أثر إمداد المغذيات الحيوية على البكتيريا المعوية لدى الأطفال حديثي الولادة المبتسرين

رسالة

توطئة للحصول على درجة الماجستير
فى طب الأطفال

مقدمة من

الطبيب/ كامل عمر عبد الكامل إمام
بكالوريوس الطب و الجراحة، ٢٠٠٧
كلية الطب – جامعة عين شمس

تحت إشراف

أ.د/ سناء يوسف شعبان
أستاذ طب الأطفال
كلية الطب – جامعة عين شمس

د/ أماني رفعت البيلي
أستاذ مشارك بمعهد تكنولوجيا الغذاء
مركز البحوث الزراعية

أ.د/ غادة عبد الواحد إسماعيل
أستاذ الباثولوجيا الإكلينيكية والكيميائية
كلية الطب – جامعة عين شمس

كلية الطب
جامعة عين شمس
٢٠١٤

The Effect of Prebiotic Supplementation on Preterm Gut Microbiome

Protocol of Thesis

Submitted for partial fulfillment of Master Degree in Pediatrics

Presented by

Kamel Omar Abd El Kamel Emam
M.B, B.Ch, 2007
Faculty of Medicine- Ain Shams University

Under Supervision of

Prof. Sanaa Youssef Shaaban
Professor of Pediatrics
Faculty of Medicine - Ain Shams University

Dr. Amany Refaat El Bialy
Associate Professor of Food Technology Institute
Agriculture Research Center

Prof. Ghada Abd El Wahed Ismail
Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams Universit

Faculty of Medicine
Ain Shams University
2014

First thanks to **ALLAH** *to whom I relate any success in achieving any work in my life.*

I wish to express my deepest thanks, gratitude and appreciation to **Prof. Dr Sanaa Youssef Shaaban.,** *Professor of Pediatric for his meticulous supervision, kind guidance, valuable instructions and generous help.*

Special thanks are due to **Prof. Dr. Ghada Abdelwahed Ismail,** *Professor of Clinical and Chemical Pathology for her sincere efforts and fruitful encouragement.*

I am deeply thankful to **Dr. Amany Refaat El Bialy** *Associate Professor of Food Technology Institute, for her great help, outstanding support, active participation and guidance. My thanks and appreciation are to my family, I owe my utmost gratitude for all their support and understanding.*

Kamel omar

INTRODUCTION

It is well known that both the establishment of an optimal microbial community immediately after birth and the maintenance of a balanced intestinal microbiota represent important factors for the development of the immune system ***(Hooper and Macpherson, 2010; Round and Mazmanian, 2009).***

The colonisation of the human gut by micro-organisms starts around birth, during birth and immediately afterwards. Bacteria from the mother and the surrounding environment colonise the infants gut. However it has been suggested recently that the colonisation process or exposure to microbial compounds may start before birth and that infants may also receive microorganisms from the mother during gestation ***(Hong et al. 2010, Jimenez et al. 2008 and Satokari et al. 2009).***

Initial colonisation follows successive steps, firstly dominated by facultative anaerobes such as Enerobacteria coliforms and Latobacilli, followed by anaerobes genera such as *Bifidobacterium acteroides, Clostridium* and *Eubacterium.* This process is influenced by several factors such as genetic background, gestational age, mode of delivery (caesarean section vs. vaginal delivery), type of feeding (breastfeeding vs. formula-feeding) and antibiotic therapy ***(Adlerberth et al., 2007; Biasucci, et al 2008; Grönlund et al. 1999; Huurre et al., 2008 and Penders et al., 2006b).***

The term ***prebiotic*** was introduced ***by Gibson and Roberfroid 1995*** **"PRE"** which means "Before".

Prebiotics are non-digestible oligosaccharides that reach the colon intact and are known for their ability to selectively stimulate the growth and activity of bacteria that exert positive health effects ***(Gibson and Roberfroid, 1995).***

The beneficial effects on the immune system are commonly ascribed to the stimulation of the growth and metabolism of protective commensal intestinal bacteria ***(Boehm et al. 2005 and Langlands et al., 2004).***

An increase in the number of beneficial bacteria will provide antimcrobial effects by direct competition with pathogenic bacteria for available binding sites on intestinal epithelium and for nutrients *Bifidobacterium* species and *Lactobacillus* species are also able to produce antibacterial substances that can inhibit the growth and survival of pathogens ***(Gibson and Wang 1994).***

Criteria for eligibility as prebiotic*: (Gibson et al., 2007).*

1) Selective stimulation of the growth and/or activity of those intestinal bacteria that contribute to health and well-being
2) Resistance to gastric acidity, to hydrolysis by mammalian enzymes, and to gastrointestinal absorption;
3) Fermentation by intestinal microflora; to produce SCFA & gas.
4) Induce luminal or systemic beneficial effects.

AIM OF THE WORK

Comparing the effect of prebiotic supplemented versus non supplemented on their gut microbiome in preterm infants.

Chapter I

PREMATURITY

Preterm birth is defined by the estimated gestational age as a proxy of maturity. Three subgroups are distinguished by the World Health Organisation (WHO): preterm (< 37 weeks gestation), very preterm (< 32 weeks), and extremely preterm (< 28 weeks) ***(WHO 2007)***

In the United States of America and several other countries a classification according to birth weight is generally used. Low birth weight infants are defined as those with a birth weight of 2, 500 g or less, which may be due to prematurity, being born small for gestational age (SGA), or both. Similarly, lower cut-off limits for weight have been used to describe more severe cases, i.e. very low birth weight (VLBW < 1, 500 g) ***(Sherry B et al., 2003; Hack M et al., 2003).***and extremely low birth weight (ELWB < 1, 000 g) ***(Valcamonico A et al., 2003).*** In very preterm and/or VLBW infants, gestational age is a better predictor of short-term survival than birth weight ***(Verloove P et al., 1986).***

Also Preterm is described by birth on or before the end of the last day of the 37^{th} week (259^{th} day) following the onset of the mother's last menstrual period ***(Engle, 2006)***.

Clinical assessment of prematurity:

Clinical assessment of neonatal gestational age can be obtained by the use of **modified Dobwitz** examination, which has been further modified to achieve greater accuracy. The newly expanded new **Ballard** score (NBS) provides of valid and accurate assessment of gestational age for extremely premature infants that were not previously available ***(Ballard et al., 1991).***

The system is used to evaluate the gestational age through recording physical criteria that might differentiate extremely premature infants from more mature infants and a final score is obtained following the addition of each category score. The system is accurate ±2weeks confirmed gestational last menstrual period and gestational age by prenatal ultra-sonography ***(Lee and Cloherty, 2004).***

Incidence and prevalence:

More than 1 in 10 of the world's babies born in 2010 were born prematurely, making an estimated 15 million preterm births (defined as before 37 weeks of gestation) ***(Blencowe et al., 2012).***

In order to have the incidence of prematurity in Egypt, we should have a national survey and this was not conducted. However from a well documented survey, the incidence is approximately 11% in Menia Governorate, and 50% in El

Qalyubia Governorate and much less in Cairo. The estimated percentage of LBW in Egypt ranged (5.9%-13%) was reported in 1997. LBW was more frequent among females (13.2%) than among males (11.2%) ***(El-Rafie, 2002 and Mansaur et al., 2002)***.

Mortality:

Prematurity compromises more than two thirds of all perinatal deaths ***(Lumel, 2003)***. Prematurity is now the second-leading cause of death in children under 5 years and the single most important cause of death in the critical first month of life ***(Liu et al., 2012).***

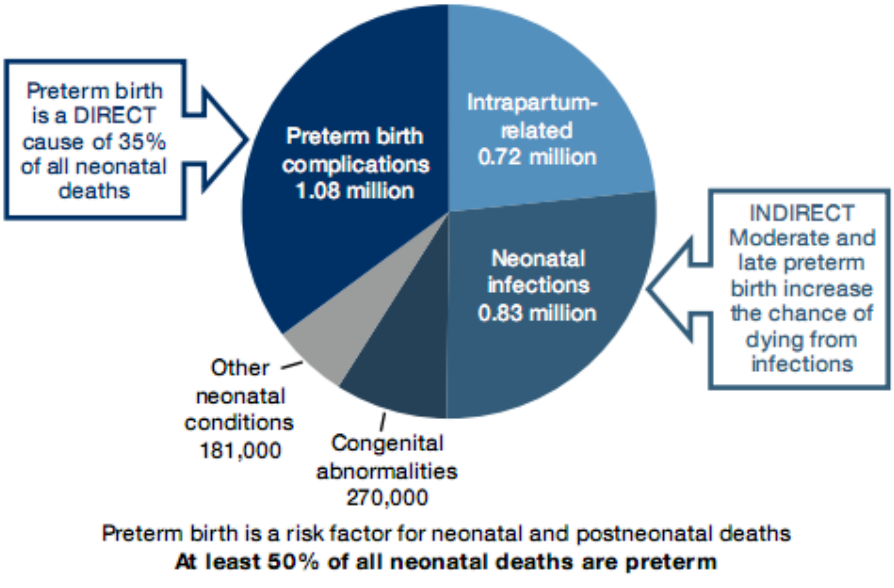

Fig. (1): Estimated distribution of causes of 3.1 million neonatal deaths in 193 countries in 2010 ***(Liu Let al., 2012.)***

The lower the birth weight the higher the neonatal mortality, as the birth weight increase from 500 gm to 3000gm, algorithmic decrease in neonatal mortality occurs ***(Kliegman, 2000)***.

Excluding the neonatal period, risk of mortality between 28 days and 1 year is still 2 times higher in the preterm group, suggesting an unmet need for continued vigilance and follow-up. This might be related to the fact that apparent life-threatening events and sudden unexplained deaths in infancy are much higher in late-preterm than term-born infants ***(Hunt, 2006)***.

Intensive care has extended the period during which a VLBW infants is likely to die from complications of perinatal diseases such as chronic lung disease, necrotizing enterocolitis or secondary infection ***(Band et al., 2000).***

Survival rate according to gestational age is:

- 21 weeks or less: 0%survival rate.
- 22 weeks: 0-10% survival rate.
- 23 weeks: 10-35% survival rate.
- 24 weeks: 40-70% survival rate.
- 25 weeks: 50-80% survival rate.
- 26 weeks: 80-90% survival rate.
- 27 weeks or more: greater than 90% survival rate.

(Ward and Beachy, 2003)

Risk factors of preterm births:

Prematurity results from three clinical conditions: medically indicated preterm birth (Iatrogenic), preterm premature rupture of membranes (PROM) and spontaneous preterm births (Idiopathic) ***(Goffinet, 2005)*.**

Epidemiological studies have identified several risk factors for preterm birth such as prior preterm birth, black race, teenage or older mothers, those with low education and of low socioeconomic status, cigarette smoking, unmarried or not living with a partner, heavy and/or stressful occupation, low maternal pregnancy body mass index and poor or excessive weight gain ***(Wen et al., 2004)***.

Medical and obstetrical complications including multifetal pregnancy, gestational or pre-existing diabetes (essential or pregnancy induced), hypertension, placenta previa and placental abruption, poly or oligohydraminos, abdominal surgery in the late second or third trimester and systemic or regional infection (such as asymptomatic bacteruria) are also strongly related to preterm birth ***(Gerstenfeld et al., 2000)***.

The obstetric precursors leading to preterm birth are: delivery for maternal or fetal indications, in which labour is either induced or the infant is delivered by prelabour caesarean section (30-35%); spontaneous preterm labour of unknown etiology (40-45%), with the remainder resulting from such obstetrical

complications as preterm premature rupture of membranes (PPROM) (25-30%). Births that follow spontaneous labour and PPROM are together designated spontaneous preterm births ***(Tucker et al., 1991)***.

Spontaneous preterm birth is most commonly caused by preterm labour in white women, but by PPROM in black women. Preterm labour is usually defined as regular contractions accompanied by cervical change at less than 37 weeks' gestation ***(Ananth and Vintzileos, 2006)***. PPROM is defined as spontaneous rupture of the membranes at less than 37 weeks' gestation at least, before the onset of contractions ***(Mercer et al., 2000)***.

Preterm births can also be subdivided according to gestational age: about 5% of preterm births occur at less than 28 weeks' (extreme prematurity), about 15% at 28-31 weeks' (severe prematurity), about 20% at 32-33 weeks' (moderate prematurity), and 60-70% at 34-36 weeks' (near term) ***(Goldenberg et al., 2008)***.

Growth in Preterm infants:

A large proportion of prematurely born infants show evidence of postnatal growth impairment irrespective of whether birth weight was appropriate or small for gestational age. The timing and magnitude of catch-up growth is very variable, with the most immature infants showing markedly delayed catch up which is often incomplete ***(Pilling et al., 2008)***. Catch up is a properly of

human growth whereby children return to their genetic trajectory after a period of growth arrest or delay ***(Ong et al., 2000)***. It may occur at any stage of growth, but is most commonly observed in the first 1-2 years of life, and pronounced catch-up growth postnatal is often seen after severe intrauterine growth restraint ***(Karlberg and Albertsson, 1995)***.

Catch up growth can be defined as a gain in weight and length greater than estimated from the intrauterine (or postnatal) growth curve. Recent studies indicate that catch up growth might be advantageous for brain development ***(Sauer, 2007)***.

Problems of prematurity:

Preterm delivery is a major cause of perinatal mortality and morbidity. Respiratory distress (RDS), persistent pulmonary hypertension, intracranial hemorrhage, as well as necrotizing enterocolitis are due to the difficulty of extra uterine adaptation due to immaturity of organ systems ***(Hohlagschwandtner et al., 2001;Watts and Saigal, 2006).***