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

Amaruddin, A.I.

CHAPTER TWO

BCG SCAR, SOCIOECONOMIC AND NUTRITIONAL STATUS: A STUDY OF NEWBORNS IN URBAN AREA OF MAKASSAR, INDONESIA

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BCG SCAR, SOCIOECONOMIC AND NUTRITIONAL STATUS: A STUDY OF NEW-BORNS IN URBAN AREA OF MAKASSAR, INDONESIA

Aldian Irma Amaruddin[1,2#], Sitti Wahyuni[1], Firdaus Hamid[3], Maisuri T. Chalid[4], Maria Yazdanbakhsh[2], Erliyani Sartono[2]
Department of Parasitology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
Department of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
Fetomaternal Division, Department of Obstetrics and Gynaecology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[#]Corresponding author
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ABSTRACT

Objective. To investigate factors that determine the response to BCG vaccination in urban environments with respect to socioeconomic status (SES), prenatal exposure to infections or new-born's nutritional status.

Methods. The study was conducted in an urban area, in Makassar, Indonesia. At baseline, 100 mother and new-borns pair from high and low SES communities were included. Intestinal protozoa, soil transmitted helminths, total IgE, anti-Hepatitis A Virus IgG and anti-*Toxoplasma* IgG were measured to determine exposure to infections. Information on gestational age, birth weight/ height, and delivery status were collected. Weight-for-length z-score, a proxy for new-borns adiposity, was calculated. Leptin and adiponectin from cord sera were also measured. At 10 months of age, BCG scar size was measured from 59 infants. Statistical modelling was performed using multiple linear regression.

Results. Both SES and birth nutritional status shape the response towards BCG vaccination at 10 months of age. Infants born to low SES families have smaller BCG scar size compared to infants born from high SES families and total IgE contributed to the reduced scar size. On the other hand, infants born with better nutritional status were found to have bigger BCG scar size but this association was abolished by leptin levels at birth.

Conclusion. This study provides new insights into the importance of SES and leptin levels at birth on the development of BCG scar in 10 months old infants.

Keywords : BCG scar, socioeconomic status, leptin, new-borns

`Introduction

Tuberculosis (TB) is known as one of the top 10 diseases causing high mortality worldwide. In 2017, with 391 new cases per 100.000 population, Indonesia was among the top 3 countries with absolute numbers of incident TB cases (1).

Bacille Calmette-Guérin (BCG) is a live attenuated *Mycobacterium bovis* vaccine. It is the only available vaccine used to protect against TB disease, in particular meningitis and disseminated TB in children (2). BCG is one of the most widely used vaccines worldwide. In Indonesia, BCG vaccination is included in the Indonesian national immunization program and it is given to new-borns at the age of 4-6 weeks. Beside its protective effects against TB, BCG vaccination has also shown to result in non-specific lower mortality and morbidity during childhood (3-5). BCG vaccination induces a memory T-helper-1 (Th-1) type response irrespective of when in life it is given (6). Studies have shown that reactions at the site of the BCG vaccination are associated with the production of Interferon gamma (IFN-γ) in response to the mycobacterial antigens. BCG scarification has been mentioned as a marker to a better survival and stronger immune response among BCG-vaccinated children living in countries with higher mortality rates (7, 8).

Immune responses to vaccines are associated with multiple factors such as economic status, parasite infestation and nutrition. Nutritional status at birth reflects new-borns adiposity and this might affect BCG vaccine response in these babies (9, 10). Adipocytes influence not only the endocrine system but also the immune response through several cytokine-like mediators known as adipokines, which include leptin and adiponectin (11, 12). Adiponectin and leptin are considered the most important hormones related to adipose depots in modulating metabolism and energy homeostasis. It is thought that leptin can directly link nutritional status and pro-inflammatory Th-1 immune responses, while adiponectin possesses *anti-inflammatory* properties (13-16).

With respect to economic status, a study on rotavirus vaccine showed that the efficacy of this vaccine was lower in low- compared to high-income-countries (17). Moreover, within low-income countries, a gradient in reduced efficacy was shown with decreasing Gross Domestic Products (GDP) (18). Low socioeconomic status (SES) has been linked with bad sanitation, hygiene (19), and higher exposure to infections (19-22). All these factors can have an impact on the immune system as shown by a study of twins in the USA where it was discerned that not only genetic but very importantly environmental factors can affect the immune system (23). This is confirmed by several studies involving low-middle-income-countries where geographical differences in immune profiles have been examined (24-27).

Indonesia with its rapid economic development bears a large diversity in population life style and SES. This study is aimed to better understand factors that determine the response to BCG vaccination. To this end, a study was conducted in three hospitals that serving high and low SES population. Maternal demographic data, socioeconomic characteristic, and infection status as well as their new-borns nutritional status, total IgE, and leptin levels were used for performing pathway analysis in order to better understand how BCG vaccine responses are shaped.

Methods

Ethics statement

The study was approved by the Health Research Ethical Committee, Faculty of Medicine, Hasanuddin University (ref.: 0685/H4.8.4.5.31/PP36-KOMETIK/2014). This study was executed based on codes stated in Declaration of Helsinki and International Ethical Guidelines for Epidemiological Studies. Written informed consent was obtained from mothers for the collection of their samples and their new-born samples.

Study population

The study population consisted of pregnant mothers and their born infants living in urban city of Makassar between January 2015 and May 2016. Pregnant women in the last trimester were recruited in government hospitals and private hospitals. Questionnaires were used to gather information regarding demographic, socioeconomic status and education. Based on Makassar minimum city wages, family income of IDR 60 million/year is used to define high (income equal/more than IDR 60 million/year) and low (income less than IDR 60 million/year) SES. Maternal characteristics such as gravidity, parity, miscarriage history or other medical conditions were also collected. Information about gestational age was estimated from last menstrual date.

Child information such as sex and delivery status were collected via the midwives or obstetricians through a questionnaire. Only infants born full term, delivered vaginally and healthy were included in the study. Birth weight and height were assessed of new-born wearing the minimum clothing, using baby weighing scale (GEA, Megapratama Medikalindo, Indonesia) and new-born length board. The weighing scale was calibrated using standardized weight as part of routine care. Weight for length z-score (Wflz) at birth, a proxy of new-born adiposity and nutritional status was calculated according to the WHO references value (28, 29).

BCG vaccination and scar measurement

Vaccination program in Indonesia requires every infant to be vaccinated with BCG at 6-weeks of age by staff from local primary health care center. The vaccine given contains live-attenuated *Mycobacterium bovis* Paris 1173-P2 strain (Biofarma, Bandung, Indonesia). The resulting BCG scar was measured at 10 months of age by same person from the research team. Mean diameter of scar size was calculated from diameters perpendicular to each other. Immunization card which proved that the newborns were BCG-vaccinated were recorded.

Blood and stool samples collection

Maternal blood samples were collected in the last trimester of pregnancy while cord blood samples were collected right after delivery. All blood samples were transported to the laboratory at Hasanuddin University Medical Research Center (HUM-RC) and processed within 4 hours after collection. Maternal stool samples were collected and stored at -80°C until further analysis.

Parasitological examination

DNA isolation from stool was performed as described elsewhere (30, 31). Two different panels of multiplex real-time PCR were used to detect and quantify soil transmitted helminths and intestinal protozoa. Panel 1 targets hookworm (*Ancylostoma duodenale, Necator americanus*), *Ascaris lumbricoides*, *Trichuris trichuria*, and *Strongyloides stercoralis* (30-33) while panel 2 targets *Entamoeba histolytica, Dientamoeba fragilis, Giardia lamblia,* and *Cryptosporidium* spp. (33-36).

Measurements of total IgE, anti-Hepatitis A Virus IgG, anti-*Toxoplasma gondii* IgG, leptin and adiponectin

Total IgE level was measured using ELISA technique as described previously (30). For this assay, maternal and cord sera were diluted 50 and 2 times in PBS containing 0.05% Tween20, respectively (Tween-20, Sigma-Aldrich, St. Louis, MO, USA). The results were expressed in International Units per millilitre (IU/ mL).

Anti-Hepatitis A Virus IgG (anti-HAV IgG) and anti-*Toxoplasma gondii* IgG (anti-Toxoplasma IgG) were measured at the clinical microbiology laboratory of the Department of Microbiology at Leiden University Medical Center as part of routine diagnostic procedures. Samples were considered to be positive for anti-HAV IgG if the signal-to-cut-off (S/CO) ratio was ≥ 1.00 while for Anti-Toxoplasma Ig-G, the samples were considered to be positive if the titre was ≥ 8 IU/ml.

Adiponectin and leptin levels were quantified using Human Adiponectin Duo Set and Human Leptin Duo Set (R&D System, Abingdon, UK) at Department of Parasitology in LUMC, according to manufacturer's guidelines (37). Cord sera were diluted 40 and 10000 times for measurement of leptin and adiponectin, respectively. The values were expressed in ng/mL and µg /mL for leptin and adiponectin, respectively.

Statistical analysis and conceptual framework

Descriptive data was presented in mean ± standard deviation for normally distributed data and median (Interquartile range, IQR) for non-normally distributed data. The correlation between two continuous data was done using Pearson correlation for normally distributed data or Spearman's rank correlation for non-normally distributed data. Prevalence was calculated as percentage of collected data. Pearson chi-square test was used to compare the prevalence of infection between two groups.

To obtain normally distributed data, maternal total IgE, cord leptin and adiponectin level were log10-transformed. Student t-test was then used to compare the mean differences between two groups. Since the levels of cord blood total IgE, anti-HAV IgG and anti-Toxoplasma IgG were not normally distributed even after transformation, the comparison between two groups was performed using Mann-Whitney U test.

The conceptual framework in Figure 1 was used to answer our research question. First, (A) we assessed whether the effect of SES to the size of BCG scar is through exposure to infections. Second, (B) we assessed the contribution of SES to the size of BCG scar is through the nutritional status pathway and whether leptin or adiponectin have a mediatory role in this path.

We primarily performed a linear regression to obtain estimate coefficients of SES on the size of BCG scar. Next, to test the contribution of SES on BCG scar through infection pathway (A), this model was adjusted for total IgE, anti-HAV IgG or anti-Toxoplasma IgG as a proxy of prenatal exposure to infection. In pathway (B), we first assessed association between SES and WfLz, a proxy of adiposity at birth. Subsequently, the effect of WfLz on BCG outcome was modelled by adjusting with leptin and adiponectin. Mediation was considered to occur if (1) all the crude association between variables tested in this pathway were significant; (2) the association disappeared and the effects attenuated when the model was adjusted for total IgE, leptin or adiponectin.

All models above were analysed using linear regression with adjustments for gestational age, sex and other variable that we found relevant to be account as confounder. Statistical test was considered significant at $p<0.05$. All data were analysed using IBM Statistical Package for the Social Sciences (SPSS) Statistics version (IBM-SPSS Inc., Chicago, IL, USA).

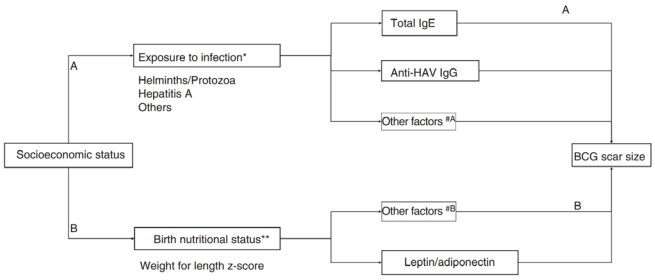

Figure 1. Conceptual framework of the study. The analysis of the association between Bacille Calmette–Guérin (BCG) scar size and SES through two different pathways. The analysis asked whether SES contributes to the outcome of BCG vaccination through (A) prenatal exposure to infection or (B) birth nutritional status pathway, using linear regression adjusted with gestational age and child gender for both pathways. *total IgE and anti-Hepatitis A IgG were measured as proxy of current or previous exposure to infections. **weight-for-length *z*-score was measured as a proxy of nutritional status at birth. $^{\#A/\#B}$ Other factors that were not assessed in pathway A or B.

Results

Characteristics of the study population

Maternal characteristics in low and high SES are presented in Table 1. The prevalence of any intestinal protozoa was not different between low and high SES. Although no significant differences in the prevalence of soil transmitted helminths was seen between two groups, the prevalence of *A. lumbricoides* (12% vs 4%) and *T. trichiura* (12% vs 6%) was slightly higher in low than in high SES mothers, respectively. The prevalence of hookworm (2%) and *S. stercoralis (2%)* was very low.

The levels of total IgE were significantly higher in low compared to high SES mothers [geomean (95% confidence interval, CI), 248.08 IU/ml (175.36-350.96 IU/ml) and 131.80 IU/ml (95.94-181.06 IU/ml), respectively; p=0.008] (Table 1).

Table 1. Baseline characteristics of the study population of Low (low SES) and High (high SES) socio-economic status

	Total		Low SES		High SES		P value[$]
	N	Results	N	Results	N	Results	
Mothers							
Age, years, mean ± SD	100	27.44 ± 4.90	50	26.71 ± 5.27	50	28.18 ± 4.42	0.135
Primigravida, N, n (%)	100	53 (53)	50	30 (60)	50	23 (46)	0.229
Intestinal parasites infection							
Any helminth, N, n (%)	100	16 (16)	50	9 (18)	50	7 (14)	0.585
Ascaris lumbricoides, N, n (%)	100	8 (8)	50	6 (12)	50	2 (4)	0.140
Trichuris trichiura, N, n (%)	100	9 (9)	50	6 (12)	50	3 (6)	0.295
Any protozoa, N, n (%)	100	20 (20)	50	17 (34)	50	13 (26)	0.517
Entamoeba histolytica, N, n (%)	100	3 (3)	50	2 (4)	50	1 (2)	0.558
Dientamoeba fragilis, N, n (%)	100	26 (26)	50	14 (28)	50	12 (24)	0.648
Giardia lamblia, N, n (%)	100	4 (4)	50	2 (4)	50	2 (4)	1.000
Cryptosporidium. spp N, n (%)	100	1 (2)	50	1 (2)	50	0 (0)	0.315
Any intestinal parasites, N, n (%)	100	42 (42)	50	23 (46)	50	19 (38)	0.418
Total IgE, IU/ ml, geomean (95%CI)	100	180.82 (142.31-229.75)	50	248.08 (175.36-350.96)	50	131.80(95.94-181.06)	**0.008**
Leptin, ng/ml, geomean (95%CI)	100	30.28 (26.40-34.73)	50	29.45 (24.00 - 36.12)	50	31.15 (25.77 - 37.65)	0.687
Adiponectin, µg/ml, geomean (95%CI)	100	3.41 (3.00-3.87)	50	3.58(3.04 - 4.23)	50	3.25 (2.67 - 3.96)	0.453

New-borns							
Sex, female N, n (%)	100	50 (50)	50	28 (56)	50	22 (44)	0.317
First born, N, n (%)	100	52 (52)	50	30 (60)	50	22 (44)	0.161
Gestational age, weeks, mean ± SD	100	39.71±1.6	50	39.69 ± 1.68	50	39.73 ± 1.53	0.902
Birth weight, grams, mean ± SD	100	3130.5±402.76	50	3194.64 ± 368.26	50	3156.67 ± 415.79	0.666
Weight-for-length z- score at birth, mean ± SD	100	-0·50±0·63	50	-0·47 ± 0·52	50	-0·46 ± 0·65	0.887
Total IgE, IU/ml, median (IQR)	100	0.07(0.07-0.21)	50	0.12(0.07-0.76)	50	0.07(0.07-0.07)	**<0.001**
Ratio S/CO anti-Hepatitis A Virus IgG, median(IQR)	98	9.60 (7.77-10.37)	49	9.93 (9.07-10.46)	49	9.33 (0.30 - 10.28)	0.088
Anti-Hepatitis A Virus IgG seroposi-tivity, N, n (%)	98	77 (78.6)	49	42 (85.7)	49	35 (71.4)	0.139
Anti-*Toxoplasma* IgG titres, IU/ml, median(IQR)	100	0.00 (0.00-41.25)	50	0.00 (0.00 - 44.25)	50	0.00 (0.00 - 37.00)	0.846
Anti-*Toxoplasma* IgG seropositivity, N, n (%)	100	35(35)	50	20 (40)	50	15 (30)	0.402
Leptin, ng/ml, geomean (95%CI)	100	10.04(8.30-12.14)	50	9.15 (7.17-11.68)	50	11.01 (8.17-14.84)	0.336
Adiponectin, µg/ml, geomean (95%CI)	100	16.44(14.35-18.83)	50	17.73 (14.68-21.41)	50	15.24 (12.48-18.61)	0.271

Data presented as number of positives (n) of the total population (N) and as percentage of total population (%), SD: standard deviation, IU: international unit; S/CO: signal-to-cut-off. [$]unadjusted; Bold: p value<0.05.

Anti-HAV IgG S/CO ratio was slightly higher in mother with low compared to high SES [median (IQR), 9.93 (9.07-10.46) and 9.33 (0.30 - 10.28), respectively; p=0.088] (Table 1). There were no differences in the levels of IgG antibody against *T. gondii* between two groups. Similarly, adiponectin or leptin levels were not different between low and high SES mothers.

Of 100 new-borns that were included in the study, no significant differences in sex ratio, nor gestational age between low and high SES group was seen. The birth weight, height and Wflz were not different between low and high SES new-borns (Table 1). The level of total IgE in cord blood was significantly higher in low (median [IQR], 0.12 IU/ml [0.07-0.76]) compared to high SES (median [IQR], 0. 07 IU/ml [0.07-0.07]) new-borns (p< 0.001) (Table 1).

The levels of leptin and adiponectin were not different between high and low SES new-borns. When gender was considered, girls either form high or low socioeconomic status tended to have higher leptin levels compared to boys (High SES: geomean [95%CI] = 15.25 ng/ml [9.76-23.84] vs 8.52 ng/ml [5.74-12.69], p=0.103; Low SES: 10.90 [8.09-14.70] vs 7.31 ng/ml [4.84-11.03], p=0.051), in girls and boys, respectively.

During follow up at 10 months of age, we were able to collect data from 59 out of 100 infants (29 from low SES and 30 from high SES). There were no differences in sex ratio between these high and low SES groups. In terms of weight, high SES infants gained more weight compare to low SES infants (mean ±SD, 5103.33 ± 534.65 vs 4777.58 ± 466.28; p=0.016). The major reason of loss to follow up was families moving out of the city. There were no differences in gestational age, sex, birth weight, Wflz, total IgE, leptin, nor adiponectin levels between those who remained in the study and those who were lost to follow up.

The effect of SES and exposure to infection on the size of BCG scar

All 59 infants that participated in the follow up time points were vaccinated. Among these infants, 89.3% (n=53/59) had developed a BCG scar, while 11.7% (n=6/59; 5 from low SES and 1 from high SES) infants did not develop BCG scar. Comparison of BCG scar size between the two groups (Figure 2) revealed that infants from high SES have bigger scar size compared to low SES (2,2 ± 0,98 mm vs 1,65 ± 1,01mm, p=0.041, respectively).

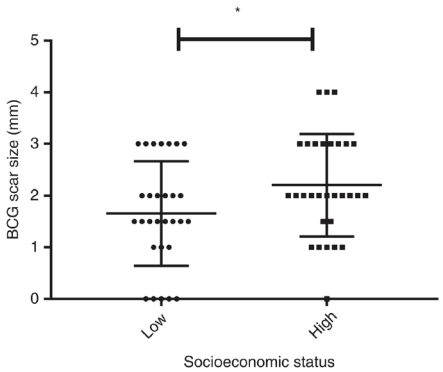

Figure 2. The BCG scar size in low and high socioeconomic status infants measured at 10 months of age. Data presented as Mean ± SD. * indicates p<0.05 (adjusted for gestational age, sex, and weight-for-length z-score)

To investigate whether prenatal exposure to infection has an effect on the size of BCG scar, we analysed the association between the size of BCG scar and total IgE, anti-HAV IgG and anti-Toxoplasma IgG. We found an inverse relationship between BCG scar size and total IgE (estimates [95%CI] = -0.33 [-0.67 - -0.004] p=0.048) but no association with either anti-HAV IgG or anti-Toxoplasma IgG (-0.015 [-0.079 – 0.048], p=0.625; and -0.003 [-0.002 – 0.008], p=0.243; respectively).

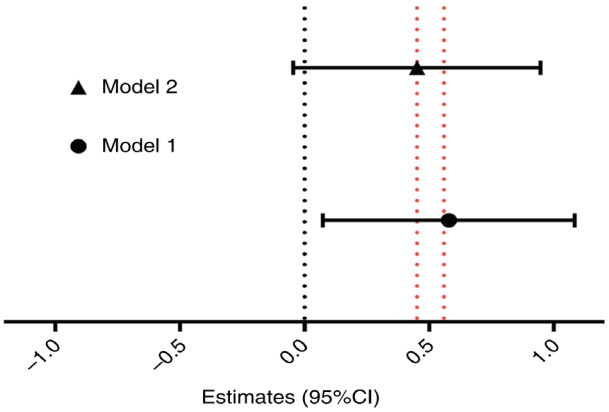

Figure 3. Effects of socioeconomic status on BCG scar size at 10 months. Model 1: crude (adjusted for gestational age, sex, and birth weight-for-length z-score); Model 2: Model 1+ cord total IgE. Data presented as beta estimate with 95% confidence interval.

Next, the association between SES and BCG scar size were analysed in multivariate analysis, adjusted with gestational age, sex, and Wflz. The results showed that the size of BCG scar remained larger in high SES compared to low SES infants (estimate [95%CI] = 0.559 [0.083-1.094], p=0.022) (Figure 3 Model 1). Interestingly, when the model was adjusted with total IgE (Figure 3 Model 2), the effect of SES on the size of BCG scar was slightly attenuated (estimates [95%CI] = 0.451 [-0.045-0.947], p=0.074) and fell short of significance in terms of predictor of BCG scar size.

Nutritional status at birth and its association with cord blood adipokines and BCG scar size

To find out whether the size of BCG scar is affected by birth nutritional status through adipokines (Figure 4), we first analysed the association between Wflz and adipokine levels. The results showed that the levels of leptin and adiponectin increased with increasing Wflz (estimates [95%CI] = 1.54 [1.16-2.06], p=0.003 and 1.43 [1.16-1.77] p=0.001, respectively). The analysis on the association between BCG scar size and adipokine levels revealed that the BCG scar size increased with increasing leptin and adiponectin levels (estimates [95%CI] = [9.46 [2.40 – 37.32], p=0.002]; [13.33 [1.82 – 97.27], p=0.012]; respectively).

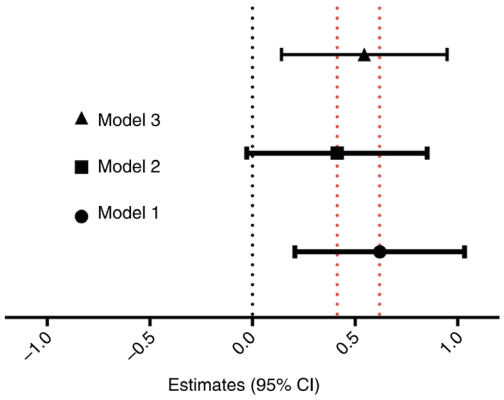

Figure 4. Pathway analysis of nutritional status at birth and BCG scar size at 10 months. Model 1: Crude (adjusted for gestational age, sex, and SES). Model 2: Model 1 + Leptin. Model 3: Model 1 + Adiponectin. Data presented as beta estimate with 95% confidence interval.

Pathway analysis on Figure 4 model 1 showed that higher Wflz was associated with larger size of BCG scar (estimates [95%CI] = 0.620 [0.206-1.034], p=0.004). Interestingly, when leptin level was also considered (model 2), the association between nutritional status at birth and BCG scar size is no longer significant and the Wflz effects to BCG scar size was attenuated from 0.620 [0.206-1.034] to 0.412 [-0.027-0.852]. However, when adiponectin level was considered (model 3), this had less effect on the effect of birth Wflz effects on BCG scar size; from 0.620 [0.206-1.034] to 0.545 [0.141-0.949] and Wflz remained as a significant predictor of BCG scar size.

Discussion

In the present study which involved mothers and their new-borns residing in an urban area of Makassar, Indonesia, we reported that both SES and nutritional status at birth determine the response towards BCG vaccination measured at 10 months of age. Regarding SES, our study found that infants born to low SES families have smaller BCG scar size compared to infants born to high SES families and that total IgE partly contributed to reducing the size of scars. Conversely, infants born with better nutritional status were found to have bigger BCG scar size but this association was abrogated by leptin levels at birth.

Similar to the finding in the current study, a study in school-aged children in Dominican Republic found positive correlation between BCG scar prevalence and an index of socio-economic factors (38). In epidemiological studies, low SES has been associated with poorer hygiene practices which in turn could increase exposure to other microorganism or parasites (39). Infection with parasitic helminths is known to induce a strong Th-2 immune response that can lead to elevated levels of IgE (40, 41). In the current study, we found no statistical significant differences in the prevalence of current helminth infections between mothers with low and high SES, however, the levels of total IgE in mothers/ new-borns with low SES were significantly higher than mothers/ new-borns with high SES. This finding indicates that previous helminth infections, or other factors might contribute to the high levels of total IgE. Our previous study in Indonesia, which looked at the development of Th-2 responses from

infancy to 4 years of age reported that children born to mothers with low education or low SES showed stronger development of total IgE responses over time compared to children born to mothers with high SES or high education. In the study, maternal helminth infection status was not the strongest factor determining the Th-2 polarisation in their children (42). Like infection with *M. tuberculosis*, BCG vaccination induces Th-1 type immune responses and cause suppression of Th-2 type responses.(43) Here we found that BCG scar size is inversely associated with the levels of total IgE which is in an agreement with Soysal A *et al* who reported that the presence of scar was associated with lower levels of total IgE (44).

The nutritional status at birth in our study population were within the normal range and no differences as a function of SES were found. Furthermore, adipokine levels were observed to be positively associated with nutritional status which is consistent with previous studies showed adiponectin and leptin levels increases with increasing body composition at birth (45-48). The finding that leptin, but not adiponectin, strongly attenuated the relationship between nutritional status and the BCG scar size suggests that leptin levels and not nutritional status at birth determine the response toward BCG scar formation. To our knowledge, our study is the first to investigate the relationship between leptin levels and BCG scar size. Regarding nutritional status, a recent study in 6-12 months old babies from Guinea Bissau found that BCG scarification was not associated with nutritional status determined by mid-upper-arm-circumference as well as weight-for-age (49).

Leptin is a 16-kD hormone mainly secreted from adipocytes (50, 51) and has been reported to be positively associated with intrauterine foetal growth (52), birth weight and total body fat content of neonates (53). Leptin is released in the circulation in proportion to the number of adipocytes and acts at the hypothalamus receptor to maintain metabolic homeostasis (51, 54, 55). Besides its role in energy homeostasis, there is also evidence for an immunomodulatory role of leptin. Leptin may shift immune responses towards Th-1 phenotype (15, 55, 56). In this study, for the first time, we observed that infants with higher neonatal leptin levels had bigger BCG scar size. Although no studies in human have been reported on the role of leptin in vaccine induced protection, in experimental mouse model, Wehrens and colleagues reported that functional leptin receptor signalling is required for mediating an effective protective response against *Helicobacter pylori* (57).

Among infants in this study, 10.16% did not develop BCG scar. The presence or absence of a scar is often used as an indicator of BCG vaccination in a clinical context as well as in health surveys to assess vaccine coverage (58). Our finding was quite similar to that observed by Rani *et al* (59) and Sara *et al* (60) where 8.6 – 9.8 % of BCG-vaccinated infants did not develop any scar. Previous studies have been reported that the development of BCG scar in BCG-vaccinated children is also influenced by several factors such as sex, seasons at vaccination, sequence of vaccinations, BCG administration techniques and BCG strain (4, 7, 61, 62).

Some limitations are worth noting. The small sample size and considerable loss to follow up, however, some interesting data were generated that should be followed up in larger studies powered to discern the role of leptin in modulating BCG scar size.

In conclusion, BCG scar size was influenced by SES and leptin levels at birth. Furthermore, total IgE partly contributed to reducing the size of BCG scar. This study would need to be followed up to determine whether leptin and total IgE affect BCG vaccine efficacy and it also provides a departure point for thinking of strategies whereby leptin levels are increased while total IgE levels are decreased in order to improve responses to BCG vaccine.

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Conflict of interest statement

We declare that we have no conflict of interest.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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