POSITION PAPER

Gut microbiota in early life and its influence on health and disease: A position paper by the Malaysian Working Group on Gastrointestinal Health

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Abstract: The role of gut microbiota in early life and its impact on gut health and subsequent diseases remain unclear. There is a lack of research and awareness in this area, especially in the Asia-Pacific region, including Malaysia. This paper reports the position of a Malaysian Working Group on some key issues surrounding gut microbiota in early life and its role in gut health and diseases, as well as experts’ stand on probiotics and prebiotics. The group reached a consensus that certain factors, including elective caesarean; premature deliveries; complementary feeding; use of antibiotics, prebiotics and/or probiotics; and exposure to the external environmental, have an impact on gut microbiota in early life. However, as evidence is lacking, especially from the Asia-Pacific region, further studies are needed to understand how gut microbiota in early life affects subsequent diseases, including allergy, inflammatory bowel disease, obesity and infantile colic. Lastly, although beneficial in acute diarrhoeal disease and probably allergic eczema, probiotics (and/or prebiotics) should be used cautiously in other gut dysbiotic conditions until more data are available.

Key words: Asia; early life; gut health; gut microbiota; prebiotics; probiotic.

As an emerging concept, ‘gut health’ encompasses multiple positive aspects of the gastrointestinal (GI) system, that is, stable composition of gut microbiota, efficient digestion and absorption of food, absence of illnesses and effective immune and metabolic status.1 In particular, gut microbiota in humans plays an important role in shaping the gut health of early life, starting from the first 1000 days and transcending beyond.2 While the research of gut microbiota in early life is progressing rapidly in the West, the same cannot be said for Asia.

Another emerging concept is the Developmental Origin of Health and Disease (DOHaD), which emphasises the central role of the maternal microbiota in shaping a stable gut microbiota in early life.3 The gut microbiota established during early life is then influenced by a variety of physiological, cultural and environmental factors (Fig. 1). Dysbiosis, a condition when the gut microbiota is disrupted, may result in various intestinal (e.g. inflammatory bowel disease (IBD)) and extra-intestinal disorders (e.g. allergy and obesity), all of which are now on the rise in Asia in proportions comparable to the West.4 Although probiotics (and prebiotics) are attractive management options for dysbiosis, their exact roles remain controversial.

The Malaysia Working Group on Gastrointestinal Health (MYGiH) is comprised of experts in gut health, with the primary aim to raise awareness among the community at large. This paper reports their views on certain key issues surrounding gut microbiota in early life, the role of gut microbiota in gut health and diseases, and their stands on probiotics and prebiotics.

Methods

Membership of the Working Group

Members of the MYGiH were invited based on the following criteria: (i) demonstrated proficiency and knowledge by publication, research or participation in national guidelines and (ii) diversity of perspective and expertise in the health-care system (including paediatricians, primary care doctors, microbiologists, gastroenterologists and immunologists). Two independent scientists with expertise in gut microbiology during early life were also part of the Working Group.

Literature search

Relevant English-language articles from the years 2010–2015 were identified using MEDLINE/PubMed and EMBASE databases. The keywords used for search purposes were: gut
microbiota, breastfeeding, formula feeding, complementary feeding, obesity, allergy, IBD, caesarean section, premature delivery, antibiotics, probiotics, prebiotics, Asia and Malaysia. A consensus on references was reached in the following order: (i) meta-analysis and systematic reviews and (ii) original articles of Asian and/or Malaysian origin, but if not available, western articles would be considered. A total of 187 articles were identified based on the above selection criteria.

Modified Delphi process

A face-to-face meeting with invited experts was first held in March 2015. They formed the Working Group, and participating members provided their input, drafted the statements and reviewed the collected evidences. An anonymous voting system using the GRADE consensus (Table 1) for each drafted statement was then conducted electronically via Google Forms. Results and feedback of voting were later collated and discussed in a second meeting. Several rounds of discussion were held thereafter to scrutinise the statements and evidence thoroughly. The final statements, levels of agreement and strength of evidence are shown in Figure 2. Statements with poor agreement levels were also limited in terms of evidence due to the lack of information available from the Asian literature. These statements will serve as grounds for future research in this region.

Results

Statement 1: Gut microbial colonisation begins in early life

Level of agreement: I, 70%; II, 30%; III, 0%; IV, 0%; V, 0%. Strength of evidence: A, 60%; B, 40%; C, 0%; D, 0%.

It is now becoming apparent that the foetus may be already seeded with maternal microbes rather than being sterile during pregnancy, with evidence of bacterial elements detected at low levels in the umbilical cord, placenta, amniotic fluid and infant meconium. Transmission of maternal microbiota at birth is also found to be programmed throughout pregnancy. Maternal bacterial isolates, such as Enterococcus and Lactobacillus, have been detected in umbilical cord blood, amniotic fluid, meconium, placental and foetal membranes. The DOHaD concept suggests that the maternal microbiome shapes the microbiome composition in the offspring. The maternal vaginal and gut microbiota typically form the first microbial inoculum at birth. Studies have shown that vaginally born infants acquire Bifidobacterium and Bacteroides from their mothers. These early colonisers have roles in human milk oligosaccharides (HMOs) metabolism and resistance as well as in the host immune and metabolic programming.
Strength of evidence: A, 10%; B, 90%; C, 0%; D, 0%.

Level of agreement: I, 80%; II, 20%; III, 0%; IV, 0%; V, 0%.

The rates of caesarean section within the Asia-Pacific region has increased dramatically over the last decade, with a reported rate of 16% in Malaysia between 2005 and 2011. Birth by caesarean section deprives the newborn of exposure to the maternal birth canal, specifically the maternal vaginal faecal microbiota. There is also delayed colonisation of *Bifidobacterium* and *Bacteroides* in caesarean-delivered infants. Instead, microbiota from the maternal skin and mouth, and also from the hospital environment have been found in bowels of caesarean-born infants. This compromised transmission of maternal microbial inoculum at birth has been associated with an impaired priming of the neonate immune system, which may lead to gut health consequences in early life.

**Statement 2: Delivery by caesarean section is associated with alteration of gut microbial colonisation in early life**

Level of agreement: I, 80%; II, 20%; III, 0%; IV, 0%; V, 0%. Strength of evidence: A, 80%; B, 20%; C, 0%; D, 0%.

The rates of caesarean section within the Asia-Pacific region has increased dramatically over the last decade, with a reported rate of 16% in Malaysia between 2005 and 2011. Birth by caesarean section deprives the newborn of exposure to the maternal birth canal, specifically the maternal vaginal faecal microbiota. There is also delayed colonisation of *Bifidobacterium* and *Bacteroides* in caesarean-delivered infants. Instead, microbiota from the maternal skin and mouth, and also from the hospital environment have been found in bowels of caesarean-born infants. This compromised transmission of maternal microbial inoculum at birth has been associated with an impaired priming of the neonate immune system, which may lead to gut health consequences in early life.

**Statement 3: Premature delivery is associated with alterations of gut microbial colonisation**

Level of agreement: I, 20%; II, 60%; III, 20%; IV, 0%; V, 0%. Strength of evidence: A, 10%; B, 90%; C, 0%; D, 0%.

According to the World Health Organization (WHO) estimates, the preterm birth (less than 37 weeks) rate in Malaysia has been reported as 12.3 per 100 births. Preterm delivery is a leading cause of perinatal death and morbidity, largely because of sepsis. Notably, the gut microbiota colonisation in preterm infants is different from term infants, especially in terms of key bacterial members, such as *Bifidobacterium* sp. Factors that worsen dysbiosis in preterm infants include delay of enteral feeding, frequent use of total parenteral nutrition, aseptic condition in intensive care unit and maternal and post-natal antibiotic administration. A high prevalence of nosocomial infections, especially *Staphylococcus* sp. and *Enterobacteriaceae* sp., have been found among preterm infants who developed sepsis or necrotising enterocolitis. Motor and cognitive disabilities and attention deficit hyperactivity syndrome have been associated as consequences of premature birth, but whether some of these complications are related to altered gut microbiota due to prematurity requires further investigation.

**Statement 4: Breastfeeding and infant formula feeding have different impacts on gut microbiota**

Level of agreement: I, 60%; II, 30%; III, 10%; IV, 0%; V, 0%. Strength of evidence: A, 60%; B, 30%; C, 10%; D, 0%.

The first 1000 days of life is a critical period for appropriate diet to promote growth and development. The National Breastfeeding Policy of Malaysia concurs with the WHO recommendation for exclusive breastfeeding until 6 months of age. Breast milk contains not only the beneficial microbiota *Bifidobacterium* but also antimicrobial factors such as immunoglobulins, cytokines, lysozyme and lactoferrin, as well as HMOS. The gut microbiota of breastfed infants is reportedly different and more stable than formula-fed infants, although there may be differences between...
different regions of the world due to environmental factors. For example, in a Korean study, the genus *Bifidobacterium* and *Lactobacillus* predominated in breastfed infants, but *Firmicutes* and *Proteobacteria* predominated in formula-fed infants. Moreover, intestinal contents of breastfed infants are generally more acidic (pH 5.0) and contain more short-chain fatty acids (SCFAs). Nowadays, the bifidogenic effects of human milk can be emulated in infant formula through the addition of prebiotic and/or probiotic supplements, but whether such approaches produce similar effects compared with human milk will require further studies.

**Statement 5: Introduction of complementary feeding has an impact on the gut microbiota**

Level of agreement: I, 50%; II, 40%; III, 10%; IV, 0%; V, 0%. Strength of evidence: A, 30%; B, 60%; C, 10%; D, 0%.

Complementary feeding introduces solid (non-milk) foods to infants typically by 6 months of age. The stability of the gut microbiota in early life is affected by the introduction of home-prepared foods that contain non-digestible carbohydrates, proteins and fibres, especially fruits and vegetables. Home-prepared foods, with its well-conserved overall micronutrients, have been shown to be inversely associated with the development of allergic diseases. Notably, complementary feeding provides new substrates that may promote the survival and dominance of certain bacterial species (e.g. *Bacteroides* and *Clostridium cocoides*), which are not supported by human milk and infant formula. However, it may be possible that the effect on microbiota of complementary feeding is also due to maturity of other gut functions as weaning progresses, including pancreatic function, small intestinal absorption and colonic fermentation, which modify the materials that reach the colon and microbiota.

**Statement 6: Antibiotic exposure during pregnancy and infancy affects gut microbial colonisation**

Level of agreement: I, 50%; II, 40%; III, 10%; IV, 0%; V, 0%. Strength of evidence: A, 60%; B, 30%; C, 10%; D, 0%.

Antibiotic exposure during pregnancy can cause teratogenic effects, whereas during infancy, it can disrupt the stability of the gut microbiota. Maternal intrapartum antibiotic use is reported to be associated with delayed colonisation of vaginal *Bifidobacterium* and *Lactobacillus* in neonates. Additionally, antibiotic use during breastfeeding may interfere with the infant’s gut microbiota composition due to antibiotic transfer into the breast milk. While antibiotics may reduce short-term complications, it is unknown for how long the use of antibiotics may have an impact on gut microbiota, and their long-term effects are yet to be determined. Studies have associated repeated exposure of antibiotics in early infancy with diseases such as obesity and allergy in later life. Perinatal exposure to antibiotics (e.g. penicillin) is linked to a higher abundance of *Enterobacteriaceae*. In infants who received oral antibiotics (e.g. amoxicillin and ciprofloxacin); a higher abundance of pathogens (e.g. *Enterobacter* sp.) have been detected in their faeces, and this effect can persist for weeks to months. Furthermore, early exposure to ampicillin and gentamicin has been found to disrupt the early colonisation of *Actinobacteria* (e.g. *Bifidobacterium*) and *Firmicutes* (e.g. *Lactobacillus*) and an overgrowth of *Proteobacteria*.

**Statement 7: External environmental exposure plays a role in the modulation of the gut microbiota**

Level of agreement: I, 40%; II, 50%; III, 10%; IV, 0%; V, 0%. Strength of evidence: A, 40%; B, 50%; C, 10%; D, 0%.

Environmental factors, including geographical region, family size and exposure to pets, are thought to shape the gut microbiota of early life. For example, in young children from China, Japan and Taiwan, *Bifidobacterium* and *Bacteroides* are the most common bacterial clusters, whereas *Prevotella* is more predominant in children from Indonesia and Thailand. In addition, infants with older siblings have higher counts of *Bifidobacteriaceae* and lower counts of *Peptostreptococcaceae*, compared with infants without older siblings. Infants living with furred pets (e.g. dogs), they may have a greater diversity of dog-derived bacterial taxa, such as *Bacteroides*, *Actinobacteria* and *Acidobacteria*. Moreover, a preclinical study showed that an early exposure to dog-associated house dust might mediate gut *Lactobacillus* enrichment and airway immune defence against allergens and virus infection.

**Statement 8: Allergic diseases are associated with alterations in the gut microbiota**

Level of agreement: I, 50%; II, 50%; III, 0%; IV, 0%; V, 0%. Strength of evidence: A, 50%; B, 40%; C, 10%; D, 0%.

Allergic diseases are on the rise globally, including Malaysia, and it is estimated that 35% of children in Malaysia suffer from allergy. Infants with allergies have a relatively higher abundance of *Enterobacteriaceae* but lower levels of beneficial *Bifidobacterium* and *Lactobacillus*. Johansson et al. suggested that early colonisation with *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus rhamnosus* might reduce the risk of developing an allergy despite allergic heredity. More recently, Arrieta et al. ascertained that gut microbiota of early life have significant influences on the development of the immune system, especially on asthma susceptibility. Moreover, infants who exhibit severe multi-sensitisation to food or aero-allergens have a significantly higher risk of developing asthma in childhood. Immunological studies have found a significant association between gut microbiota and the development of immunoglobulin A (IgA)-, IgG- and IgM-mediated sensitisation in allergy, asthma and atopic dermatitis.

**Statement 9: IBD is associated with alterations in the gut microbiota**

Level of agreement: I, 60%; II, 20%; III, 20%; IV, 0%; V, 0%. Strength of evidence: A, 60%; B, 20%; C, 20%; D, 0%.

There is increasing prevalence of IBD within the Asia-Pacific region, including Malaysia, with figures comparable to the West. The lack of diversity of beneficial gut microbiota in early life is thought to be an important cause. Beneficial gut microbiota (e.g. *Bifidobacterium* and *Lactobacillus*) protects the intestinal mucosa of early life by neutralising pro-inflammatory cytokines.
and chemokines released by harmful pathogens.\textsuperscript{53} Infants with less-beneficial microbes may therefore have a higher susceptibility to IBD.\textsuperscript{52} The loss of microbial diversity in IBD at a later age has been attributed to fewer members of the \textit{Firmicutes} phylum.\textsuperscript{54} Furthermore, the success of probiotic mixture (VSL\#3), \textit{Faecalibacterium prausnitzii} and \textit{Bacteroides fragilis} in IBD suggests a causal role of dysbiosis in IBD.\textsuperscript{55,56}

**Statement 10: Obesity is associated with alterations in the gut microbiota**

Level of agreement: I, 20\%; II, 40\%; III, 40\%; IV, 0\%; V, 0\%.
Strength of evidence: A, 10\%; B, 60\%; C, 30\%; D, 0\%.

Paediatric obesity is a major public health concern in Malaysia. The Malaysian National Health and Morbidity Surveys in 1996 and 2006 reported a three-fold increase in the prevalence of obesity among adolescents, increasing from 4.4\% to 14.0\% over a decade.\textsuperscript{57} A recent study reported that obese individuals harboured fewer \textit{Bacteroides} and more \textit{Firmicutes} compared with non-obese individuals.\textsuperscript{58} A high fat diet may contribute to imbalances in the gut microbiota and disrupt the gut barrier integrity, leading to increased endotoxaemia and metabolic diseases.\textsuperscript{59} Additionally, the efficiency of food conversion in obese individuals is higher and thus provides the host with a greater amount of usable energy in the form of SCFAs, which contribute to adiposity, insulin resistance and type 2 diabetes.\textsuperscript{60} Increased production of SCFAs in obese children also implies more efficient colonic fermentation that may be involved in the aetiology of childhood obesity.\textsuperscript{58}

**Statement 11: Infantile colic is associated with alterations in the gut microbiota**

Level of agreement: I, 0\%; II, 30\%; III, 50\%; IV, 20\%; V, 0\%.
Strength of evidence: A, 0\%; B, 50\%; C, 40\%; D, 10\%.

Infantile colic is a non-specific disorder with a possible behavioural or functional origin.\textsuperscript{61} In Pakistan, it has been shown that 21.8\% of their infants are affected by this condition, mostly within the first 12 weeks of life.\textsuperscript{62} Although Párty et al. and de Weerth et al. have shown that the stability of gut microbiota in infants with colic were different from those without colic, the factors or mechanisms accounting for these different microbial signatures are not clear.\textsuperscript{63,64} Furthermore, effectiveness of probiotics in randomised controlled trials of infantile colic have been inconclusive.\textsuperscript{65} More studies are needed to determine the role of gut microbiota in the pathogenesis and management of infantile colic.

**Statement 12: Prebiotics and probiotics play a role in modulating the gut microbiota in early life**

Level of agreement: I, 50\%; II, 40\%; III, 10\%; IV, 0\%; V, 0\%.
Strength of evidence: A, 40\%; B, 50\%; C, 10\%; D, 0\%.

Prebiotics

‘Prebiotics’ are selectively fermented ingredients (usually indigestible oligosaccharides) that result in specific changes in the composition and/or activity of the gut microbiota, thus conferring benefits to host health.\textsuperscript{66} Some prebiotics (e.g. galacto-oligosaccharides or inulin-type fructans) in prebiotic-supplemented infant formulas exert similar functions as the HMOS in early life.\textsuperscript{67} More recently, the World Allergy Organization (WAO) recommends the use of prebiotic supplementation in non-exclusively breastfed infants.\textsuperscript{68} In addition, prebiotics improve stool consistency and transit, thereby reducing constipation.\textsuperscript{69} Other gut health benefits of probiotics in early life include alleviation of GI discomforts in functional GI disorders (but it may cause bloating) and reduction in the risk of immune-related diseases.\textsuperscript{70} The use of prenatal probiotics may increase maternal intestinal \textit{Bifidobacterium} and enhance glucose metabolism, hence reducing the risks of gestational diabetes and pre-eclampsia.\textsuperscript{70}

**Statement 13: Probiotics should be recommended in diseases associated with gut dysbiosis**

Level of agreement: I, 10\%; II, 40\%; III, 40\%; IV, 10\%; V, 0\%.
Strength of evidence: A, 10\%; B, 40\%; C, 40\%; D, 10\%.

Acute diarrhoeal disease in early life is a leading cause of significant morbidity and mortality in developing countries. Randomised controlled trials have shown that specific probiotic strains are useful to treat acute diarrhoeal illness. For example, \textit{Lactobacillus ruminis} is used for cases of moderate to severe infective diarrhoea,\textsuperscript{76} while \textit{Saccharomyces boulardii} is used for cases of rotavirus-associated diarrhoea.\textsuperscript{77} Allergic eczema is another condition that is closely related to gut dysbiosis. Based on the recommendation of the WAO and also from available evidence, probiotics may provide a net benefit in reducing the risk of eczema in pregnant women at high risk of having an allergic child, in women who breastfeed infants at high risk of developing eczema and in infants at high risk of developing eczema.\textsuperscript{76,79} In addition, probiotic supplementation with \textit{L. paracasei F19} during the weaning period has been shown to reduce the incidence of atopic dermatitis in infants.\textsuperscript{80}

**Conclusion**

The Working Group ascertained that a number of early life factors are involved in modulating the gut microbiota, and these factors affect subsequent gut health. However, further studies are needed, especially in the Asia-Pacific region, to understand how the gut microbiota in early life affects subsequent diseases, including allergy, IBD, obesity and infantile colic. The Working Group also cautions the use of probiotics and prebiotics in early life due to current limited evidence. It is hoped that through this position paper, there will be greater awareness and research interest in the area of gut microbiota in early life from the Asia-Pacific region.
Fig. 3 Mechanism of action of probiotics. (Adapted from Bermudez-Brito et al.74 and Gogineni et al.,75 with permission.)

Acknowledgements
An unrestricted educational grant was received from Danone Dumex (M) Sdn. Bhd. We also thank Yong Fung Lin, Audra Paliany and Joslyn Ng from MIMS Medica Sdn. Bhd, for their voting and manuscript preparation. The authors disclose no financial arrangements with the funding company. The sponsor did not participate in the collection of literature, discussion, voting and manuscript preparation.

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