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BIOLOGICAL SAMPLE COLLECTION

Blood samples

Blood samples will be collected for analyses of DNA, protein, and exosomes and isolation of Peripheral Blood Mononuclear Cells (PBMCs). The mothers will be asked to be fasting for 8 h or more before the blood draw. Blood samples will be kept at room temperature for 30 min before centrifugation. For DNA analyses, a blood sample of 2ml will be collected at gestational week 24-28 into EDTA tubes and stored at -80°C until use. For protein analyses, the serum will be centrifuged at 1000g for 15 min at room temperature and the supernatants will be aliquoted and stored at − 80 °C until use. For PBMC isolation, PBMC will be purified as previously described, and stored in liquid nitrogen for use [1]. For exosome analyses, 5ml of blood will be collected into EDTA tubes and stored at 4°C for 4 h or overnight. Plasma will be separated by centrifugation at 5000×g for 5 minutes at 4°C. Cell-free, platelet poor plasma will be collected, aliquoted and stored at − 80 °C until use[2].

At birth, the venous and arterial cord blood will be collected from the umbilical cord into EDTA tubes using a syringe. Blood components will be processed and stored as detailed above for analyses of DNA, protein and exosomes. An additional 3mL arterial cord blood will be drawn and stored at room temperature for flow cytometric analysis.

At the year of two, children will be asked to test for serum specific allergens including house dust mite, cat dander, birch pollen, grass pollen, milk, and egg. If the parent consent to having their children's blood drawn, 2ml blood will be collected into tubes containing clot activator and centrifuged at 1000g for 15 min. The supernatant will be aliquoted into 500ul aliquotes and stored at − 80 °C until use.

Urine samples

The first catch midstream urine of mothers will be collected using a sterile cup, centrifuged and added to sterile tubes and stored at –80 °C until analyzed.

Placenta

After delivery, the placenta will be sampled for analyses of histology, DNA, methylation, RNA and protein. First, a cross-section 2cm away from the cord insertion will be sampled and stored in formalin to be fixed for histological examination. Next, twenty pieces of villous tissue on the maternal aspect measuring 3*3*3mm will be taken at 2-4cm away from cord insertion and stored in five cryo-tubes, among which two will be prefilled with RNAlater. Villous samples will be snap-frozen in liquid nitrogen and then stored at -80°C. Third, villous tissue on the fetal aspect will be sampled and stored likewise.

Breast milk

Breast milk will be collected with a breast pump at locations where postnatal follow-ups take place. At follow-up, 30ml of breast milk will be collected with sterilized RNase-free tubes, with the first 500ul disposed. For exosome analysis, 15 ml breast milk will be centrifuged at 4°C for 10 minutes at 1500 g to remove cells and the cream layer. The supernatant will then be transferred to new tubes and centrifuged again at 12,000 g at 4°C to remove remaining cells and cream. The supernatant will then be immediately processed or frozen at -80 °C until use[3]. The remaining 15 ml breast milk sample will be aliquoted and stored at -80 °C directly until use.

Feces

The fecal samples will be self-collected by participants. For 16s RNA sequencing, participants will be provided with a sterile feces collection and preservation kit (ML-001A, Shenzhen Dayun Gene Technology Co., Shenzhen, China). For metabolomics analyses, participants will be provided with a sterile feces collection device with a spatula. Participants will be instructed to collect stool specimens of approximately 10ml within 2 h before each visit and bring them to the visits. Collected feces will then be snap-frozen in liquid nitrogen and stored at -80 °C until use [4]. Meconium of 2ml will be sampled and stored likewise.

Vagina swab

Vaginal swabs (4520CA, COPAN Flock Technologies, Brescia, Italy) will be collected from the posterior fornix by obstetricians. A speculum will be placed in the absence of lubrication. All specimens will be collected by swirling a sterile swab for 30 seconds, withdrawing the swab without contamination from other sites, and transferring the specimen into a sterile tube and stored at -80 °C until use.

Skin swab

Skin specimens will be collected from the cheek, anterior forearm, and lesional sites of the children. The forehead, anterior forearm, and cubital fossa of the mother will be sampled likewise. These sites will be swiped vigorously for 50 times over an area of 4 cm2 using a sterile swab (4520CA, COPAN Flock Technologies, Brescia, Italy). The swab will be premoistened with DNA free saline. Swab specimens will be inserted into a sterile tube and stored at -80 °C until use.

Tape stripping

For each child, skin lipids will be collected by tape stripping from the same body sites as to where skin swabs are sampled from the children. The first layer will be discarded, and the second to fourth layers at the same place will be retained and placed separately in a glass tube

with 5 mL of methanol, then stored at -80 °C until use. The vernix will be sampled from the back of the newborn likewise.

Tongue dorsum swab

Subjects will be instructed not to eat, drink (except water), or brush their tongue during the 12 h period before sampling and not to brush their teeth during the 2 h period before sampling. Specimens will be collected from the central part of the tongue dorsum by swiping for 15 seconds using a sterile swab (4520CA, COPAN Flock Technologies, Brescia, Italy).

Zhao M, *et al. BMJ Open* 2022; 12:e048083. doi: 10.1136/bmjopen-2020-048083

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Table S1. Specific Recommendations on Baby Skincare Practices

Part 1. Bathing and Moisturising	
1	The duration of a baby bath should not exceed 10 minutes.
2	Babies should be bathed no less than twice per week and more frequently during summer to reduce sweet irritation.
3	Liquid cleansers with a neutral or mildly acidic pH could be used. Soap-based cleansers should be avoided.
4	Scrubbing and exfoliation should be avoided.
5	Bath temperature should be set to 38-40 °C for newborns and <38 °C for infants.
6	Emollients should be used after bathing, preferably within 5 minutes.
7	Emollients could be used liberally at multiple times to alleviate xerosis.
8	Liquid cleansers and emollients should be fragrance and dye free.
9	Emollients are recommended to be used for the whole body
10	Lotions are recommended for use in hot summer months whereas creams are recommended for use in winter months.
Part 2. Sun Protection	
1	Sun protection A-B-C: Avoid the sun between 10am and 4pm; Block harmful sun rays with a broad-spectrum sunscreen; Cover up with clothing and sunglasses
2	Sunscreen can be used for infants older than 6 months.
Part 3. Commonly Used Topical Agents During Infancy	
1	Topical corticosteroids are the first line therapy for eczema.
2	Topical corticosteroids do not induce premature puberty.
3	Topical corticosteroids used properly will not cause weight gain.
4	Topical corticosteroids used properly will not cause osteoporosis nor hamper the physical development of a child.
5	Prolonged use of topical corticosteroids is associated with higher risk of skin infection, excessive hair growth and skin redness.
6	Topical antibiotics and astringents can be used in eczematic skin.
7	Topical corticosteroids used properly will not cause addiction nor resistence.
Part 4. Atopic dermatitis and Atopic March: An Overview	
1	Atopic dermatitis is one of the most common skin condition during infancy.
2	Significant itch and extensive skin lesions may impose substantial disease burden on the affected child and family.
3	Barrier repair with emollients is the mainstay of treatment for atopic dermatitis.
4	Avoidance of triggers and control of inflammation and infection should be implimented following physician's instructions.
5	Topical corticosteroids are the first line therapy for control opf inflammation in eczematic children.
6	Children with atopic dermatitis are at greater risk for developing othe atopic disorders.

Table S2. Responses of a 12-item Knowledge survey

Survey questions	Score	Number answered (n=395), n (%)
1. I should apply body wash every time I bathe my baby.	1	17 (4.30)
	2	61 (15.44)
	3	61 (15.44)
	4	124 (31.39)
	5	132 (33.42)
2. Moisturizer should be applied all over my baby's body.	1	27(6.84)
	2	86(21.77)
	3	76(19.24)
	4	128(32.41)
	5	78(19.75)
3. Moisturizer should be used no more than once a day.	1	55 (13.92)
	2	109 (27.59)
	3	121 (30.63)
	4	91 (23.04)
	5	19 (4.81)
4. I only use moisturizer after I bathe my baby.	1	25(6.33)
	2	65(16.46)
	3	79(20.00)
	4	170(43.04)
	5	56(14.18)
5. Massage oils can be used as a baby moisturizer.	1	32 (8.10)
	2	96 (24.30)
	3	112 (28.35)
	4	112 (28.35)
	5	43 (10.89)
6. Moisturizer is not necessary for my baby at summer time.	1	10(2.53)
	2	36(9.11)
	3	72(18.23)
	4	160(40.51)
	5	117(29.62)
7. Sweat is an irritant to the baby's skin.	1	39 (9.87)
	2	54 (13.67)
	3	95 (24.5)

	4	105 (26.58)
	5	102 (25.82)
8. I'm concerned to use topical corticosteroids ointment on my baby.	1	103(26.08)
	2	81(20.51)
	3	149(37.72)
	4	37(9.37)
	5	25(6.33)
9. Topical corticosteroids will make the baby fat.	1	43(10.89)
	2	53(13.42)
	3	124(31.39)
	4	105(26.58)
	5	70(17.72)
10. Topical corticosteroids will induce premature puberty in babies.	1	69(17.47)
	2	47(11.9)
	3	138(34.94)
	4	88(22.28)
	5	53(13.42)
11. If I had to use topical corticosteroids on my baby, I'd be concerned that my baby will become addicted to the drug.	1	93(23.54)
	2	67(16.96)
	3	140(35.44)
	4	59(14.94)
	5	36(9.11)
12. If I had to use topical corticosteroids on my baby, I'd be concerned that my baby will become resistant to the drug.	1	88(22.28)
	2	89(22.53)
	3	135(34.18)
	4	57(14.43)
	5	26(6.58)