Gut environment and socioeconomic status: a study of children in urban area of Makassar

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CHAPTER SIX
SUMMARIZING DISCUSSION

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WHAT WAS ALREADY KNOWN ABOUT THE RELATIONSHIP BETWEEN CHILD HEALTH AND SOCIOECONOMIC STATUS

The rise of communicable and non-communicable diseases, together with socioeconomic health inequalities are still a problem in developing countries. Indonesia is now facing the triple burden of disease, namely, communicable diseases, non-communicable diseases, and re-emerging diseases [1]. The upsurge of Indonesian economic development is also widening the gap in the health inequality between the rich and the poor.

A large study examined development and growth in young children across socioeconomic status (SES) in four developing countries (Indonesia, India, Peru and Senegal) and reported that children from wealthiest households had higher development scores, determined by EASQ (Extended Ages and Stages Questionnaire) and better growth, indicated by LAZ (length for z-scores) than children from the poorest households, while controlling for maternal education and relevant covariates [2]. The association between SES and child health is also reported in developed countries, for example, a study in Germany showed that children from low-SES report lower quality of life and adopt less healthy lifestyle, while wealthiest children have better health [3]. In this thesis, the focus has been on studying vaccination, allergies, the microbiome and gut health in children of high and low SES in Indonesia to gain more insight into how socioeconomic discrepancy might affect certain biological processes. Therefore, in the following sections, the background to these focus areas will be sketched.

Vaccination

Tuberculosis, one of vaccine-preventable diseases, is found to be more prevalent among the poor. Despite massive efforts to prevent this disease, tuberculosis remains a significant cause of morbidity and even mortality especially in the low-income countries [4]. Until now, BCG is the only vaccine licensed for prevention of tuberculosis. Vaccine response and effectiveness has been reported to be lower in low- compared to high-income countries [5]. A comparative study on immune responses following BCG vaccination in the UK versus Malawian infants reported that immune responses induced by BCG vaccination differ in both profile and magnitude between the two settings and these might be due to factors related to maternal, nutritional and environmental factors [6]. One explanation of the weakened protection from the vaccine has been suggested to be the influence of chronic helminth infection of the mother before [7] or during pregnancy [8] which may affect the developing immune system of the child. However, a double-blind placebo-controlled trial performed in Ugandan pregnant mothers showed that anthelmintic treatment had no effect on the immune response of their infants to BCG, tetanus or measles immunization [9]. Indeed, more factors other than helminth infections that occur in similar environments might be responsible for poor BCG responses.

Allergies

The changes in lifestyle and environment, characterized by increasing sanitation, hygienic measures and urbanization in developed countries in the past decades have been linked to the increase in the prevalence of allergic disease [10, 11]. Developing countries, such as India [12] and China [13] are now also rapidly facing the allergic epidemic. With regards to

the urban and rural differences, there is strong evidence from many parts of the world reporting higher prevalence of allergic disorders in urban compared to rural areas [14-16]. Different environmental exposures along with genetic factors could account for the observed variability [17]. Environmental exposure can include exposures to microbes, parasites such as helminths, and different lifestyles [18]. Helminths have coevolved with human immune system and they have developed numerous survival strategies which can modulate the host immune system through direct secretion of excretory/secretory molecules [19] [20]. These molecules then interact with the immune system and influence it directly or indirectly, which can be through the regulation of the microbiome [21, 22]. One type of allergy that is life-threatening is venom allergy, which has mostly been studied in temperate or subtropical countries. The prevalence of Hymenoptera venom sensitization has been reported to vary from 3.66 to 41.6% [23-27], whereas prevalence of systemic allergic reactions to venoms has been estimated to be between 0.3 and 16.0% [25, 28-31]. Little information is available regarding the prevalence of venom allergies in the tropics. Several epidemiological studies in Indonesia have reported an inverse relationship between aeroallergens and helminth infections [32, 33], which spark the question whether the same relationship applies to venom allergy in Indonesia.

The microbiome

In many observational studies it has been shown that the gut microbiota affects numerous aspects of human physiology [34]. The imbalance of microbiota composition, richness or diversity, also known as dysbiosis, may induce inflammation, metabolic condition, or other pathologies [35, 36]. Microbiota diversity is a measure of how many different species exist in the community and, depending on the diversity indices, how evenly distributed they are. In the tropics, intestinal parasites, such as helminths or protozoa co-exist in the gut with gut microbiota. There are several factors that might determine gut microbiota profile in a population. Environmental factors are known to predominantly shape the composition of gut microbiota instead of genetic factors [37]. Large amount of data has been gathered by a study across ethnic groups in the United States [38] as well as studies of populations with varied ethnic origins either living in different geographical areas [39] or in the same location [40]. However, little is known about the gut microbiota profile of Indonesian children living in urban areas where socioeconomic disparities exist with the resulting differences in exposure to helminth infections, hygienic practices and diet. So far, few investigations have been conducted regarding the interaction between SES, intestinal parasites, and gut microbiota. A study in a group of indigenous people in rural Malaysia has observed that helminth colonization is associated with higher microbiome diversity and that *Trichuris trichiura* infection drives the higher abundance of *Paraprevotellacae* in this population [41]. Moreover, among this indigenous population in Malaysia, it was reported that serum zinc and iron levels were affected by helminth infection status and also associated with an abundance of specific microbial taxa. It was also mentioned that the majority of microbiota that were associated with the changes in zinc levels, belonged to *Bacteroidales* order while the predominant microbiota associated with changes in iron levels belonged to *Clostridiales* order [42]. Since helminths and intestinal microbiota share the same niche, it would be interesting to investigate their relationship in populations living in urban Indonesia with inequalities in SES.

Gut health

Several studies have established the importance of gut barrier function including intestinal permeability in gut health and development of diseases [43, 44]. The intestinal barrier is semipermeable with critical function for nutrient absorption and as a barrier against pathogens [45]. To maintain this intestinal barrier function, short chain fatty acids (SCFAs) and

other microbial metabolites, end-products resulting from fermentative metabolism of fibers or complex polysaccharides by intestinal microbiota, are needed [46-48]. Therefore, the interaction between intestinal parasitic infections, gut microbiota and intestinal cells should be investigated.

As far as we know, very little data are available on the direct effects of intestinal parasites on human gut permeability [45]. A previous study indicated that residents of a tropical country have higher level of gut permeability compared to residents living in temperate or subtropical areas suggesting the importance of environmental factors [49]. Exposure to microorganisms and parasites as well as hygienic lifestyle might contribute to this variation but further research is needed to disentangle the relationships that influence intestinal permeability, as a determinant of health disparity in populations with distinct SES.

The gap

Most studies on child health and SES have been performed by comparing health status of low- and high-income countries or by assessing rural versus urban areas within one country. However, very few studies have been conducted on the health status of children within one urban area but with different SES. In order to investigate the impact of SES on child health we conducted studies in Makassar, an urban center in Indonesia with a population living under very different socioeconomic conditions.

HOW DID OUR STUDIES ADVANCE THE FIELD?

In this thesis, the complex association between SES and several outcomes that can affect health, such as responses to BCG vaccine, venom sensitization and gut barrier function have been investigated. Here, we conducted a study in children with different socioeconomic backgrounds living in the same urban area in Makassar, Indonesia. Information regarding the presence of intestinal helminths and protozoa as well as gut microbiota characteristics were included to gain information on factors that might play a role in how SES shapes child health.

The impact of socioeconomic status on responses to BCG vaccination

Vaccines are among the most cost-effective preventions against the rise of infectious diseases. However, it is known that vaccine responses can vary across populations [50, 51]. For example, BCG vaccine has shown very distinct immunogenicity and efficacy in different parts of the world, in particular between high-income countries and low- and middle-income countries [5, 6] including Indonesia.

In **Chapter 2**, we provide evidence that both SES and nutritional status at birth determine the response to BCG vaccination measured at 10 months of age in Indonesian children. Our results showed that low-SES children have smaller BCG scar size compared to high-SES children in the same city, which is in line with another study in children from Dominican Republic [52], where lower socioeconomic index was associated with smaller BCG scar size. We went further to investigate whether exposure to helminths and/or nutritional status might be responsible for this observation.

Infections with helminths is associated with Th-2 immune response characterized by increased production of IL-4, IL-5 and IL-13 cytokines, polyclonal and specific IgE as well as eosinophilia [53, 54]. BCG vaccination induces a Th-1 type immune response and can be in an equilibrium with Th-2 type responses [55] and therefore a strong Th-2 response could be inversely associated with response to BCG. In agreement with this notion and a study conducted in Turkey [56], in **Chapter 2,** we report that the larger size of BCG scar was associated with lower IgE levels observed in high-SES infants. However, here through examining helminth infections we found that although IgE levels were higher in low- compared to high-SES mothers and their newborns, this could not be related to the presence of helminth infections in pregnant women taking part in the study. This could either be due to previous helminth infections of mothers that had an imprinted immune system towards Th-2, or contribution of other environmental factors to skewing of the immune system and elevated IgE antibodies.

As nutritional status might affect responses to BCG vaccine [52], we also assessed whether BCG scar size was influenced by z-weight-for-height at birth as a proxy of newborns' nutritional status and adiposity. We observed z-weight-for-height to be positively associated with larger size of BCG scar through the leptin pathway. Leptin has been shown to drive immune responses towards Th-1, [57, 58], for example, Mattioli et. al [57] have shown that leptin down-regulates IL-10 production by dendritic cells and drives naive T cell polarization toward Th1 phenotype. In **Chapter 2,** we found that leptin, and not adiponectin, strongly attenuated the relationship between nutritional status and BCG scar size in infants. This finding suggests that higher leptin levels at birth determine the development of larger BCG scar in vaccinated-infants. Emerging evidence shows that lower leptin levels can be associated with poor vaccine responses in the general population by reducing the differentiation of human follicular T helper cells (T_{FH}), which are a subset of T cells that facilitate B cell antibody production [59]. Thus, leptin levels might not only be important in B cell dependent vaccines but also in vaccine responses such as BCG that hav an important cell mediated efficacy against *Mycobacterium tuberculosis*. [60, 61].

In summary, the size of BCG scar as a proxy of immune response to BCG vaccination, was affected by SES and leptin levels at birth. Additionally, total IgE, partly contributed to the reduction in BCG scar size.

Bee- and wasp venom sensitization in schoolchildren with different socioeconomic status

The global magnitude of venom allergy is not completely mapped as the majority of studies have been conducted in temperate or subtropical regions of the world. Despite the high sensitization to bee and wasp venoms in Europe, most of those individual have had no systemic reactions to bee and wasp stings [62] and this could be due to cross-reactive carbohydrate determinants (CCDs, alpha 1,3-fucosylated N-glycans) [63]. Apart from true sensitization, IgE against CCD has been seen in plants and invertebrate glycoproteins and show low clinical relevance [64-66]. In areas where helminth infections are prevalent, a number of studies have shown that high levels of cross reactive IgE to allergens are not biologically functional [67]. Therefore, we aimed to gather more information on bee venom allergy in Indonesia, to assess how SES and factors associated with it, affect the prevalence of this allergic sensitization.

In **Chapter Three,** we showed that skin sensitization against bee- (14.3%) or wasp-venom (12.7%) is quite prevalent among schoolchildren in Indonesia and that the prevalence was different when SES of the children was considered. Similar to the responses against aeroallergens, SPT reactivity to bee- and wasp venom was more prevalent in high compared to

the low-SES children, while the proportion of specific IgE positivity to venom allergens was higher in the low compared to the high-SES children. Interestingly, the sensitizations to Hymenoptera venoms appeared to have poor clinical relevance as they rarely translated into clinical symptoms.

The observation of discordance between SPT and sIgE to venom allergens in our study can have two reasons. Firstly, when SPT reactivity is seen in the absence of sIgE, it might suggest that skin reactivity to Hymenoptera venom is not through IgE but might be through non-IgE mediated mechanisms [68, 69] since venom components such as peptide-401 may provoke mast cell degranulation [70] and directly result in a positive skin reaction in some children [71]. Secondly, the lack of skin reaction with detectable sIgE, may suggest the presence of sIgE with poor function, for example cross reactive IgE, unable to induce mast cell degranulation [72, 73]. However, the low report of clinical venom allergy and SPT, might also be the result of desensitization as consequence of frequent stinging [74, 75].

Despite interesting results provided in this thesis; some limitations are worth noting. Cross sectional design used in **Chapter Three** does not allow us to determine causality and time of exposure. It is known that sensitization peaks several weeks after the sting and subsides gradually. [62, 76]. This chapter examined past responses to venom and current sensitization, instead of tracing responses after sensitization and sting. In addition, the involvement of helminths in the lack of skin reaction despite the presence of sIgE in the low-SES could not be discerned.

Bacterial gut microbiota and socioeconomic status

In the past decades, plethora of studies have tried to disentangle the association between environmental influences and human health through examining gut microbiota [77-79]. Several reports showed that the gut microbiota is highly variable among individuals and is determined primarily by environmental factors such as diet, hygiene level, physical activity, disease status and medication, rather than genetics [37, 80]. In **Chapter Four**, we showed that schoolchildren living in urban Makassar share a core microbiota irrespective of their SES. These core microbiotas consisted of *Bifidobacterium, Collinsella*, and multiple members of the *Lachnospiraceae* and *Ruminicoccaceae* families, but the relative abundance of these taxa varied greatly among children. SES has been found to be the main driver of differences in gut microbiota composition. Several genera of bacteria showed different abundance in children with high and low SES, including *Escherichia-Shigella, Prevotella,* and *Lactobacillus.* Similar to our study, Chong and co-workers [81] showed lower microbial diversity in wealthier children as compared to economically deprived children living in the same rural area in Malaysia. Furthermore, the study also reported that the presence of parasitic infections exerted a significant but only a small influence (explained 5% variance) on the elevated gut microbial diversity [41]. The finding in our **chapter four,** presented that helminth infections were prevalent in low-SES children, and although this was positively associated with *Olsenella, Enterorhabdus, Lactobacillus*, and *Mogibacterium* abundance, it was negatively associated with the relative abundance of *Prevotella*. Additionally, infection with protozoa was prevalent in low-SES children, and was also negatively associated with the relative abundance of *Prevotella*. In contrast to the study by Chong and co-workers [81], our results observed no clear association between helminths or protozoa with microbiota diversity. Such variations in microbiota structure at high and low-SES are most likely caused by lifestyle differences.

Diet has been considered as a major driver of the composition of bacterial gut microbiota [82]. Thus, observed variations in microbiota composition between high- and low-SES children can be reflecting variations in their diet. The high abundance of *Bifidobacterium* and

Lactobacillus among the wealthier children could be related with dairy products and probiotics consumption [83]. Meanwhile the higher relative abundance of *Prevotella* observed in the low-SES has been linked with vegetable-rich diet [81]. A study in Thai schoolchildren reported a contrasting microbiota type between urban (Bangkok) and rural (Khon Kaen) population. Using dietary intake questionnaires, they found that children in Bangkok who eat much less vegetables and fruits, tended to have the BB- (*Bifidobacterium/Bacteroides*) type microbiota while children living in rural have the P- (*Prevotella*) type microbiota [84].

The study performed in **chapter four** did not collect information on food intake, therefore we are not able to assess the influence of diet on microbiota composition

In **Chapter Five**, we showed that irrespective of helminth infection status, or SES, albendazole did not affect bacterial gut microbiota diversity. However, the composition of gut microbiota was altered 4 weeks after albendazole administration in low-SES uninfected and high-SES groups. This finding is similar to a study in Kenya where no changes were observed in gut microbiota diversity 3 weeks after albendazole treatment, but the microbiota composition was altered with a significantly decreased *Aeromonodales (Gammaproteobacteria)* [85]. It should be noted that the study did not distinguish the effect of albendazole between helminth infected and uninfected groups. In our study, following albendazole treatment, we observed changes in the relative abundance of several gut microbiota taxa, but specifically in the uninfected children of low-SES and the high-SES children. We also observed some alteration in several short chain fatty acid (SCFA)-producing bacteria in the uninfected low- and high-SES children. *Faecalibacterium* and *Prevotella* wer found to be decreased, meanwhile the relative abundance of *Lactobacillus, Streptococcus, and Clostridiales* were increased in the low-SES uninfected and high-SES children following treatment. Moreover, some alteration also observed in the uninfected group of low-SES only with a decreased relative abundance of *Dialister, Succinivibrio*, and *Rikenellaceae,* and increased relative abundance of *Bifidobacteriaceae* and *Bifidobacterium*. This indicates that these alterations were more SES-related and not associated with helminth infection status of the children. The data would suggest that albendazole has an effect on the microbiome, either directly or indirectly through influencing protozoa. However, as our study was not placebo-controlled, the changes might reflect natural variation over time in the uninfected group.

Soil-transmitted helminth infections still remain a burden especially in the tropics, and despite the high efficacy of albendazole in reducing *Ascaris lumbricoides*, it is less effective against *Trichuris trichiura* [86], even our approach by administering 400 mg albendazole given on 3 consecutive days, did not eliminate *T. trichiura* (Chapter five). The inability of albendazole to reduce helminths might be one reason why no alteration was observed in the gut microbiota composition in the helminth-infected group. Several strategies are currently being investigated to lessen the prevalence of *T. trichiura* infections, such as increasing the dose of albendazole to 800 mg [87] or by combination of ivermectin and albendazole [88]. Interestingly, a recent study has suggested that the gut microbiota composition can affect the efficacy of anthelminthic treatment on hookworms and *T. trichiura* [89]. The complexity of microbiome studies in their own right, let alone when considering the effect of helminths and treatment, indicates that better designs and larger studies are needed to fully address the question we tried to tackle.

Intestinal permeability, parasite infections and socioeconomic status

The gut barrier consists of epithelial cells and a mucus layer which create a barrier between tThe gut barrier consists of epithelial cells and a mucus layer which create a barrier between the lumen of the digestive tract and systemic circulation [90]. Under certain circumstances,

gut barrier could be impaired leading to a disrupted permeability in the nutrient absorbing areas of the intestine. Therefore intestinal integrity can be assessed by measuring the transcellular and paracellular transport of orally administered high- and low-molecular-weight sugars across the gastrointestinal tract [91]. Lactulose and mannitol, of all sugars, are most frequently used in studies of gut permeability [90] and can measure the permeability of the small intestine [92]. The Lactulose-Mannitol Ratio (LMR) is beneficial to use as both sugars are not actively absorbed from the intestine, not metabolized, and excreted unaltered in urine corresponding to the quantities absorbed [93]. Mannitol, the smaller molecule, is presumed to permeate transcellularly through the water pores of the membrane, whereas lactulose, the larger molecule, is assumed to have paracellular permeation that it transverse through the tight junctions [94]. With increased intestinal permeability, lactulose would passes through the paracellular spaces, cleared by glomerular filtration, not undergo tubular reabsorption, and later on presented in the urine at high concentrations, leading to an elevated LMR. Other markers for compromised intestinal barrier integrity are LBP and I-FABP. LBP is a protein manufactured by enterocytes and liver cells [95] in response to bacterial translocation of endotoxin (lipopolysaccharide, LPS) [96] from the intestinal microbiota to the bloodstream [97]. LBP binds LPS and presents LPS to CD14, promoting immune responses [98] that in turn activate Toll Like Receptor 4 (TLR-4). Higher LBP levels may reflect the leakage of LPS out of the gut and into the bloodstream [96]. The other marker, I-FABP is a protein which is expressed in the small intestinal epithelium. When intestinal damage or injury occurs, I-FABP is released into the circulation. Both LBP and I-FABP markers has been used as a non-invasive predictor of intestinal injury [99, 100].

In **Chapter five,** we observed different levels of gut barrier function differed between high- and low-SES schoolchildren. Both LMR and I-FABP were higher, while LBP was lower in the low-SES group. High LMR and I-FABP indicate that intestinal barrier function and integrity may be compromised in children with low-SES compared to those with high-SES.

The differences in LMR could not be attributed to the high prevalence of helminth infections in the low-SES. In previous studies, *A. lumbricoides* has been associated with high LMR [101, 102] and this is similar to what we observed in the crude analysis. Yet, when we include SES in the multivariate analysis, SES is the main driver in determining the LMR level. Moreover, after anthelminthic administration, LMR was decreased in the group of children who were uninfected at baseline. Therefore, factors other than *A. lumbricoides* might contribute to the high level of LMR, such as recurrent gastrointestinal infections [103] that are not surveyed in this study. Another study conducted in several developing countries discovered that a higher LMR was associated with low-SES, recent diarrheal illness, and enteropathogen load in infants [104]. High physical activity such as walking to school might also contribute to the higher LMR [105] in our low-SES population. Following albendazole administration, I-FABP was increased in the low-SES-infected group which might suggest that worm expulsion induces gut inflammation and therefore leads to the release of I-FABP into the circulation.

The LBP level, a marker for microbial translocation, was shown to be lower in the low-SES children, where the intestinal barrier was more compromised, compared to the high-SES group. Helminth infections are thought to be associated with elevated levels of several microbial translocation markers [106], however, in **chapter five**, we observed no differences in LBP levels between helminth infected and uninfected group. Furthermore, no alteration in LBP levels was observed after albendazole administration. Similar findings have been reported in a previous study conducted in Southern India where they observed no significant differences in LBP levels between hookworm-infected and uninfected and no alteration in LBP levels after anthelminthic administration [107]. This suggests that LBP is not linked to helminth infection. The higher LBP levels in children from higher socioeconomic backgrounds

may be consistent with other findings linking elevated LBP levels to obesity, weight gain, or a high carbohydrate and high fat diet [108-110]. However, in our study, the differences were maintained even after adjusting for zBMI, indicating that additional variables play a role. Another factor to consider is that LPS translocation occurs not only via paracellular leakage but also via transcellular transport [111]; however, the clinical significance of this transcellular pathway remains unknown. Importantly, while we observed some changes in microbiota composition following albendazole administration, the differences in gut integrity markers could not be explained by the composition of intestinal microbiota.

DIRECTION FOR FUTURE RESEARCH

This thesis contributes to our current knowledge of the extent to which SES and a number of associated factors affect BCG vaccine response, venom and aeroallergen sensitization, gut microbiome and gut permeability, which might affect child health. The necessity of larger well designed cohort studies to establish the complex relationship between intestinal parasitic infections, gut microbiota and host responses is needed. Specifically, a number of directions to focus on in the near future are indicated below:

Innovative and alternative approaches to TB prevention, including vaccine development and improving their efficacy, are necessary. Therefore, a larger scale study would be needed to investigate whether leptin and total IgE have an effect on the efficacy of BCG vaccine. The data might help develop a strategy whereby leptin levels are increased while tIgE levels are decreased in order to improve responses to BCG and any future vaccines.

Previous studies have shown the important role of gut microbiota in effectiveness of immunotherapy by modulating the tumour immuno-microenvironment [112, 113]. In the field of neglected tropical diseases, the first step has been taken towards understanding possible interaction between gut microbiota and anthelminthic drugs [89], but we also need to look at its effects on vaccine responses. Given that gut microbiota is highly dynamic and demonstrates substantial inter and intra-individual variation, larger and more in-depth studies in different geographical settings are needed. Such studies should also take into account SES and other indicators such as food intake in terms of food quality, quantity and diversity; hygienic lifestyle, and also physical activity, which could be driving the associations observed. Any intervention, would benefit from including a placebo arm in the trials, if possible.

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