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

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CHAPTER ONE
GENERAL INTRODUCTION

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Indonesia oscillates between being graded as upper middle or lower middle-income country [1], with great variation not only in the socioeconomic status of its population but also the degree of urbanization that its population faces. Socioeconomic status (SES) is a well-known determinant of health [2]. However, SES indicators often do not impact health directly, but instead serve as proxies for other determinants. Indeed, many intermediate factors have been highlighted in the relationship between SES and health outcomes, such as poor environmental conditions that lead to high exposure to microorganisms and parasites. In addition, factors such as gut microbiota composition or diversity can also be intermediates. One mechanism by which SES may put forth its effects on health is through differential activation of the immune system. Several studies have shown the maternal environment such as exposure to helminth infection during pregnancy and maternal gut microbiota might affect child immune responses soon after birth [3-5]. This in turn is thought to determine the subsequent immune responses of a child later in life against pathogens, vaccines, venoms or allergens.

In Indonesia, low socioeconomic status or residence in rural areas have been shown to increase the risk of getting helminth infections compared to those with higher SES living in urban centers [6, 7]. Poor sanitation, low degree of hygienic lifestyle, low levels of maternal education, distance from clean water source are important contributors to infection with helminths [8, 9]. Although there are reports that helminth infections can be associated with poor nutritional status [10, 11], anemia [12] and cognitive development [13], there are also reports from meta-analysis that refute this [14, 15]. There are even reports of helminth infections having protective effects against allergy or diabetes [16, 17]. “The Old Friends” hypothesis that was coined by Rook in 2003 stated that humans co-evolved with their “old friends”, among them parasitic helminths, can act as inducers of immunoregulatory circuits [18, 19]. Hygienic lifestyles and western medicine lessen the exposure to the “old-friends” and other organisms from the natural environments that humans co-evolved with. Therefore, the human immune system must have evolved to act optimally under a very different condition and any change in these conditions might alter the immune system and its downstream effects on disease outcomes.

Over the past decades, it is becoming increasingly clear that the microbiome can be important for the gut function and human health [20-26]. The gut microbiota profile is predominantly affected by environmental factors such as diet and exposure to infection [27, 28]. The gut microbiota is considered as an important factor as derived metabolites might maintain gut barrier function. Short chain fatty acids, including butyrate, which are produced by certain gut microbiota are thought to have a protective effect on the intestinal lining and thus associate with intestinal barrier health [29]. Therefore, imbalance of gut microbiota composition might aggravate gut inflammation, affect nutritional absorption and consequently lead to growth faltering in children [30-32]. Therefore, one of the ways human health is wired by the socioeconomic status, environment and lifestyle factors can be through the variation in the microbiome.

Environmental factors are thought to affect immune responses to vaccines. With respect to economic status, the response to *Bacille Calmette-Guérin* (BCG) vaccine [33] and the efficacy of rotavirus [34] are lower in the low compared to high-income countries. These differences might be due to varied exposure to infection, and host characteristics, including diet and nutritional status.

The geographical differences are also observed in the prevalence of allergic diseases [35]. In this context, most intensively studied allergic diseases are asthma, rhinitis, atopic dermatitis and food allergies. Allergic reactions to bee- and wasp-venom can be life-threatening [36], but they are less studied. Most studies of venom allergy, have so far been conducted in developed western countries. Prevalence of Hymenoptera sensitization varies among regions, with some of the studies performed in the community while others were performed in groups at risk. There is, however, very little information on the magnitude of venom allergy in tropical parts of the world. The location of Indonesia near the equator provides a warm and humid climate which is favorable for Hymenopterans like bees and wasps to live. Therefore, further information of the sensitization to Hymenoptera in low-to-middle-income countries such as Indonesia would aid understand the extent of Hymenoptera allergy not only in the temperate but also in tropical areas and also the risk factors associated with such allergic disease [6].

Scope of the thesis

The main objective of this thesis is to improve our understanding of the impact of life style and socioeconomic status on the immune response of children living in an urban area of Indonesia. We started by studying responses to a vaccine. To this end, we assessed lifestyle and socioeconomic factors that determine the response to BCG vaccination. Data on maternal infection status, demographic and socioeconomic characteristics, as well as their infant's nutritional status, total IgE and leptin levels of newborn were studied and pathway analysis was performed to understand the effect of different variables on response to BCG vaccine. Next, we conducted a study in schoolchildren with different socioeconomic backgrounds living in the same urban area and assessed their allergic response to bee and wasp venom. Baseline data on sociodemographic, anthropometric and intestinal parasitic infection status were collected. The skin prick test reactivity and reported sting-related symptoms to bee and wasp were recorded. The profile of bacterial gut microbiota and its association with environmental factors were also assessed as a function of SES. The markers of intestinal permeability and intestinal damage before and after albendazole treatment were measured to assess the association between SES, intestinal parasitic infections and markers of intestinal barrier function and taking into account the alteration of gut microbiota after treatment.

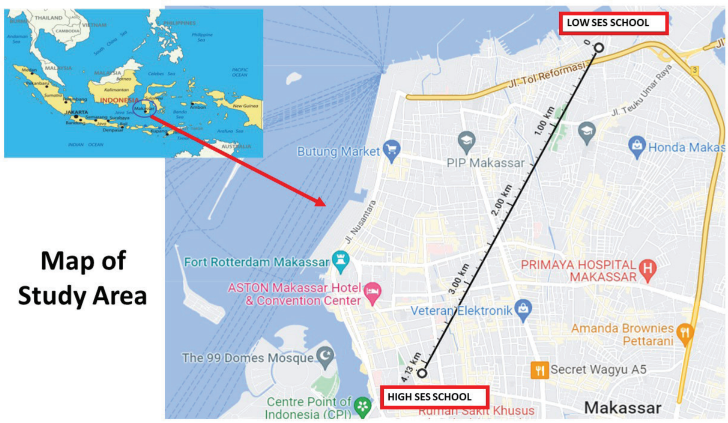
The following questions were addressed:

What is the effect of SES on the outcome of BCG vaccination in young infants?

BCG is one of the vaccines that is given early in life to infants in Indonesia. This vaccine has been known to have protective effect against meningitis and miliary tuberculosis (TB) in children [37] and also known to have non-specific effects unrelated to TB during childhood [38]. One way to measure the strength of the immune response to BCG vaccine is by assessing BCG scarification. In **Chapter Two**, differences on BCG scar size and factors contributing to the development of the scar were investigated in high and low-SES newborns. To this end, we recruited pregnant mothers at the third trimester of pregnancy and followed up the babies at birth and at 10 months of age. Pathway analysis was used to investigate factors contributing to the development of BCG scar, in particular, exposure to infection during gestational period (third trimester) and nutritional status of the baby at birth. In addition, we also explored whether the size of BCG scar is affected by nutritional status at birth through measuring adipokines.

What is the impact of SES on atopic sensitization, bacterial gut microbiota and intestinal barrier function in schoolchildren?

Study area



Map of Study Area

In **Chapter Three**, Allergic reactions and sensitization to Hymenoptera venoms and aeroallergens were compared between high and low-SES schoolchildren and the question whether helminth infection might be involved in these reactions. In addition, we also assessed the agreement between SPT and sIgE positivity to allergens.

Together, **Chapter two** and **Chapter three** investigate how SES, nutritional status and exposure to infection might affect the immune responses of Indonesian children living in an urban area.

It is important to investigate how intestinal parasites [39], gut microbiota [40], and gut permeability [41], which can influence the immune system, differ in high versus low SES schoolchildren. In **Chapter Four, an** exploratory analysis was conducted of gut microbiota of Indonesian schoolchildren living in an urban area but with distinct SES. We compared the diversity and composition of bacterial gut microbiota in children from high and low-SES schools, taking into consideration the intestinal parasitic infections and the nutritional status of the children. In **Chapter Five,** the differences in intestinal permeability (determined by LMR, lactulose-mannitol ratio) between high and low-SES children was investigated. In addition, markers of intestinal cell injury (I-FABP, intestinal fatty acid binding protein), bacterial translocation and inflammatory markers (LBP, LPS-binding protein), were measured. Furthermore, we also asked whether presence of intestinal parasites and composition of bacterial gut microbiota contributed to the association between SES and intestinal permeability.

In **Chapter Six**, the findings of this thesis are discussed and can be used as a starting point for further research to understand the complex association between SES, intestinal microorganisms and parasites, gut barrier function, and immune responses in children.

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