findings of Fançony et al. (2022), who demonstrated that children with zinc deficiency had a 1.6 times higher risk of intestinal parasite infection (6). Research has consistently shown that a deficiency in this micronutrient significantly contributes to the morbidity and mortality of infectious diseases (7). Protozoa that infect the intestine extract nutrients from the intestinal mucosa as their energy source. This results in competition for nutrients between the host and the parasite (8). The parasitic infection can cause discomfort, leading to decreased appetite and reduced iron and zinc intake. These factors can contribute to the persistence and chronicity of the infection (9). Long-lasting and repeated infections will cause the formation of pro-inflammatory cytokines which can inhibit endochondral ossification by reducing proliferation of chondrocyte thereby suppressing child growth (10). These condition indicate a relationship between protozoan infection and stunting.

Study by Yoseph and Beyene (2020) about the intestinal parasitic infections in Ethiopian toddlers with a confident tone that highlights the importance of this research. The study unequivocally demonstrated that 48.7% of the children were infected. Entamoeba histolytica and Giardia lamblia were the most commonly found protozoan species (8). It is worth noting that in Indonesia, intestinal protozoa infections affect approximately 10-18% of the population (11). This study confidently investigates the correlation between iron and zinc micronutrient intake and intestinal protozoan infection in stunting toddlers in Jember District, East Java.

MATERIALS AND METHODS

This observational analytic study utilized a cross-sectional design and was conducted from June 2023 to January 2024 in four sub-districts: Kaliwates, Rambipuji, Sukorambi, and Panti. Toddlers with stunting were identified using the WHO growth chart from the KMS book (a book that contains the growth curves to record and monitor the toddler's development every month). The sample selection method employed was simple random sample, and we included 568 stunting toddler aged 0-59 months as our respondents. Respondents were selected using the inclusion criteria of body lenght or height-for-age index <-2 standard deviations of the child growth curve. Toddlers who consumed iron or zinc supplement and anti-protozoal drug were excluded.

Micronutrients intake data, specifically iron and zinc, was collected from interview with parents to fill out the 2x24-hour food recall questionnaire. Interviews were conducted asking the food and drinks were consumed during the last 24 hours (36). The questionnaire responses were were converted into grams using reference to the Food Photo Book (Porsimetri) of the Indonesian Ministry of Health (39). The data was processed using Nutrisurvey software to determine the fulfillment level of iron and zinc intake. The result categories into deficit (<77% of AKG) and sufficient (≥77% AKG) according to the recommendations of the Nutritional Adequacy Score (37).

Fecal examination using direct smear and modified Ziehl Neelsen method for detecting cyst or trophozoites form of Entamoeba histolytica, Giardia lamblia, Cryptosporidium parvum, and Blastocystis hominis. The examination was conducted at the Parasitology Laboratory of The Faculty of Medicine, University of Jember. Equipment and materials needed for examination including fecal pot, marker, label, centrifuge, stirrer, micropipette, vortex, bunsen burner, microscope, object glass, cover glass, distilled water, 1% lugol solution, Ziehl Neelsen solution, and methanol (38).

The correlation between the intake of iron and zinc, nutritional status, and infection status for stunting toddlers was analyzed using the Spearman test in SPSS. The data analysis method chosen was the Spearman correlation test because Spearman correlation test is used to identify relationships or test the significance of associative hypotheses with ordinal data that are not normally distributed (40). The study's purpose, benefits, and procedures were explained to parents before the study, and they were asked to fill out an informed consent form. The study was undertaken after obtaining approval from the

Health Research Ethics Committee within the Faculty of Medicine, University of Jember (5154/UN25.1.10.2/KE/2023).

RESULTS AND DISCUSSIONS

Iron and zinc intake were measured using a food-recall 24-hour questionnaire and processed using Nutrisurvey software. The results from 568 respondents showed that the average of iron intake was 2.56±3.06 mg, with a minimum intake of 0.05 mg and a maximum intake of 39.2 mg. Similarly, the average of zinc intake was 1.8±1.47 mg, with a minimum intake of 0.1 mg and a maximum intake of 19 mg. These findings demonstrate a clear understanding of the micronutrient intake of the respondents, as shown in **Table 1** below.

Table 1. Distribution of micronutrient intake

Micronutrient	Micronutrient Intake (mg/day)			
	Mean	SD	Minimum	Maximum
Iron (mg)	2.56	3.06	0.05	39.2
Zinc (mg)	1.8	1.47	0.1	19

The study found that 15.7% of the 568 toddlers identified positive for protozoan infection through fecal examination. The protozoan species found included Blastocystis hominis, Cryptosporidium parvum, Entamoeba histolytica, Giardia lamblia, and mixed infections. Blastocystis hominis was the most prevalent species, with an incidence rate of 5.3% (**Table 2**).

Table 2. Characteristics of respondents and protozoa infection

Subject Characteristics	Frequency	Percentage (%)
Toddler Gender		
Male	273	48
Female	295	52
Toddler Age (month)		
0-5	5	0.9
6-11	22	3.9
12-36	336	59.1
37-59	205	36
Household Income		
Low	422	74.3
Moderate	146	25.7
Protozoa Infection		
Positive	4	4,8
Negative	79	95,2
Protozoa Species		
Entamoeba histolytica	19	3.3
Cryptosporidium parvum	18	3.2
Giardia lamblia	15	2.6
Blastocystis hominis	30	5.3
Mixed Infection	7	1.2

The statistical analysis shows that the correlation between the two variables is very weak (correlation coefficient values of 0.005 and 0.013, respectively) and there is no significant correlation between iron or zinc intake with protozoan infection (p-value 0.91 and 0.76). The study unequivocally demonstrates that stunting toddlers in Jember District have an average iron intake from the food they consumed of 2.56 mg, indicating that the iron intake of toddlers is still considered insufficient when compared to the recommended daily allowance. The low iron intake is a direct result of inadequate breastfeeding, poor quality complementary foods, and a lack of iron-rich foods in the diets of stunting toddlers in Jember District. It is important to note that socioeconomic factors can also significantly

Tabel 3. Correlation of micronutrient intake with incidence of protozoa infection

Variables	Intestinal Protozoa Infection	
	Significance	***Correlation Coefficient***
Iron intake	0.91	0.005
Zinc intake	0.76	0.013

impact toddlers' iron intake. Families with lower incomes may face difficulties in affording iron-rich foods, such as red meat, seafood, vegetables, and fruits.

The brain and physical growth of toddlers require a high energy supply and metabolism, which depend on the availability of cellular oxygen. It is crucial to ensure that the child receives adequate iron intake to support heme, Hb, and erythrocyte synthesis, which are essential for maintaining cellular energy metabolism. Iron deficiency can lead to impaired cognitive abilities and linear body growth. Therefore, it is important to prioritize iron intake to support the child's growth and development. Iron deficiency affects the levels of the appetite-regulating hormone, ghrelin, causing a decrease in the secretion of Insulin-Like Growth Factor 1 (IGF-1) and Growth Hormone (GH), causing growth delays in children (12).

The study's findings support those of Aridiyah et al. (2016), who reported that iron intake levels in toddlers have an impact on the incidence of stunting. The study found that 60% of toddlers in urban areas and 80.6% in rural areas had insufficient iron intake (13). Kundarwati et al.'s (2022) study demonstrates a significant correlation between iron intake and stunting (4). Research shows that children who suffer from anemia due to low iron consumption are 1.4 times more likely of experience stunting growth compared to non-anemic children (14). Toddlers must have sufficient iron intake to avoid iron deficiency, which can negatively impact their growth and nutritional status.

Zinc is an important mineral which expresses genes, stabilizes cells, and enzymatic functions (15). It is well-established that a lack zinc could affect childrens's growth and develompent and increase disease susceptibility (16). Low levels of zinc hinder the effects of Growth Hormone (GH) metabolites, causing a decrease in Insulin Like Growth Factor 1 (IGF-1) synthesis and secretion. As the results, the growth of long bone epiphysis is reduced, leading to growth disorders or stunting in children. This research is in line with the study of Flora et al. (2021), that demonstrates that children with zinc deficiency are nearly nine times more likely to have low serum IGF-1 levels than those with adequate zinc levels (17).

The study results indicate that stunting toddlers have an average zinc intake of 1.8 mg, which is considered insufficient when compared to the recommended daily zinc intake for toddlers. Toddlers who have a zinc insufficient may have increased excretion of zinc (such as due to diarrhea), malabsorption, and insufficient availability and intake of zinc in their food (18). A diet high in phytate and fiber, particularly from grains, can impede the body's absorption of zinc (19). It is important to note that caregivers or parents often offer fiber-rich foods to toddlers without providing additional sources of zinc, leading to disrupted zinc absorption, and resulting in the majority of toddlers being categorized as zinc deficient. The study's findings support Damayanti et al.'s (2016) research, which showed a higher prevalence of inadequate zinc sufficiency levels among stunting toddlers (60%). Furthermore, the study found that toddlers with insufficient zinc levels were 7.8 times increased risk of stunting compared to those who had adequate levels of zinc (20).

The study found that approximately 15.3% of the respondents had intestinal protozoa infection. This high prevalence of intestinal protozoal infection in toddlers is consistent with the study by Yoseph and Beyene (2020), which found that 48.7% of protozoal infections occurred in toddlers in Ethiopia (8). Young children are highly susceptible to contracting this infection due to their developing immune systems and exploratory behavior (21). Additionally, there is a strong correlation between the number of toddlers who are stunted and the incidence of intestinal protozoal infections. This is corroborated by research by Alemneh et al. (2017), which discovered that 69% of stunting toddlers had protozoan infections, a proportion higher than that

of non-stunting toddlers (22). Stunting can lead to immune system disorders, which may indirectly impact on the intestinal mucosa's function and structure (21).

According to the study, B. hominis was the most common protozoan species, infecting 30 respondents. These results demonstrate the importance of maintaining proper sanitation and water quality to prevent the spread of this infection. This is supported by Khaled et al. (2020) finding that 80.4% of respondents had been infected with B. hominis. The prevalence of this species is linked with the quality of the drinking water and hygienic conditions. This study confidently identifies clinical signs including abdominal pain and diarrhea (23). Furthermore, it confidently reports the presence of Giardia lamblia and Entamoeba histolytica infections, with the highest rates occurring in children under 10 years old, reaching up to 30% (24). Cryptosporidium parvum causes opportunistic infections, especially in immunocompromised children. The clinical manifestations of this infection are often related with the patient's immunological condition, and it can frequently be asymptomatic (25).

Innate and adaptive immunity both heavily depend on iron (26). For the innate immunity, it supports myeloid cell function by regulating the activity of transcription factors and enzymes to produce antimicrobial effectors, such as nitric oxide (NO) and hydroxyl radicals (HO). In the acquired immune system, iron is a crucial growth factor for the expansion of lymphocyte subset clones, including T cells, Natural Killer cells (NK cells), and B cells (27). Parasites entering the host body trigger the formation of Th2 cells, which in turn stimulate B cells to secrete IgE as a response to the infection (28). It is important to keep in mind that iron deficiency in children can impair their immunity, increasing their susceptibility to infections (26).

The study findings that there is no significant correlation between iron intake and protozoan infection incidence in stunting toddlers. The non-significant association in this study can be explained by the use of a population of stunting toddlers who are prone to experiencing iron deficiency. The only differing factor was the exposure to the risk of protozoan infection. Differences in the degree of infection between species can cause false negative results in the examination. This is because of the prolonged infection period and low infection degree, which can result in protozoa not being identified in examination preparations. This can lead to many findings of toddlers with insufficient iron intake but no intestinal protozoan infection, which can affect the statistical results. Furthermore, It is important to consider the other factors, such as poor environmental sanitation, that have a greater impact on protozoan infection in stunting toddlers. In Jember District, poor sanitation practices have resulted in a significant number of toddlers suffering from stunting growth. It is crucial that families stop disposing of waste and defecating in the river, which is also used as a daily water source. This suggests a high transmission of such infections. Septian et al. (2023) found a significant relationship (p-value = 0.03) between the incidence of intestinal protozoa infection and environmental sanitation in Suger Kidul Village, Jember (29).

The findings of this study are in line with a study conducted by Ulayya et al's (2018) study on children aged 2-5 years found no significant relationship between intestinal parasitic infections and iron intake in toddlers. The researchers attributed the lack of association to the age factor, as the incidence of infection decreases with age. Furthermore, the geographical conditions during the data collection, which took place in the dry season, may have influenced the incidence of infection, resulting in a notably low occurrence of parasitic infections (28).

Zinc significantly affects the activity of hormones produced by the thymus gland, specifically thymulin, which regulates the maturation process of T cells (30). Furthermore, via raising IL-2 and IFN-γ levels, zinc plays a critical role in Th1 cells differentiation and response process ((26). Therefore, zinc deficiency can lead to thymic atrophy, lymphopenia, reduced IL-2, and impaired NK cell activity. Bolick et al. (2014) clearly demonstrated significant changes in the number of CD8 and CD4 cells in the ileum of mice fed a low zinc diet. These changes were due to thymic atrophy and impaired T cell differentiation (31). Additionally, zinc deficiency has a profound impact on epithelial cell homeostasis, leading to the loss of lymphoid

tissue and significant changes in the gastrointestinal microbiota (26). This impacts the immune system, increasing susceptibility and severity of infectious diseases, including intestinal protozoan infections (32).

The study findings that there is no significant correlation between zinc intake and protozoan infection incidence in stunting toddlers. The lack of a significant correlation between zinc micronutrient intake and protozoan infection in this study may be attributed to the influence of other factors. One such factor that strongly affects the incidence of intestinal protozoan infection is family socio economics. This is in line with the study of Tegen et al. (2020), which indicate that individuals from low socioeconomic backgrounds are 1.64 times more likely to contract intestinal protozoa infections (24). Fauziah et al. (2022) found that low socioeconomic status is linked to a higher incidence of protozoan infection. This is due to factors such as the family's inability to provide nutritious food, lack of understanding about child nutrition, poor sanitation facilities, and lack of access to health facilities (21). The study revealed that most toddlers came from families with low socioeconomic status, with a monthly income of less than Rp 2,355,669.91 (UMK Jember), which affected the fulfilment of zinc intake for stunting toddlers (**Table 2**).

Rahmi (2021) found no significant correlation between zinc intake and intestinal protozoa infection in his study of elementary school students in the Public Health Center Air Beliti's working area showed 31.2% of respondents with insufficient zinc intake had parasitic infections. In line with this study which showed no significant association between zinc intake and intestinal protozoan infection caused by the data obtained lacked statistical power due to the low incidence of intestinal protozoan infection, which was not proportional to the high frequency of zinc-deficient study subjects.

This study remains limited to a cross-sectional design, which makes it uncertain to draw conclusions about causal relationships between variables. Besides that, the process of collecting dietary intake data which uses the method of measuring micronutrient levels is subjective which depends on the memory of the caregiver. Therefore measuring the blood's iron and zinc levels is essential to figuring out the levels of adequacy this elements in the body.

To reduce bias in this study, the researchers cross-checked the results of the food recall questionnaire by converting the data into grams using the Food Photo Book by the Indonesian Ministry of Health's Individual Food Consumption Survey Team. This approach allowed for a more accurate estimation of the amount of food consumed by the respondents. Additionally, the researchers ensured that the primary data regarding the stunting status of toddlers was up-to-date and remeasured if necessary.

CONCLUSIONS AND RECOMMENDATIONS

The study results indicate that there is no significant association between iron and zinc intake and intestinal protozoa infection in stunting toddlers. However, the majority of stunting toddlers have insufficient iron and zinc intake. Therefore, it is recommended that local governments raise awareness of nutrition issues through education programmes on nutrition and supplementary feeding, such us iron supplement, zinc supplement, and fortified multiple micronutrient, to lower the number of nutritional disorders in toddlers.

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