Excessive infant crying

New insights in the role of parental factors and long-term stress through hair cortisol analysis

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Excessive infant crying

New insights in the role of parental factors and long-term stress through hair cortisol analysis

ACADEMISCH PROEFSCHRIFT

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Als het huilen niet meer te dragen,

Zijn er altijd dagen.

Waarop lichtjes in jouw ogen verschijnen,

En het verdriet lijkt te verdwijnen.

MH

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GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

A case report of one of our families with an excessively crying infant

Boy A, was seen at the age of 3 weeks due to "excessive crying" together with his mother and father at the outpatient clinic of a secondary hospital. He was the first child of the parents and was born at 39 weeks with a normal birth weight of 3740 grams, and a good start, Apgar 9/10. The mother experienced the delivery as very stressful. The crying began a week after birth, and at the time of the consultation, the parents reported inconsolable crying for 12 hours per day. He vomited small amounts after each feeding, grimaced, and overstretched, and the parents were convinced that he was in pain. He had a normal defecation pattern, no skin abnormalities, and no fever. After a short period of breastfeeding he was fed formula thickened with nutriton, and the parents started swaddling him. These interventions had no effect, and both parents were desperate. The paediatrician saw a healthy, well-fed baby, regurgitating and crying during both the history, and the physical examination. After checking the urine, which showed no signs of infection, the paediatrician tried to reassure the parents. Despite an extensive explanation about the aetiology of infant crying, the parents were convinced that pain before and during the vomiting required medication. Surprisingly, the paediatrician prescribed a proton pump inhibitor and called the parents two weeks later. The parents said that the crying was progressive, and because the family history showed allergic rhinitis in both parents and an allergy to cow's milk in the father when he was a baby, the paediatrician advised to stop the proton pump inhibitor and to change to a protein hydrolysate formula. The next evening, the parents phoned the hospital in crisis ("we can no longer handle this"), and their son was hospitalized for seven days. During the admission, their son slept a lot, cried two hours per day, was easily comforted. Despite this clinical observation the mother remained concerned. One evening, she told one of the nurses, about her troubles with sleeping, her history of depression and her progressive depressive symptoms at this moment. After this, she was diagnosed by the consultant psychiatry with postpartum depression. After the admission of her son to the paediatric ward, intensive home treatment began. Unfortunately, the mother became more and more depressed, and a month later, she was admitted with her son to the Mother-Baby Unit, a specialized psychiatric unit of the hospital. She was treated for her depression with a serotonin reuptake inhibitor and for her sleep problems with a benzodiazepine. The mother reported bonding difficulties and mother-child interaction therapy was started during admission. After an intensive therapy trajectory of three months, mother felt much better and her son cried only one hour per day and they could be discharged from the Mother-Baby Unit. During follow-up, parent–child therapy was continued at home, as the father at this time also reported bonding difficulties, and both parents and child recovered well.

Based on the medical history, clinical evaluation and treatment as described, both parents and healthcare professionals addressed several important questions:

- "Did the mother's history of depression, and the psychological vulnerability that arises from this, trigger the crying and vomiting of her baby?"
- "How about the feelings of father? Are they influenced by the excessive crying of his son and/or by the depression of the mother?"
- "When the baby started crying, it caused stress in this family. Are there ways to measure chronic stress?"

- "Is the amount of stress experienced by the mother and her son affected by the fact that she had been dealing with psychiatric disease in the years before?"

Unfortunately, scientific evidence is lacking to provide parents and healthcare professionals with answers to these important questions, many of which are necessary to optimally guide this family through this challenging period. The introduction of this thesis will shed some light regarding the current knowledge on excessive infant crying, parental factors, and hair cortisol analysis.

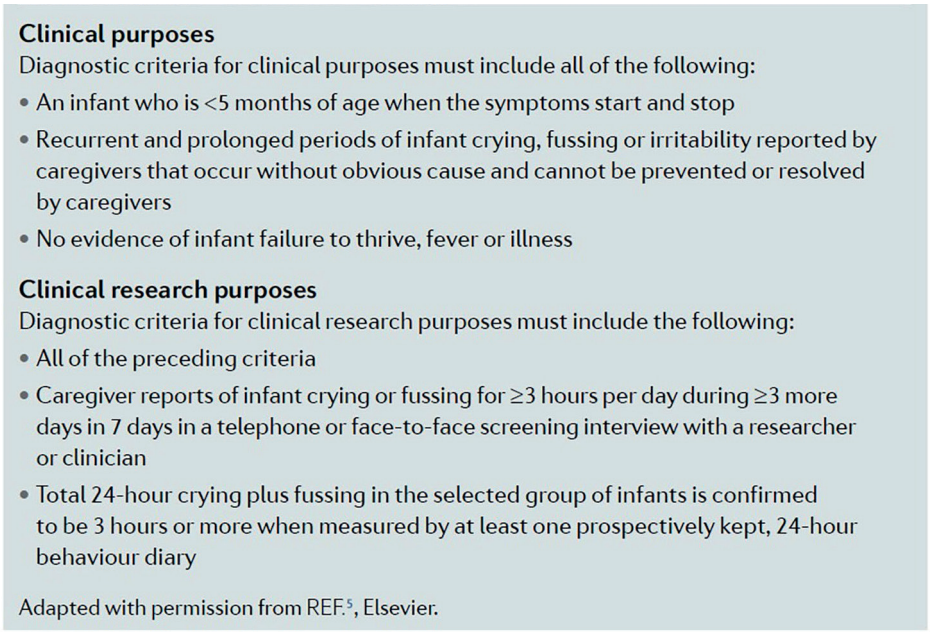
DEFINITION AND BACKGROUND

Excessive infant crying or infant colic (IC), as formulated by the Rome IV committee in 2016 (1), is one of the most distressing challenges for new parents. The crying of an infant is one of the earliest and most powerful forms of communication and is essential for protection and nurturing and this evolutionary selected behaviour likely increases the infant's chances of survival. This is why it is one of the most appealing and stressful sounds which exist; one of the most stress-inducing tapes used in the torture of prisoners in the United States-run military detention centre in Guantánamo Bay, Cuba, were recordings of babies crying inconsolably.(2) The crying stresses parents, and they search the internet and ask their family and friends what to do and are eventually referred to a paediatrician. Excessive crying accounts for 10% to 20% of paediatric consultations during the first weeks of an infant's life (3,4) and affects between 4% to 20% of infants worldwide, depending on geography and the definitions used. (5–9) Parents are often concerned that the recurrent and prolonged periods of crying arise from an organic cause, which is found in only 5% of cases. (10) A thorough and careful history and physical examination of the infant and information on the normal "crying curve" can help to reassure parents and support them through this challenging period. (11) To be able to reassure and support both parents, information on family stress, which is rated as an important factor in infant colic (12), is essential.

Most of the available definitions for excessive crying focuses on the duration of crying and increasingly on the effect it has on parents. (11,13) The crying typically presents in the second or third week of life, peaks around six weeks, and usually resolves by the age of twelve weeks. (14,15) The affected infant has periods of inconsolable crying, irritability, and screaming without an obvious cause, and during these episodes of fussiness, which occur more frequently in the evenings, there is a classically red-faced appearance, flexion of the hips, and clenching of the fists. These additional characteristics have led to the suggestion that the crying might be related to abdominal discomfort, and therefore the term "infant colic," derived from "kolikos", the Greek term for colon, is often used. (16–18) A systematic review on definitions and outcome measures in trials of IC reported great differences and variability in defining IC, which parallels the non-uniformity of measuring IC. (19) In this review, 20 different definitions for IC were used in 39 trials, and most of these definitions were based on either Wessel's criteria (20) or the Rome III criteria. (21) Wessel's criteria, also known as the "rule of threes", defines excessive crying as paroxysms of irritability, fussing, or crying lasting ≥3 hours per day on ≥3 days per week in any one week with paroxysms for >3 weeks in an otherwise healthy baby aged two weeks to four months. However, over the years, the consensus has grown that in daily practice, the

parental experience of recurrent and prolonged crying appears to be more important than the exact measurement of crying hours. Therefore, the most recent diagnostic criteria of IC for clinical purposes, formulated by the Rome IV committee in 2016, are recurrent and prolonged periods of crying without an obvious cause or evidence of failure to thrive or illness in infants younger than five months. (1) For clinical research purposes, to fulfill the definition of colic these episodes of crying or fussiness should last at least three hours per day, for a minimum of one day when measured by a prospectively kept 24 hour behavior diary or three days per week according to a parental interview. See Figure 1.

Figure 1. Rome IV criteria for infant colic



Clinical purposes

Diagnostic criteria for clinical purposes must include all of the following:

- An infant who is <5 months of age when the symptoms start and stop
- Recurrent and prolonged periods of infant crying, fussing or irritability reported by caregivers that occur without obvious cause and cannot be prevented or resolved by caregivers
- No evidence of infant failure to thrive, fever or illness

Clinical research purposes

Diagnostic criteria for clinical research purposes must include the following:

- All of the preceding criteria
- Caregiver reports of infant crying or fussing for ≥3 hours per day during ≥3 more days in 7 days in a telephone or face-to-face screening interview with a researcher or clinician
- Total 24-hour crying plus fussing in the selected group of infants is confirmed to be 3 hours or more when measured by at least one prospectively kept, 24-hour behaviour diary

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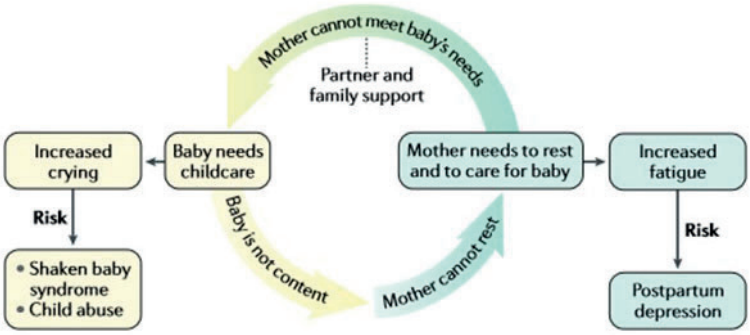
Although benign and self-limiting, only 5% of distressed infants have identifiable medical explanations for their crying. (10) If not, this condition can be highly frustrating and stressful for parents, and infant crying is the strongest risk factor for infant abuse, including abusive head trauma and shaken baby syndrome. The shape of the age-specific incidence curve for shaking/abusive head trauma is similar to that of the normal crying curve. (22,23) In addition, excessive crying can lead to exhaustion and ultimately to postpartum depression in mothers (Figure 2).

Figure 2. The vicious circle of infant crying and maternal fatigue and adverse events



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Indeed, parental factors play an import role in IC: family stress and quality of life are rated as two of six most important outcomes in IC. (12) The term “infant colic” is used predominantly in paediatric and paediatric gastroenterological literature and the terms “excessive (infant) crying” and “regulation disorder” are used in psychiatric, obstetric, and infant mental health literature. To increase multidisciplinary readability, I preferably use the term “excessive (infant) crying” in this thesis.

PATHOPHYSIOLOGY

The pathophysiological mechanisms underlying excessive infant crying are poorly understood. It is likely to be a multifactorial condition (11), and proposed causes include gastrointestinal factors, parental factors, and neural factors.

Gastrointestinal factors

Several gastrointestinal factors have been proposed to contribute to the pathogenesis of excessive crying in infants. First, the causative role of an allergy to cow’s milk has been studied in several randomized controlled trials in infants with excessive crying. These studies have shown that the use of protein hydrolysate formulas and a maternal milk-free diet decrease infant crying time (24–27); however the found benefits of these dietary modifications are inconsistent and therefore cannot be recommended due to research with small sample sizes and poor quality. (28)

Second, the role of lactose intolerance has been postulated as a possible causative factor for excessive crying. Carbohydrate malabsorption leads to the colonic fermentation of sugars and an increase in levels of hydrogen. Studies investigating breath hydrogen levels in infants with excessive crying versus hydrogen levels in infants without excessive crying have shown conflicting results. (29–31) The authors of a recent Cochrane review (28) concluded that although lactase enzyme supplementation has no adverse effects, they were unable to conduct a meta-analysis due to the significant heterogeneity of outcome reporting and limited data within the reports. In addition, the studies were cross-over

trials that did not report data from before washout.

Third, there are two studies on the possible role of gut hormones in the pathophysiology of excessive crying. One study showed significantly higher levels of motilin and ghrelin, which are hormones that affect gastric emptying and intestinal peristalsis, resulting in intestinal pain and hyperperistalsis in excessive crying infants compared to infants without excessive crying. (32) Another study showed significantly higher urine levels of a metabolite of serotonin, which possibly affects infant gastrointestinal motility, pain conduction, and pain sensation, in infants with excessive crying compared to infants without excessive crying. (33)

Fourth, a poor feeding technique has been suggested to be related to excessive crying. An improper feeding technique, such as underfeeding or overfeeding, or infrequent burping might play a role in excessive crying in ineptitude (primiparous) parents.

Fifth, gastro-oesophageal reflux (disease) (GER(D) has been proposed to have a relationship with excessive infant crying due to overlapping symptoms in both entities, such as crying, irritability, and restlessness. (34,35) As only one study supports the contribution of GER(D) and several studies argue against this contribution, the evidence of a cause–effect relationship between GER(D) and infant colic is weak. (36,37) The maternal perception of infantile gastrointestinal symptoms like GER is strongly influenced by psychosocial stress factors and plays an important role in the decision to seek medical care. (1) A mixed-methods study showed that mothers with a psychiatric disorder were nearly five times more likely to have a baby admitted with complaints of GER in the first year after birth. (38) In addition, maternal psychopathology played a significant role in feeding problems in 39 children with GERD when compared with 39 control children up to three years of age. (39) Recently, it was shown that infants of mothers with postpartum-elevated depressive symptoms had an increased risk of physician-diagnosed GER. (40) Although mothers with a psychiatric history have an increased risk for maternal depression during and after pregnancy (41,42), experience more parenting stress and have more problems with bonding with their infants during the first months postpartum in comparison with mothers without psychiatric problems (43), it is uncertain whether their infants are more at risk for GER(D) and what the role of mother-infant bonding and maternal depressive symptoms is.

In **Chapter 1** we evaluate the presence of GER in infants of mothers with and without a history of a psychiatric disease. As infants often present with a combination of other gastrointestinal symptoms such as constipation and crying (1,44), we have also investigated the presence of functional constipation and excessive crying in infants of mothers with, and without a history of psychiatric disease. In addition, we evaluated the association of infant GER, functional constipation and excessive crying with maternal depressive symptoms and the possible mediating role of bonding.

There is growing evidence that among the gastrointestinal factors the intestinal microbiota in infants plays an important role in the pathophysiology of excessive crying. The microbiota of excessively crying infants differs from the microbiome of healthy controls as higher levels of anaerobic bacteria, such as coliform and Escherichia coli, and microaerophilic bacteria such as helicobacter pylori and a lower concentration of lactobacilli have been reported in infants with excessive crying. (11,45–48) In addition

to this intestinal dysbiosis, gut inflammation, altered hepatic bile acid production and regulation, and enteric nervous system immaturity, may play a role in infants with excessive crying. Crying behaviour as the result of this interplay would be facilitated by the microbiota–gut–brain axis that links the brain with peripheral intestinal functionalities in a bidirectional manner. (11,49–51) Through this axis, intestinal dysbiosis can affect central and enteric neuronal functions, such as the detection of pain in infants (50,52), which could potentially play a role in excessive crying. (53) Further research is necessary to reveal these theories and to further unravel the potential underlying mechanisms.

Parental factors

Whether crying is defined as a problem is mainly dependent on the perception of the parents regarding what they experience as excessive crying. Indeed, in recent years, there has been a shift from the traditional focus on the infant to increased attention on the impact of crying on parents as well as parental vulnerabilities that may lead to non-optimal or harmful responses. (54) Feelings of powerlessness to comfort and help their infants can cause desperation (55), make parents feel frustrated (56), and create a vicious cycle in which crying leads to parental stress, which negatively affects the parent-infant relationship and often leads to more crying. (57) Previous research has consistently shown that excessive crying is highly associated with both maternal postpartum depression and anxiety. (58–60) In addition, excessive crying results in an elevated risk of developing postpartum depression, even after the crying has decreased. (61) Surprisingly, there is limited research on the impact of excessive crying on fathers, which is remarkable because in the past decade, fathers have increasingly become involved in early childcare. There are good reasons to assume that paternal involvement could exert significant influence on both the developing child and the mother. Indeed, it has been shown that depressive symptoms of fathers during maternal pregnancy are associated with excessive crying. (62) Excessive crying is also associated with increased paternal anger, doubt, and increased paternal depressive symptoms. (63) In addition, in one study, fathers with an excessively crying infant showed less optimal father-child interaction in a video recording carried out during the infant's feeding, and diaper change and a discussion between the parents. (64)

In **Chapter 2** we assess stress, depression, anxiety, and bonding problems in fathers and mothers presenting at the outpatient clinic of a secondary hospital with an excessively crying infant and compare these data with those of parents with infants of the same age who did not cry excessively, who were recruited predominantly in the surrounding infant welfare centres.

Other parental factors involved in excessive crying are parental smoking (65–68), younger maternal age, being a single mother (60) , ethnicity (69) , low access to advice, information, material resources and practical services during pregnancy, and mothers whose job involves the use of their mind rather than the use of their hands or physical strength, also referred to as non-manual occupations. (70) Having a job with high demands and high decision latitude at work act synergistically with a non-manual occupation, yielding even higher odds ratios for an infant with excessive crying.

Neural factors

There are different hypotheses proposed that the brain contributes to excessive infant crying. Neurobiological, neurodevelopmental and neurohormonal mechanisms are described in the next section.

Neurobiologically, crying can be considered a normal behaviour. It serves survival and can be characterized as proximity-promoting behaviour and a primary means of communication between infant and parent(s) in the first months of life. (54) It has been suggested that excessive crying may be considered the upper end of a spectrum of individual differences in normally developing infants. (71) It is striking that this difference can already be noticed in the reaction of very young infants on day one or two in the maternity ward, of whom some can then be perceived as likely candidates to become excessively crying infants. These infants are sensitive with increased irritability, prone to intense reactions and less adaptable, and take longer to calm down with the usual measures of being held and cuddled. (4) These infant reactions are referred to in older literature as "temperament", a set of characteristics related to the type of response to stimuli, adaptive capacity (ability to calm down in stressful situations), and activity level. These individual differences in infants' abilities to regulate the crying state suggest that intrinsic differences in central nervous system functions contribute to the regulation of crying in young infants. Indeed, there is evidence that the sensory processing in excessively crying infants differs from that of infants who do not cry excessively; the infant crying following a neurobehavioral assessment was triggered more easily and there was a harder-to-soothe response in the excessive crying group. (72) Furthermore, a controlled clinical trial reported that infants with excessive crying were less able to regulate their crying and were less effectively soothed following a sucrose stimulation than control infants without excessive crying. (73) One of the hypotheses is that the reduced effect of sucrose tasting is due to an altered endogenous opioid system in infants with excessive crying. (74)

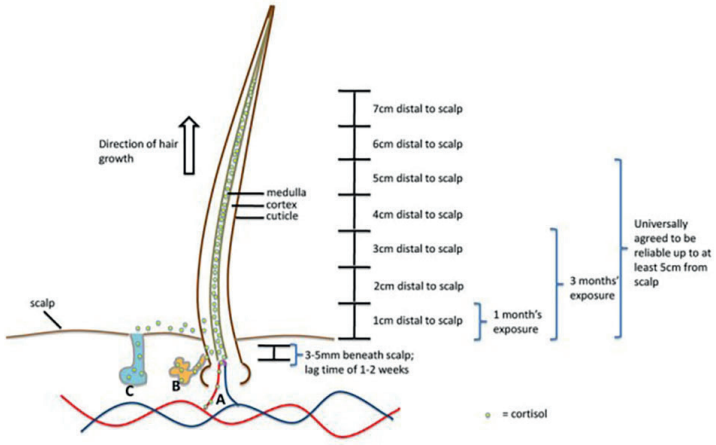
Neurodevelopmental maturity also plays a role in infant crying. This is supported by the fact that infants born with a central nervous system (CNS) impairment, cry more intensely (3), and infants born premature or small for the gestational age show an increased risk of excessive crying during the first months of life. This hypothesis is based on the fact that these groups are also at increased risk of an developmental delays, which may be related to the immaturity of the CNS as well as the digestive system. (4)

Neurohormonally, it is hypothesized that the circadian system plays a role in infant crying. The circadian system, regulated by the hypothalamus, affects physiological activities, such as sleep time, body temperature, and feeding, and the production of hormones such as melatonin and cortisol, and matures during the first three months of life. (75) Melatonin is secreted by the pineal gland in a circadian pattern, with the highest amount released during the night. Although no change in total hours of sleep has been demonstrated in infants with excessive crying, they show a more differentiated pattern in random eye movement sleep and a fragmented sleep pattern. (76) Breastfeeding probably plays a protective role: infants with exclusive breastfeeding have less excessive crying, and nocturnal breast milk contains substantial amounts of melatonin. (77) In the process of waking from sleep in the morning, cortisol, the glucocorticoid hormone secreted by the adrenals after the activation of the neuroendocrine HPA axis, peaks and has a daily rhythm with a nadir around midnight. The HPA axis is a major homeostatic system that is activated in response to stress. It is hypothesized that crying in the first months signals the activation of the HPA axis and adrenergic neuronal circuitry in response to perceptions of discomfort or threats. Excessive crying could result from positive feedback loops in the HPA and adrenergic systems. (78) Limited studies support the association between cortisol, measured in saliva, and excessive infant crying. (76) In one study, the psychological wellbeing of mothers of 24 excessively crying infants was predicted by the infant morning salivary cortisol levels and crying intensity. (79) Another study, including 20

excessively crying infants and 20 controls showed a blunted circadian rhythm of cortisol in the excessively crying group. (80) This may be an important finding, because in general, flatter diurnal cortisol rhythms across the day are associated with poorer health (81); however, cortisol measurements in saliva and blood represent short-term stress responses and momentary stress. (82) Long-term cortisol measurements are needed to study experienced chronic stress of parents dealing with an excessively crying infant.

In the last decade, hair cortisol concentrations (HCC) have been increasingly studied as biomarkers for HPA activity over weeks and months instead of minutes or hours. (83–85) Hair offers unique benefits and characteristics, which include non-invasive sampling and a long-term retrospective reflection of cumulative cortisol concentrations.

Figure 3. Proposed mechanisms for incorporation of cortisol into hair via blood (A), sebum (B), and sweat (C)



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These features make the use of scalp hair beneficial for both paediatric clinical practice and research. To date, limited studies of reference intervals of cortisol concentrations in children have been conducted (86–88), and no extended studies of reference intervals using the state-of-the-art method of liquid chromatography-tandem-mass spectrometry (LC-MS/MS) (89) have been published. Hair growth velocity is also essential in the interpretation of HCC in hair. Although it is generally accepted that hair in adults grows at a fairly constant rate of approximately 10 mm per month (90), meaning that the first centimeter proximal to the scalp is assumed to contain cortisol incorporated during the month prior to sampling, data of children's hair characteristics and hair growth patterns needed for the correct interpretation of their hair cortisol concentration are limited. (91,92) A study in 20 healthy children aged 3−9 years showed a growth rate of 9.6 mm per month. (93) To our knowledge, studies on the hair growth rate in healthy infants aged 0−2 years are lacking.

In **Chapter 3,** we aim to establish age-adjusted reference intervals for hair cortisol using LC-MS/MS in healthy children aged 0-18 years. In addition, we assess hair growth velocity in healthy children aged 0-2 years.

Excessive crying lasts for weeks to months and can be very stressful for parents; no HCC have been reported in excessively crying infants. To date, studies have shown increased HCC in relation to chronic stress exposure, especially when this was still ongoing at the time of the study. (93,94) In contrast, decreased levels of HCC have been found in anxiety disorders, such as posttraumatic stress disorder. (93,95) In children, HCC in relation to stress and/or trauma exposure and other psychosocial factors (96) has heterogeneous results. This may point to difficulties in defining and measuring "stress" as well as the fact that the HPA axis might be more sensitive to influences during certain developmental periods. Persistent stress, such as maternal distress, has been shown to be related to elevated hair cortisol in young children (97), and studies on fathers are lacking. It is also interesting to investigate objective methods to measure the severity of the stress experienced by parents and their babies suffering from excessive crying. (98)

In **Chapter 4,** HCC in infants, mothers and fathers of excessively crying infants is compared to HCC in control families without an excessively crying infant. In addition, we evaluate the association of parental HCC and experienced stress, depression, anxiety, and bonding problems.

Infants of mothers with psychiatric disorders during pregnancy are at a high risk for developing physical and psychiatric disorders later in life. (99,100) Psychiatric disorders are associated with alterations in basal cortisol levels and a disturbed variability of the stress response due to a dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis. (101,102) Accumulating research suggests that a placental transfer of maternal cortisol might play a mediating role in the effects of maternal psychopathology on the neurocognitive and physical development of the fetus (103), and increased prenatal maternal cortisol levels have been repeatedly linked to adverse child outcomes in the short- and long-term, such as lower birth weight, small for gestational age, intellectual disability and behavioural problems (104,105) as well as a "difficult" temperament. (106) As most previous research has focused on less-affected individuals, we aim to explore the effect of severe and long-lasting psychiatric disorders during pregnancy on early HCCs in mother–infant dyads.

In **Chapter 5,** perinatal HCC in mother-infant dyads with severe psychiatric disorders versus healthy controls are explored.

TREATMENT

For the majority of infants excessive crying is a self-limiting condition and should primarily be treated with empathy and reassurance**.** Reassurance that the infant is not sick is of major importance, and it may require multiple paediatric consultations with experienced caretakers before parents become confident with this idea. (26) The goals of management are to help the parents cope with the crying and to prevent long-term damage in the parent-infant relationship as well as child abuse (107); however, parents are often in a state of crisis and want action to be taken, leading to frequent hospital emergency visits, often on weekends and at night, and unnecessary therapeutic measures, such as feeding changes and medication. Parents also seek help for their excessively crying infant in nutritional and probiotic supplements, physical treatments such as massages and manipulation, and complementary medicines such as reflexology and acupuncture. (108)

Parental reassurance and education

The first step is to take time, be empathic, and listen to the parents carefully. It is essential for the paediatrician to acknowledge parental stress and fatigue, to identify the insecurities of the caregivers about their nurturing abilities, and to reassure the parents that these feelings are normal. (11) This is followed by a detailed physical examination and discussion of differential diagnoses with the parents. It might be helpful to assure parents that an organic cause is found in only 5% of infants with excessive crying. Next, the parents should be educated on the normal crying curve (10,14) and crying as a form of communication. (109) Parental education materials compared to control materials lead to higher knowledge about early infant crying and the dangers of shaking and to sharing information about walking away if frustrated. (110,111) In addition to parental reassurance and education, parent training using behavioural modification programs of infant care and environmental routines should be considered. A meta-analysis of three studies including 157 participants showed that parent training compared to routine care decreased infant crying time by 113.58 minutes per day. (28) The training program consisted of counselling to show how to be responsive to an infant, to understand the cues and needs of the baby and to use the appropriate responses. Furthermore, 1.8 hours per day of crying reduction was achieved in one study comparing kangaroo care versus a control group which was instructed to rock their infants in the crib. (112) Another study showed crying reduction in a group receiving family-centered treatment consisting of intensive support by a behavioural paediatrician and a mental health clinician compared to a control group receiving a brief office visit by their own healthcare provider. (113) Furthermore, counselling parents concerning more effective responses to infant crying reduced crying time more than the elimination of soy or cow's milk protein from the infant's diet. One Dutch RCT of 398 EC infants compared a parent training intervention with the same parent training plus swaddling training. The parent training consisted of regularity, predictability, and stimuli reduction in infant care. No differences were found between the two treatment groups, and infants of both groups demonstrated a 42% reduction of crying in one week of intervention. (114) In addition to the effects on infant crying and effective parental responses, some parent training programs reduced parental stress levels compared to routine care. (115,116)

Complementary therapies

The shortage of treatment options for excessive crying, in combination with a lack of confidence in traditional medicine and searches on the internet/social media may lead parents to seek complementary and alternative medicine (CAM).

At first, herbal formulations, such as fennel, lemon balm, and chamomile, have been suggested to relieve pain due to their anticholinergic and antiadrenergic activities. (117,118) Some evidence suggests that compared with a placebo or no treatment, herbal agents may reduce crying time; however, because the quality of these studies is very poor and the extent of the benefit observed was variable, these results should be interpreted with caution. (119,120)

Second, oral sucrose, which produces a calming effect or yields pain relief, might also have weak effects on reducing crying time in excessively crying infants. (11,73,119,121,122)

Third, manipulative therapies might alleviate biomechanical distress that originates during the birth process, which might have led to excessive crying symptoms by cranial moulding or cervical dysfunction. A Cochrane review (123) examining the effectiveness

of manipulative therapies (chiropractic, osteopathy and cranial manipulation), including six trials with 325 infants showed that it is impossible to achieve a definitive conclusion about the efficacy of manipulative therapies due to methodological shortcomings despite a greater proportion of parents reporting fewer hours crying per day than parents whose infants did not receive the therapy.

In addition, reflexology was evaluated in two small RCT's (124,125) with 75 infants, and both showed a reduction in crying, the most recent published one also showed reduction in "colic symptoms", similar to the study of Icke. (126) Definitive conclusions about the effectiveness of reflexology are difficult because evidence is scarce, and the methodology is impaired.

Lastly, acupuncture might attenuate symptoms of EC due to its inhibiting effect on somatic and visceral pain and its effect on the autonomic nervous system (11), although the acupuncture needles induce some treatment pain in many infants. Recent published systematic reviews concluded that acupuncture should not be recommended as available trials were small and presented conflicting results without clinically important differences between infants receiving acupuncture and no acupuncture. (108,127)

Probiotics

Because there is growing evidence that the intestinal microbiota in excessively crying infants is different than that in control infants, such as the concentration of anaerobic bacteria and lactobacilli, and that is plays a role in the pathogenesis, another promising option for the treatment of excessive crying may be probiotic supplementation. (11,128,129) Probiotics are non-pathogenic live strains of bacteria that when administrated in adequate amounts, exert beneficial effects on the host. In infants with excessive crying, it is postulated that probiotics promote conditions in the intestinal microbiome that activate protective host factors and inhibit the pathogenic effects of bacteria. Probiotics are substances that promote the growth of probiotic bacteria, playing a vital role in the modulation of the gut microbiome.

Probiotics consist mostly of strains of the genera Bifidobacterium and Lactobacillus, and the strains of Bacillus, Pediococcus and some yeasts have been introduced as suitable candidates for the treatment of excessive infant crying. These strains play an important role in the protection of the host against pathogen microorganisms and also strengthen the host's immune system in the maintenance of the intestinal microbial balance. (130,131) New trials and reviews are published almost monthly, and the probiotic Lactobacillus reuteri DSM 17938 has been used in the majority of treatment trials. This strain was found to significantly reduce crying time in breast-fed excessively crying infants compared to a placebo and could be considered as a treatment option. (11,128,129) The effect of the Lactobacillus reuteri DSM 17938 in formula-fed infants, and the data on other probiotic strains, such as Lactobacillus rhamnosus GG (LGG), Bifidobacterium and symbiotic containing Lactobacillus casei, L. rhamnosus, Streptococcus thermophilus, Bifidobacterium breve, L. acidophilus, Bifidobacterium longum subsp. infantis, Lactobacillus delbrueckii subsp. Bulgaricus, and fructooligosaccharides, varied, and they were more or less successful in their effect on infant crying time. (11,129,132,133) Research on the use of probiotics in preventing excessive crying showed no clear evidence that probiotics are more effective than a placebo in preventing IC; however, daily crying time appeared to be reduced with probiotic use compared to a placebo. (46)

Pharmacological treatment

Simethicone is a mixture of dimethicone and SiO2, and it is claimed to act as a topical barrier for protecting the gut mucosa against irritants. It is not absorbed and is virtually non-toxic. While its use in diagnostic procedures is well-established, the evidence of therapeutic effects in excessive crying does not reach the threshold of significance. (119) Despite the absence of evidence for beneficial effects, simethicone is widely used for the treatment of excessive crying in some countries. (134)

Physical treatments

Baby massage is frequently used as a soothing technique as it may improve the mother–child relationship and provides sensory stimulation inducing pacifying effects in infants. A Cochrane review concluded that baby massage alleviated crying/fussing, and improved sleep duration and mother-infant interactions in the massage group versus the control group; however, the quality of the included studies was poor, and many studies did not address the biological mechanism by which the change might be achieved. Therefore, more high-quality studies are needed. (135)

Prognosis

In the majority of infants with excessive crying, the crying typically resolves spontaneously after three months of age. (11,18) However, several studies have linked excessive crying to child behavioural problems, such as aggressiveness and attention deficit hyperactivity disorder, and maternal anxiety and depression. (59,61,136) It has also been linked to migraine (137–140) and functional abdominal pain and family functioning later in life. (117,136,141) The association between excessive crying and the development of asthma or atopic disease during childhood remains uncertain as results of prospective studies are not unequivocal and show inconsistent and sometimes conflicting results (117,142,143)

CONCLUSION

In summary, excessive crying is a common and distressing problem during early infancy with negative effects on the infant, parents, and paediatricians. The pathophysiological mechanisms underlying excessive crying are likely to be multifactorial and gastrointestinal factors, parental factors, and neural factors have been proposed to contribute. Parental reassurance and support are the most important cornerstones in the management of excessive crying. In this thesis, parental influences and the involvement of the HPA axis by measuring hair cortisol are examined in families with excessively crying infants.

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PART 1

Infantile gastrointestinal symptoms and parental feelings

1

Gastrointestinal symptoms in infants of mothers with a psychiatric history and the role of depression and bonding

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ABSTRACT

Objectives

Gastroesophageal reflux (GER), excessive crying, and constipation are common gastrointestinal symptoms in infancy of multifactorial origin in which psychosocial stress factors play an important role. The aims of this observational study were to investigate the presence of gastrointestinal symptoms in infants of mothers with or without a history of a psychiatric disorder, their association with maternal depressive symptoms, and the possible mediating role of bonding.

Methods

One hundred one mothers with a history of a psychiatric disorder and 60 control mothers were included. Infant gastrointestinal symptoms, maternal depressive symptoms, and mother-infant bonding were assessed using validated questionnaires and diagnostic criteria at 1.5 months postpartum.

Results

The mean total score on the Infant Gastroesophageal Reflux Questionnaire Revised reported in infants of mothers with psychiatric disorder (13.4 standard deviation 5.4) was significantly higher than that in infants of control mothers (10.8 standard deviation 5.4; P=.003). No significant differences were found in the presence of excessive crying (modified Wessel criteria and subjective experience) and constipation (ROME IV criteria) between both groups. Infant GER was associated with maternal depressive symptoms (P=0.027) and bonding problems (P=<0.001). Constipation was related to maternal depressive symptoms (P=0.045), and excessive crying (Wessel and subjective criteria) was associated with bonding problems (P=0.022 and P=0.002, respectively). The effect of maternal depressive symptomatology on infant GER symptoms and excessive crying was mediated by bonding problems.

Conclusion

Maternal psychiatric history is associated with infant gastrointestinal symptoms, in which mother-infant bonding is a mediating factor.

INTRODUCTION

Gastroesophageal reflux (GER), excessive crying, and constipation are common gastrointestinal symptoms in infancy. (1) These symptoms may occur in up to 50 percent of infants (2), often leading to consultation of a healthcare professional and high costs. (3,4) Underlying organic causes are rare and a multifactorial origin is assumed in the majority of cases, among which psychosocial stress factors, such as parenting stress, play an important role.

It is known that mothers with a history of psychiatric disorder (PD) often experience more parenting stress and problems with bonding to their infant during the first months postpartum in comparison to mothers without psychiatric problems. (5) In addition, mothers with a history of PD have an increased risk for maternal depression during and after pregnancy. (6,7)

Maternal psychiatric diagnosis has been associated with infant gastrointestinal symptoms. (8,9) A recent study showed that mothers with a PD were nearly 5 times more likely to have a baby admitted with complaints of GER in the first year after birth. (9) When GER leads to bothersome symptoms, such as growth failure and hematemesis, it is defined as gastroesophageal reflux disease (GERD). (10) Maternal psychopathology is shown to play a role in feeding problems in children with GERD. (11) Maternal depression is also associated with excessive crying and infant feeding problems. (12–14)

Therefore, we hypothesize that GER, excessive crying, and constipation occur more often in infants of mothers with a psychiatric history than in healthy control mothers and that maternal depressive symptoms are associated with more gastrointestinal symptoms in infants. Little is, however, known about underlying pathways in the association between maternal psychiatric history and infant gastrointestinal symptoms. Therefore, in the current study we explore if bonding problems are a mediator in the association between maternal depressive symptoms and gastrointestinal symptoms in infants.

METHODS

Study design and sample

In this prospective study, data were analyzed from 2 groups of mother-infant dyads: mothers with PD and healthy control mothers. The inclusion criteria for the mothers with PD in this study were the actual presence or a history of psychiatric disease.

Mothers with PD were all recruited at specialized Psychiatry-Obstetrics-Pediatrics (POP) outpatient clinics from 1 tertiary referral hospital (Erasmus Medical Center) and 6 secondary hospitals and 2 specialized mental health care clinics within the Netherlands. At these POP clinics, pregnant women with a history of PD received a consultation by a multidisciplinary team consisting of a psychiatrist, obstetrician, and pediatrician. The presence or history of a PD was clinically assessed during the POP consultation by a clinical psychiatrist with perinatal expertise, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 2000).

Two thirds of the mothers with PD were included in one of the secondary hospitals (St. Antonius Hospital) in a standardized follow-up care program from May 2014 to August 2016. They were asked to participate in this research project and written informed consent was obtained from all participating mothers. Because these participants were not subject to procedures and were not required to follow rules of behavior, the medical ethical commission deemed that this study was not subject to the Dutch Medical Research Involving Human Subjects (Act WMO). One third of the PD mothers and all control mothers were recruited in the context of the INCAS study: a multicenter observational study on maternal parenting capacity and infant development in mothers with a severe mental illness between February 2013 and December 2014. The presence or history of a PD was additionally established using a structured clinical interview administered by a trained interviewer. (15) Mothers in the control group were recruited during pregnancy at several midwifery practices in the Netherlands. Mothers were excluded from the control group when the presence of postpartum psychopathology was indicated by a General Severity Index in the clinical range on the Brief Symptom Inventory. (16,17) When they scored in the clinical range, for example because they did not seek help for their symptoms, they could not be considered as control in the sense of not having a PD. The INCAS study was approved by the Erasmus MC medical research ethics committee and written informed consent was obtained from all participating mothers (NL42662.078012).

For the current study, data regarding infant gastrointestinal symptoms, maternal depression, and bonding from PD mothers at 6 to 7 weeks (6.7 standard deviation [SD] 1.5) postpartum (n=101) were compared to data from the control group (6.1 weeks SD1.3) (n=60). Mother-infant dyads were excluded from the current analysis if the infant had a gestational age of ≤36 weeks or suffered from a serious medical condition.

Measures

Demographic and background information of the mothers and their infants was gathered by self-report questionnaires.

Infant Gastrointestinal Symptoms

GER symptoms were assessed by maternal report using the Dutch version of the Infant Gastroesophageal Reflux Questionnaire Revised (I-GERQ-R) (18,19) which consists of 12 items that can be rated either in the yes/no or the Likert scale fashion. The I-GERQ-R is a

reliable and valid measure of infant GERD symptoms during the past 7 days. Item scores were summed to obtain a total score for GERD symptoms (range: 0–42). Infant GERD has a cut off level of 16 and both the dichotomized score (present/absent) and the total score were used for analysis of infant GER symptoms.

Excessive infant crying was assessed by self-report in 1 question based on the modified Wessel's criteria (crying >3 hours per day during 3 or more days in the last week). (20–22) In addition, a second question on mothers judgment on duration of infants' crying was added, based on the final core outcome set for infant colic (23) (''Do you think your child cries a lot?''). For the current analysis these 2 items were dichotomized (present/absent). Both outcomes (modified Wessel and subjective experienced excessive crying) were included in the analysis.

The presence of functional constipation was investigated by using 4 questions ≤2 defecations per week; excessive stool retention; painful or hard bowel movements; large fecal mass) based on the ROME IV criteria for infants and toddlers (1). Functional constipation was considered present if at least 2 of these symptoms were reported. These data were only available for the infants of PD mothers from the INCAS study (n=34) and for the infants of control mothers (n=60).

Maternal Postpartum Depressive Symptoms and Bonding

The Dutch version of the Edinburgh Postnatal Depression Scale (EPDS) was used to assess maternal depressive symptoms (24,25). In this 10-item self-reporting questionnaire a mother reports subjective emotional distress during the past 7 days. All items are rated on a 4-point scale, score range is 0 to 30 and a higher score indicates more postpartum depressive symptoms.

The Dutch version of the Postpartum Bonding Questionnaire was used to assess problems in the mother-infant relationship (26,27) by self-report. The Postpartum Bonding Questionnaire includes 25 items rated on a 6-point Likert scale yielding scores on 4 factors: a general factor (scale 1), rejection and pathological anger (scale 2), anxiety about the infant (scale 3), and incipient abuse (scale 4). Item scores were summed up to obtain a total score (range: 0–125). A high score indicates maternal problems with bonding. These data were only available for PD mothers from the INCAS study (n=34) and for control mothers (n=60).

Statistical Analysis

Differences in the prevalence of gastrointestinal symptoms between both groups of infant-mother dyads were tested using regression analysis. Subsequently, we estimated the relationship between maternal depressive symptoms and postpartum bonding problems, and infant gastrointestinal symptoms using linear (reflux) and logistic (excessive crying and functional constipation) regression analysis. We presented regression coefficients and 95% confidence intervals of the unadjusted and fully adjusted analysis (all potential confounding variables).

Finally, we tested whether postpartum maternal bonding problems mediated the relationship between depressive symptomatology of the mother and infant gastrointestinal symptoms. The total score of the I-GERQ-R, excessive crying according to Wessel's criteria, and functional constipation were used as outcome measures. The size and significance of all direct and indirect relationships between depressive

symptomatology and gastrointestinal symptoms were estimated using structural equation modeling. Robust weighted least squares estimation was used to allow the inclusion of continuous and dichotomous variables into the model. (28) SEM analyses were conducted using MPlus version 7.4. (29)

Demographic and clinical differences between PD and control mothers and their infants were tested using T tests and Chi square tests. Correlations between the infant gastrointestinal symptoms were calculated with Spearman rho correlation tests.

Cases with <30% missing data on outcome measures were imputed using series mean. Cases with >30% missing data on outcome measures were excluded from the analysis. Data were tested for normal distribution and checked for outliers. Analyses were conducted using SPSS (IBM SPSS statistics 24) unless otherwise specified.

RESULTS

Between February 2013 and July 2016, 678 pregnant women met the criteria of inclusion and exclusion, 205 provided informed consent, of whom 161 women participated until the end of the study. There were 44 patients lost to follow-up, 3 mothers in the control group were excluded because of the Brief Symptom Inventory clinical range, 5 children were excluded because of prematurity, and 36 mothers were excluded because the questionnaires were not completed. The 161 infants had a mean age of 6.5 weeks (SD 1.5) and consisted of 44% girls, mean age of their mothers was 32.1 years (SD 4.6). In the PD group 55% of the mothers had a history of depressive disorder, 28% of anxiety disorder, 5% of a psychotic disorder, and 24% of the mothers had a personality disorder, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, 2000).

Baseline characteristics of the infants and mothers included are presented in Table 1. PD mothers were significantly more often unemployed, used tobacco more often and reported higher levels of postpartum depressive symptomatology. Infants of PD mothers were less often breast-fed and approximately 2 weeks younger at birth. In addition, infants of PD mothers were more often admitted to the hospital in the first week postpartum due to routine observation because of maternal medication use.

PD dyads in the INCAS group (n=34) were more often unemployed and reported lower educational levels than the other PD dyads (n=67). In addition, infants of the PD dyads in the INCAS group showed higher gestational age (M=39.2 weeks; SD 1.9) than infants in the other PD dyads (M=37.2; SD 1.0), and INCAS mothers reported higher levels of depressive symptomatology (M=10.1; SD 5.9) than other PD mothers (M=6.6; SD 5.4). No differences were observed for the remaining baseline characteristics.

Prevalence of Infant Gastroesophageal Symptoms

The presence of infant gastrointestinal symptoms is given in Table 2. The mean total score of I-GERQ-R in infants of PD mothers (M=13.4; SD 5.4) was significantly higher than that in infants of control mothers (M=10.8; SD 5.4). There were no significant differences between infants of mothers with PD and control mothers with regard to the presence of infant GER and excessive crying. A trend was found toward a higher presence of functional constipation in infants of PD mothers (15%) in comparison to infants of control mothers (3%) (P=0.058).

Relationship Between Infant Gastrointestinal Symptoms and Maternal Postpartum Depressive Symptoms and Bonding Problems

As depicted in Table 3, a significant positive relationship between infant GER and both maternal depressive symptoms and postpartum bonding problems was found after adjustment for confounders. A significant positive relationship was found between excessive infant crying and postpartum bonding problems and between infant functional constipation and maternal depressive symptoms in both unadjusted and adjusted analyses.

Table 4 shows the results of the path analysis. The effects of maternal depressive symptomatology on infant reflux symptoms and excessive crying were mediated by maternal bonding problems. A direct path between depressive symptomatology and infant reflux symptoms and excessive crying was not found. Functional constipation, on the contrary, was directly affected by maternal depressive symptomatology, and this

association was not mediated by maternal bonding. In line with the results reported in Table 2, the total impact (directly and indirectly through bonding) of maternal depressive symptoms was significant for reflux and functional constipation, but not for excessive crying.

Table 1. Sociodemographic and clinical characteristics of psychiatric disorder mothers and control mothers and their infants (n=161)

	PD group (n=101)	Control group (n=60)	Test
Mother			
Age (mean [SD])	31.9 (4.7)	32.4 (4.8)	T(158)=0.97; *P*=0.561
Dutch ethnicity	90.7%	85.0%	$Chi^2(1)$=1.03; *P*=0.311
Educational level (%Post vocational)*	36.1%	50.8%	$Chi^2(1)$=3.06; *P*=0.080
Employment (%Yes)	63.1%	80.0%	$Chi^2(1)$=4.78; ***P*=0.029**
Tobacco use during pregnancy	23.3%	8.5%	Chi2(1)=5.36; ***P*=0.021**
EPDS (Mean; SD)	7.8 (5.7)	3.2 (3.3)	T(156)=-5.57; ***P*<0.001**
Infant			
Age (weeks) (mean [SD])	6.7 (1.5)	6.1 (1.3)	T(157)=-2.35; ***P*=0.020**
Primiparous	56.4%	43.9%	$Chi^2(1)$=2.31; *P*=0.129
Sex (% male)	58.4%	53.3%	$Chi^2(1)$=.40; *P*=0.529
Breast feeding >50% of intake	37.3%	66.3%	$Chi^2(1)$=12.57; ***P*<0.001**
Gestational age at birth in weeks (mean [SD])	37.9 (1.6)	39.8 (1.4)	T(158)=7.80; ***P*<0.001**
SGA <p10	5.0%	6.8%	$Chi^2(1)$=0.24; *P*=0.628
Hospital admission in first week	21.4%	5.0%	$Chi^2(1)$=7.80; ***P*=0.005**

EPDS = Edinburgh Postnatal Depression Scale; PD = psychiatric disorder; SD = standard deviation; SGA = small for gestational age.

*Secondary vocational education and academic education. All P values ≤ 0.05 in bold.

Table 2. Prevalence of gastrointestinal symptoms in infants of psychiatric disorder and control mothers

	Infants of PD mothers (N=101)	Infants of control mothers (N=60)	Regression coefficient
Reflux			
GER (% [n / ntotal])	28 (27/97)	18 (10/57)	OR: 1.8(0.8-4.1); *P*=0.152
IGERQ-[a] total (mean [SD])	13.4 (5.4)	10.8 (5.4)	β: 0.2 (0.9-4.3); ***P*=0.003**
Excessive crying			
Modified Wessel's (% [n / ntotal])	6 (6/98)	14 (8/59)	OR: 0.4 (0.1-1.3); *P*=0.122
Subjective (% [n / ntotal])	18 (17/96)	12 (7/60)	OR: 1.6 (0.6-4.2); *P*=0.312
Functional constipation*			
(% [n / ntotal])	15 (5/33)	3 (1/60)	OR: 5.2 (0.95-28.4); *P*=0.058

IGERQ-R = Infant Gastroesophageal Reflux Questionnaire Revised; PD = psychiatric disorder; SD = standard deviation.

* Only data available from INCAS study. All P values ≤ 0.05 in bold.

Table 3. Relationship between maternal postpartum depressive symptoms (Edinburgh Postnatal Depression Scale) and postpartum bonding problems (PBQ) and infant gastrointestinal symptoms

	EPDS Regression coefficient (95% C.I.) Unadjusted	EPDS Regression coefficient (95% C.I.) Fully adjusted[a]	PBQ Regression coefficient (95% C.I.) Unadjusted	PBQ Regression coefficient (95% C.I.) Fully adjusted[a]
Reflux - *IGER-Q*	β: 0.26 (0.10-0.41); ***P*=0.001**	0.21 (0.02-0.40); ***P*=0.027**	β:0.27 (0.16-0.38); ***P*<0.001**	0.27 (0.15-0.39); ***P*<0.001**
Excessive crying *Modified Wessel's*	OR: 1.07 (0.98-1.17); *P*=0.111	1.05 (0.92-1.95); *P*=0.472	OR: 1.07 (1.00-1.15); ***P*=0.038**	1.12 (1.02-1.24); ***P*=0.022**
Subjective	OR: 1.09 (1.01-1.18); ***P*=0.029**	1.07 (0.96-1.20); *P*=0.226	OR: 1.13 (1.05-1.22**); *P*=0.002**	1.21 (1.07-1.36); ***P*=0.002**
Functional constipation[b]	OR: 1.16 (1.04-1.31); ***P*=0.010**	1.31 (1.01-1.71) ; ***P*=0.045**	OR: 1.04 (0.97-1.12); *P*=0.266	1.05 (0.95-1.17); *P*=0.349

CI = confidence interval; EPDS = Edinburgh Postnatal Depression Scale; PBQ = Postpartum Bonding Questionnaire. [a] Adjusted for age infant at visit, gestational age at birth, infant admission in first week, breast-feeding, employment status mother, smoking during pregnancy. [b] Only data available from INCAS study. All P values ≤0.05 in bold.

Table 4. Path model: Standardized direct and indirect effects and standard error of maternal postpartum depressive symptoms and bonding problems on infant gastrointestinal symptoms

	Reflux[a]		**Excessive crying**[b]		**Functional Constipation**	
	Estimated	SE	Estimated	SE	Estimated	SE
Depressive Symptoms						
Direct effect	-0.09	0.09	-0.25	0.24	0.49*	0.20
direct effects						
Depressive Symptoms-Bonding-Gastrointestinal symptoms	0.31**	0.08	0.32**	0.09	-0.07	0.11
Total	0.22***	0.08	0.07	0.19	0.42*	0.17
Bonding Problems						
Direct effect	0.50**	0.11	0.52**	0.14	-0.11	0.18

[a]: Assessed using the total score of the Infant Gastroesophageal Reflux Questionnaire Revised (I-GERQ-R).

[b]: According to Modified Wessel's criteria

* p <0.05,

** p<0.001.

*** p<0.01.

DISCUSSION

This study shows a higher reported mean reflux score in infants of mothers with a history of a PD, irrespective of the presence of actual depressive symptoms, than in infants of control mothers. The overall presence of excessive infant crying and constipation did not significantly differ between groups, although a trend was seen toward a higher prevalence of functional constipation in the PD group. Furthermore an association was found with maternal depressive symptoms and both infant GER and constipation and not with excessive infant crying. Lastly, maternal depressive symptoms were mediated by maternal bonding in their association with infant reflux symptoms and excessive crying.

Our results are in line with 3 small studies showing an association between maternal depression and GER. (11,30,31) In 2 Australian studies including 27 and 100 infants with diagnosis of ‘‘infant distress syndrome’’ severe enough to warrant admission, there was a high percentage of infant GER (44% and 36%) and clinical levels of major depressive disorder in their mothers (48% and 39%). Nevertheless, the clinical impression that infant reflux appeared to be associated with maternal anxiety and depression could not be confirmed in these studies. (30,31) As case-control study by Karacetin et al (11) found significantly higher depression and anxiety scores in 39 mothers of children with GERD versus a matched control group.

Several hypotheses exist concerning the association between maternal depression and GER. First, it is possible that depressed mothers misinterpret normal infant signals and infant facial expressions (8,32,33), leading to suspicion of GER and subsequently health care professional visits and infant medication. Second, the reverse causation hypothesis is that maternal psychopathology is not a risk factor for reflux symptoms, but that infant gastrointestinal symptoms could result in the development or exacerbation of maternal psychopathology, for example through negative infant-mother interaction patterns. (34,35) This hypothesis is supported by the mediating role of bonding we found in the association between maternal depression and infant GER symptoms and excessive crying. More insight into the causal mechanism underlying this association could improve treatment in clinical practice.

Unexpectedly, we found no significant association between excessive infant crying, defined by both modified Wessel criteria and maternal depression which is in contrast with abundant literature showing maternal postpartum depression to be correlated, and even a consequence of, excessive infant crying. (13,36) We speculate that the main reason for this finding is that our study was underpowered; a post hoc analysis showed that our sample size was able to significantly detect medium-sized differences (Cohen delta: 0.5 and odds ratio of 1.8), with a significance level of 0.05 and power of 0.80.

Although functional constipation was reported 5 times more often by mothers with PD (15%) than that in the control group (3%), this difference was not statistically significant, possibly due to the small number of women in the PD group who filled out the constipation questionnaire. This finding is, however, in line with a study by Krause et al (32) in which no association between reported infant constipation and maternal anxiety and/or depression prior and during pregnancy was shown. It should be noted, however, that Rome criteria were not used to diagnose functional constipation. Larger studies with validated questionnaires may shed more light on the possible association between infantile constipation and depression in mothers.

A limitation of our study is that we did not correct for functional gastrointestinal symptoms in the mothers because there is transference of functional symptoms between parents and children, which might interact as a confounder between maternal depressive symptoms and GER. (37) Another potential confounder is the use of medication in the group of infants with gastrointestinal disorders; in the Netherlands, however, effective over-the-counter medication for functional gastrointestinal symptoms in infants is unavailable and the prescription of reflux medication to infants has dropped significantly due to a more stringent national guideline.

The findings in our study have several implications for clinical practice. First, obstetricians should consider offering pregnant women with a (history of) PD preventive strategies, like a ‘‘surviving crying’’ package which has been shown to significantly reduce depression and anxiety in parents and reduce crying of the baby by increasing parental confidence and parental sleep. (38) Moreover, it is important that physicians are aware of the mental wellbeing of mothers who visit their practice with an infant with gastrointestinal symptoms. They should be encouraged to ask about feelings of depression and bonding to gain insights in possible negative cycles in which maternal and infant problems could exacerbate each other. (39)

In conclusion, infants of mothers with a PD are at an increased risk for developing gastro intestinal symptoms, which are related to maternal postpartum depressive symptoms and bonding problems. The underlying mechanisms of transference seem to differ depending on the specific gastrointestinal symptom. It is of importance that health care professionals take the maternal psychiatric history and maternal actual wellbeing in account when treating their infants with gastrointestinal symptoms, and refer to mental health professionals if necessary.

Author Contributions

Ineke de Kruijff contributed to conceptualization, investigation, data curation, formal analysis, methodology, writing - review and editing.

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Distress in fathers of babies with infant colic

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ABSTRACT

Aim

The aim of this case-control study was to compare parental stress, depression, anxiety and bonding problems between fathers and mothers of babies with infant colic and parents of control infants.

Methods

Parents of 34 infants with infant colic and 67 control dyads were included. Parental feelings were assessed using validated questionnaires.

Results

Fathers as well as mothers of infants with infant colic showed significantly higher mean scores compared with controls on stress (20.9 ± 5.8 and 25.5 ± 7.2 vs 16.4 ± 6.1 and 14.7 ± 7.0), depression (5.6 ± 4.0 and 9.1 ± 4.8 vs 2.9 ± 2.9 and 4.0 ± 3.1), anxiety (41.9 ± 9.2 and 46.0 ± 10.2 vs 32.4 ± 8.4 and 32.2 ± 9.3) and bonding problems (16.1 ± 8.1 and 13.7 ± 5.9 vs 8.7 ± 6.3 and 5.0 ± 4.4). In fathers, after adjustments for infant and parental confounders and maternal negative feelings, depression and anxiety were significantly increased in the infant colic group (difference of 2.7 (p = 0.017) and 8.6 (p = 0.002)).

Conclusion

In fathers of infants with infant colic, the experienced distress is strongly associated with maternal distress, except for depression and anxiety. Paediatricians should be aware of these paternal feelings as parental reassurance and support is one of the cornerstones in the treatment of infants with colic.

INTRODUCTION

Excessive infant crying or infant colic, defined as recurrent and prolonged periods of infant crying, fussing or irritability reported by caregivers in an otherwise healthy infant under 5 months of age (1), is one of the most distressing challenges for new fathers and mothers and a common reason to visit a paediatrician. (2) Parents are often concerned that the recurrent and prolonged periods of crying in their infant arise from an organic cause, which is found in only 5% of cases. (3) A thorough and careful history and physical examination of the infant and information on the normal 'crying curve' can help to reassure parents and support them through this challenging period. (4) In order to be able to reassure and support both parents, information on family stress, which is rated as an important factor in infant colic(5), is essential.

Previous research has consistently shown that infant colic is highly associated with both maternal postpartum depression and anxiety. (6,7) Surprisingly, there is limited research on the impact of infant colic on fathers, which is remarkable since in the past decade; attention has been increasingly paid to mental health of fathers, and there are good reasons to assume that this involvement could exert significant influence on both the developing child and the mother. (8) Depressive symptoms of fathers during maternal pregnancy have been associated with infant colic. (9) Infant colic is also associated with increased paternal anger, doubt and depressive symptoms. (10) Additionally, in one study, infants with colic showed less optimal father child interaction in a video recording carried out during the infants feeding, nappy change and discussion between the parents. (11) To the best of our knowledge, the relation between infant colic and paternal stress and anxiety has not been studied yet. It is important to take paternal distress into account because caring for an excessively crying infant can be challenging for both parents and their reactions and thus potential interventions might be different. Therefore, the objective of this study was to assess stress, depression, anxiety and bonding problems in both fathers and mothers of infants with infant colic in comparison to control families. We aimed to answer the following questions: (a) do fathers and mothers with babies with infant colic experience more distress than controls? (b) do fathers and mothers with babies with infant colic experience similar amounts of distress? (c) Do fathers and mothers with babies with infant colic experience distress independent of the amount of distress of the partner?

PATIENTS AND METHODS

Study design and setting

In this cross-sectional case-control study data were analysed from two groups of infants and their parents: infants with colic and control infants. Infants who presented with excessive crying according to the parents' perception and fulfilled the diagnostic criteria for infant colic (1) were recruited at the outpatient clinic of the Department of Pediatrics of the St Antonius Hospital, a teaching hospital in the Netherlands. The Rome IV diagnostic criteria for infant colic must include all of the following: (1) An infant who is <5 months of age when the symptoms start and stop, (2) recurrent and prolonged periods of infant crying, fussing or irritability reported by caregivers that occur without obvious cause and cannot be prevented or resolved by caregivers and (3) no evidence of infant failure to thrive, fever or illness. For clinical research purposes, a diagnosis of infant colic must meet the preceding diagnostic criteria and also include both of the following: (1) Caregiver reports infant has cried or fussed for three or more h per day during three or more days in 7 days in a telephone or face-to-face screening interview with a researcher or clinician (2) Total 24-h crying plus fussing in the selected group of infants is confirmed to be 3 h or more when measured by at least one prospectively kept, 24-h behaviour diary. For the control group, infants of the same age were recruited mostly at surrounding infant welfare centres, 25% of the controls were recruited at the outpatient clinic of the St. Antonius Hospital, when they received routine ultrasound of the hip after breech birth. Recruitment of excessively crying and control infants took place between August 2015 and March 2017. Exclusion criteria were insufficient knowledge of the Dutch language and a gestational age < 36 weeks. Control infants who cried excessively based on parental perception ('Does your child cry excessively?') were excluded.

Because the participants were not subject to procedures and were not required to follow rules of behaviour, the medical ethical commission deemed that this study was not subject to the Dutch Medical Research Involving Human Subjects Act (WMO). Written informed consent was obtained from all participating mothers and fathers.

Measures

Infant behaviour

For three consecutive days, the infant behaviour was registered by the parents in a diary. (12) For this study, behavioural states (crying, fussing, nursing, playing with parent or alone, feeding and sleeping) lasting for at least 10 minutes were registered. As crying intensity may reflect greater physiological or psychological stress rather than duration of crying, we additionally asked parents, when the infant was crying, to record the crying volume and intensity on scales ranging from 1 to 9 representing, respectively, barely audible to very loud crying and no facial expression to painful grimacing while crying. (13)

Parental stress, depression and anxiety

Stress experienced by parents was assessed using a Dutch translation of the Perceived Stress Scale (PSS). (14) This 14-item instrument aims at measuring the degree to which situations in life are experienced as stressful. Participants rate items according to how much they have been applicable in the last month on a 5-point scale ranging from 'never' to 'very often', and a sum score is calculated. Higher scores on the PSS indicate a higher level of experienced stress. Parental depressive symptoms were assessed using the Dutch

version of the Edinburgh Postnatal Depression Scale (EPDS). (15,16) In this 10-item self-reporting questionnaire, parents are asked about depressive symptoms during the past 7 days. All items are rated on a 4-point scale, yielding a sum score range of 0 to 30, and a higher score indicates more postpartum depressive symptoms. A cut-off score ≥9 identifies women who are at risk for depression, and a total score ≥12 is considered as indicative of a clinically relevant depression. (16) Although the EPDS was originally developed to investigate depressive symptoms in mothers, it has proven to be reliable and valid in screening for depressive symptoms in fathers as well. (17)

Symptoms of anxiety were assessed using the short form of the Spielberger State-Trait Anxiety Inventory (STAI) which is a widely used self-report instrument for measuring anxiety. (18,19 The six items) are answered on a four-point scale, and a sum score is calculated. A high sum score indicates an increased level of anxiety.

Parent infant bonding

The Dutch version of the Postpartum Bonding Questionnaire (PBQ) was used to assess problems in the parent-infant relationship. (20,21) The PBQ includes 25 items rated on a 6 –point Likert scale yielding scores on four factors: a general factor (scale 1), rejection and pathological anger (scale 2), infant-focused anxiety (scale 3) and incipient abuse (scale 4). Item scores were summed up to obtain a total score (range: 0–125). A high score indicates problems with bonding. A total score ≥26 results in a specificity of 0.61 and sensitivity of 0.84 for detecting mothers with suboptimal bonding.20 The PBQ was originally validated for measuring mother-infant bonding, but for this study, we also used the PBQ for fathers because of comparability.

Confounders and mediators

The following characteristics were obtained using standardized questions and considered as potential confounders: demographic characteristics, lifestyle and course and experience of pregnancy and delivery. These last items were questioned by: 'How did you experience the pregnancy/delivery?' The answer could be reported on scales ranging from 1 to 5 representing, respectively, very positive to very negative.

Furthermore, pre-existing contributing factors to the parents' current emotional state, such as recent stressful events, emotional problems during pregnancy and current psychiatric treatment, were assessed using standardised questions.

Statistical analysis

Data are presented as means (±SD), medians (range or IQR) or count (%), as appropriate. For each infant behavioural state, an average was calculated over the three recorded days from the diary. For crying volume and intensity, the minimum and maximum noted values were extracted, and a mean calculated. Sum scores for the four questionnaires were obtained, and an average calculated for both mothers and fathers. A maximum of 33.3% missing data within one subject was accepted for these questionnaires and corrected for by taking the participant's mean of the completed questions.

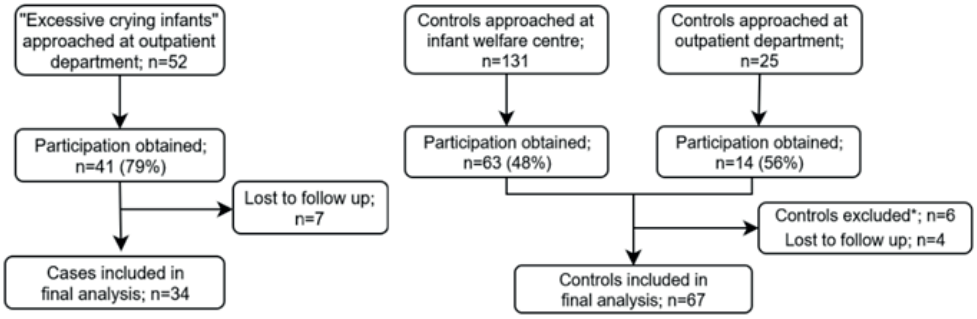
Sociodemographic characteristics and characteristics of crying behaviour were compared between the case and control groups using the Chi-square test and independent samples T test or the Mann-Whitney U test in cases of non-parametric data. Mean sum scores of the questionnaires were compared for both mothers and fathers between the case

and control groups using an independent samples T test or Mann-Whitney U test. Differences in scores of the questionnaires between mothers and fathers within the case group were tested with paired T test. For the EPDS and PBQ questionnaires, dichotomous variables were created based on the previously mentioned cut-off scores. Multivariate linear regression analyses were used to identify differences in the influence of infant crying behaviour on stress, depression, anxiety and bonding scores for both mothers and fathers between the case and control groups with and without adjustments for potential confounding factors. Potential confounders were identified by a 10% change-in-estimate criterion for estimated associations. A p-value of <0.05 was considered statistically significant. All analyses were performed using IBM SPSS (IBM Statistics SPSS version 25).

RESULTS

A total of 208 infants and their parents were approached for participation, of whom 34 infants with infant colic and 67 control infants were included in the final study protocol (Figure 1).

Figure 1: Flowchart of inclusions



*Controls excluded because one of the parents experienced their infants as excessively crying based on the questionnaire

Analyses of participants lost to follow-up showed no significant differences in parental or infants' age, infants' gender or birth characteristics. The sociodemographic characteristics of the participating families are shown in Table 1.

Infants in the infant colic group, compared to the control group, had a longer median duration of crying during the day (88 [range 68–127] vs. 18 [7–33] minutes, $p<0.001$), a higher mean crying intensity (6.4±1.8 versus 3.8±2.0, $p<0.001$) and a higher mean crying volume (6.4±1.7 vs. 4.1±2.2, $p<0.001$). Additionally, the infants in the infant colic group were reported to sleep for fewer minutes at night (470 [range 417–517] vs. 560 [505–610], $p<0.001$).

The mean reported levels of stress, depression, state anxiety and bonding for both parents in the infant colic and control group are depicted in Table 2. The differences in infant crying behaviour related to the parental states were adjusted for potentially relevant confounding factors of the infant (age and medication use), the parents (current psychiatric treatment) and the feelings of the other parent.

After adjusting for confounders, mothers and fathers with colicky infants reported significantly more symptoms of stress, depression, anxiety and bonding problems compared to controls. Adjustment for the same feelings in the other parent had no effect on the differences in the maternal feelings. However, all differences in fathers' feelings, except for depression ($p = 0.017$) and anxiety ($p = 0.002$), lost their significance (see Table 2). The differences in levels of paternal stress and bonding behaviour between fathers of excessively crying versus control infants declined by 43% and 46%, respectively, after correcting for maternal feelings. Paternal depression and anxiety levels changed much less (12% and 14%, respectively).

Within the infant colic group, there was a significant difference between mothers and fathers in stress (25.5 ± 7.2 vs. 20.4 ± 6.1, n = 28, p = 0.003), EPDS (9.1 ± 4.8 vs. 5.8 ± 3.6, n = 26, p = 0.008) and anxiety (46.0 ± 10.2 vs. 41.1 ± 9.6, n = 29 p = 0.043) but not in bonding problems (13.7 ± 5.9 vs. 17.2 ± 8.9, n = 4, p = 0.075).

Mothers with babies with infant colic experience higher amounts of distress than fathers do.

Based on their EPDS cut-off scores, seventeen (56%) mothers within the infant colic group were at risk for developing depression versus eight (12%) mothers in the control group (p < 0.001). Nine (27%) mothers in the infant colic group already had experienced a clinically relevant depression versus one (1.5%) mother in the control group (p < 0.001). Six (21%) fathers in the infant colic group were at risk for developing depression versus two (3.1%) in the control group (p = 0.010). In each group, one father appeared to have a clinically relevant depression before (p = 0.524).

Table 1. Sociodemographic characteristics of the participating families

	Infant colic; n= 34	**Control**; n= 67	**P value**
Infants' age in week (range)	8.0 [2-16]	10.0 [4-18]	0.01
Male gender (%)	22 (65)	33 (49)	0.14
Gestational age at birth in week (SD)	39.0 (1.3)	39.3 (1.3)	0.34
Birth weight in gram (IQR)	3362 [2950-3752]	3342 [3000-3670]	0.79
Feeding status (%) Exclusive breastfeeding Formula only Breast milk and formula	 7 (21) 27 (79) 0 (0)	 30 (45) 28 (42) 9 (13)	 0.001
Admission during first week (%)	9 (27)	8 (12)	0.06
Use of medication (%) Acid reflux treatment	15 (44) 13	2 (3) 1	<0.001
Firstborn (%) *Mother* *Father*	 13 (38) 12 (40)	 31 (46) 28 (42)	 0.44 0.82
Age in year (SD) *Mother* *Father*	 31.3 (3.1) 34.3 (4.5)	 32.0 (4.7) 34.4 (4.9)	 0.37 0.90
Ethnicity (Dutch- Caucasian) (%) *Mother* *Father*	 34 (100) 33 (97)	 60 (90) 63 (94)	 0.09 0.66

Table 1. Sociodemographic characteristics of the participating families (Continued)

Educational level (%)			
Mother			
Secondary school or less	2 (6)	4 (6)	0.01
Secondary vocational education	16 (47)	11 (16)	
Higher professional education	10 (29)	29 (43)	
University	6 (18)	23 (34)	
Father			
Secondary school or less	6 (20)	6 (9)	0.31
Secondary vocational education	6 (20)	22 (33)	
Higher professional education	11 (37)	20 (30)	
University	7 (23)	18 (27)	
Emotional/psychiatric problems during pregnancy (%)			
Mother	9 (27)	8 (12)	0.06
Father	3 (12)	1 (2)	0.06
Current psychiatric treatment (%)			
Mother	5 (15)	2 (3)	0.04
Father	2 (8)	1 (2)	0.19
Experienced stressful events (%)			
Mother	18 (53)	25 (38)	0.15
Father	17 (61)	28 (42)	0.11
Negative experience of pregnancy (%)			
Mother	5 (15)	4 (7)	0.27
Father	2 (8)	1 (2)	0.19
Negative experience of delivery (%)			
Mother	4 (12)	10 (15)	0.77
Father	6 (24)	2 (3)	0.006

Table 2. Reported levels of stress, depression, state anxiety and bonding behaviour in relation with perceived infant crying

Variable	**Infant colic**	**Control**	**dif[a], (p-value)**	**dif[b], (p-value)**	**dif[c], (p-value)**
Stress					
Mother	25.5 ±7.2 N=28)	14.7 ±7.0 (N=71)	10.9 (<0.001)	10.4 (<0.001)	9.6 (<0.001)
Father	20.9 ± 5.8 N=23)	16.4 ± 6.1 (N=70)	4.4 (0.003)	4.7 (0.008)	2.7 (0.178)
Depression					
Mother	9.1 ±4.8 (N=26)	4.0 ±3.1 (N=55)	5.1 (<0.001)	4.9 (<0.001)	4.6 (<0.001)
Father	5.6 ± 4.0 (N=23)	2.9 ± 2.9 (N=62)	2.6 (0.001)	3.1 (0.001)	2.7 (0.017)
State Anxiety					
Mother	46.0 ± 10.2(N=29)	32.2 ± 9.3 (N=71)	13.8 (<0.001)	9.5 (<0.001)	8.1 (0.002)
Father	41.9 ± 9.2 (N=25)	32.4 ± 8.4 (N=70)	9.4 (<0.001)	10.0 (<0.001)	8.6 (0.002)
Bonding behaviour					
Mother	13.7 ± 5.9 (N=14)	5.0 ± 4.4 (N=68)	8.7 (<0.001)	8.8 (<0.001)	6.9 (<0.001)
Father	16.1 ± 8.1 (N=13)	8.7 ± 6.3 (N=61)	7.3 (0.001)	7.2 (0.002)	3.9 (0.125)

Note: Values are mean ±SD;

[a]difference between infant colic and control group

[b]difference between excessive crying and control group corrected for infant factors (age, medication use) and parental factors (current psychiatric treatment) ;

[c] difference between excessive crying and control group corrected for above PLUS feelings of the other parent

DISCUSSION

Our study clearly demonstrates that fathers, just like mothers, experience significantly more negative feelings when their infants are crying excessively. Both parents report more stress, depression, anxiety and disturbed bonding with their infant than parents in the control group. Fathers with a baby with infant colic have lower stress, depression and anxiety scores as mothers have. Whereas maternal feelings in relation to infant colic were independent of their partners' feelings, most of the fathers' negative feelings were strongly associated with those of the mothers. Only depression and anxiety were increased independently from maternal feelings in fathers of colicky infants.

Our results strengthen the already available evidence concerning the association between infant colic and feelings of depression and anxiety in mothers. (7,22) However, to our knowledge, this study is the first which investigated levels of anxiety and stress in fathers of colicky infants. An increasing amount of studies have focussed on paternal feelings in the postnatal period in general. These studies showed that during the first weeks after birth, fathers experience significantly more anxiety and stress, which both decline when their infants become older. (23,24) In these studies, however, no association between paternal feelings and infant crying was investigated. Only few studies (9,25) have reported on feelings of depression in fathers taking care of excessively crying infants. These studies showed higher depression scores in fathers of infants experiencing crying problems, but these scores were not adjusted for maternal depressive symptoms and the infant crying was not registered in a behavior diary.

Since our study has a cross-sectional design, we can only hypothesise about the causality of the higher anxiety scores in fathers of colicky infants. First, their anxiety may have been caused by the crying itself; for example they may have feared serious illness in their infants. Another possibility is that paternal anxiety is a predictive risk factor for development of excessive infant crying, similar with the finding that paternal depressive symptoms during pregnancy are related to excessive infant crying. (8) Finally, the higher scores may have been caused by selection bias. We investigated a group of parents who consulted a paediatrician, which in the Netherlands is only possible after referral by a primary care physician. This could have selected a group of parents in whom one or both parents were more anxious about organic causes of infant colic, experienced more stress and perceived infant crying as more unpleasant. This hypothesis is supported by the fact that the parents of the investigated group of colicky infants not only registered more crying h with a higher volume, they also registered a higher crying intensity, probably reflecting greater physiological or psychological stress. (13)

Fathers of colicky infants experienced more impaired bonding compared to controls. These results are in line with a small study by Räihä (10) in which father-infant interaction was suboptimal in thirteen items out of 65 (20%) versus suboptimal mother-infant interaction in six items out of 65 (9%) in a group of thirteen severely excessive crying infants. Fathers often reported that the quality and quantity of the spend time and experienced bonding with their infant considerably decreased when they lacked confidence in their ability to care for their infant. (26)

The higher prevalence rates of depression, anxiety and stress in mothers compared to fathers within the group of infant colic could be explained by the fact that the psychological state of mothers has shown to be affected more strongly by negative infant-

related factors. (27)

In contrast, feelings of men tend to be more influenced by their partners. Our results confirm for stress and bonding problems that fathers are more negatively influenced by emotional problems experienced by their wives, than by their crying infant. (28)

The experienced feelings of depression and anxiety in fathers of babies with infant colic, however, existed independently of these feelings in mothers of these infants. It is hopeful that by introducing integrated care for families managing infant colic, the vicious cycle of infant crying and parental stress can be broken. In one study, family-based treatment reduced the amount of crying faster than did standard paediatric care. (29) In another study, assisting parents who sought help because of excessive infant crying with a support package not only led to reductions in reported crying, but was also associated with substantial reductions in parental frustration, anxiety, depression and contact with professionals. (30)

Our findings suggest that we might want to intervene differently for mothers and fathers with an excessively crying infant. Integrated care which reduces negative feelings of stress and bonding in mothers might also have positive effects on these feelings in fathers. The treatment for depression and anxiety in fathers and mothers, however, should probably be individualised.

Our study is limited by the cross-sectional design, which prevents us from establishing causality. Moreover, only half of the parents approached for the control group were willing to participate and 25% of them were recruited when they received routine ultrasound of the hip after breech birth, which may have contributed to selection bias. Both the infant colic and the control group consisted of a highly educated and mainly Caucasian population. Therefore, caution should be taken in generalising the results to low-income populations and populations of different ethnicities.

In conclusion, our results demonstrate that there is a high burden of stress, depression, anxiety and bonding problems in both fathers and mothers dealing with infant colic. We therefore recommend paying attention to the emotional state of both parents when taking care of excessively crying infants. By acknowledging and eventually treating accompanying feelings of frustration, distress, anxiety or depression in parents, the health care professional may be better able to lead them and their infant through this challenging period.

Author contributions

Ineke de Kruijff contributed to conceptualization, investigation, data curation, formal analysis, methodology, writing - review and editing.

Moniek S. Veldhuis contributed to investigation, data curation and writing - review and editing.

Ellen Tromp contributed to conceptualization, data curation, formal analysis, methodology, writing - review and editing.

Arine M. Vlieger contributed to writing - review and editing.

Marc A. Benninga contributed to conceptualization, writing - review and editing.

Mijke P. Lambregtse-van den Berg contributed to conceptualization, formal analysis, methodology, writing - review and editing.

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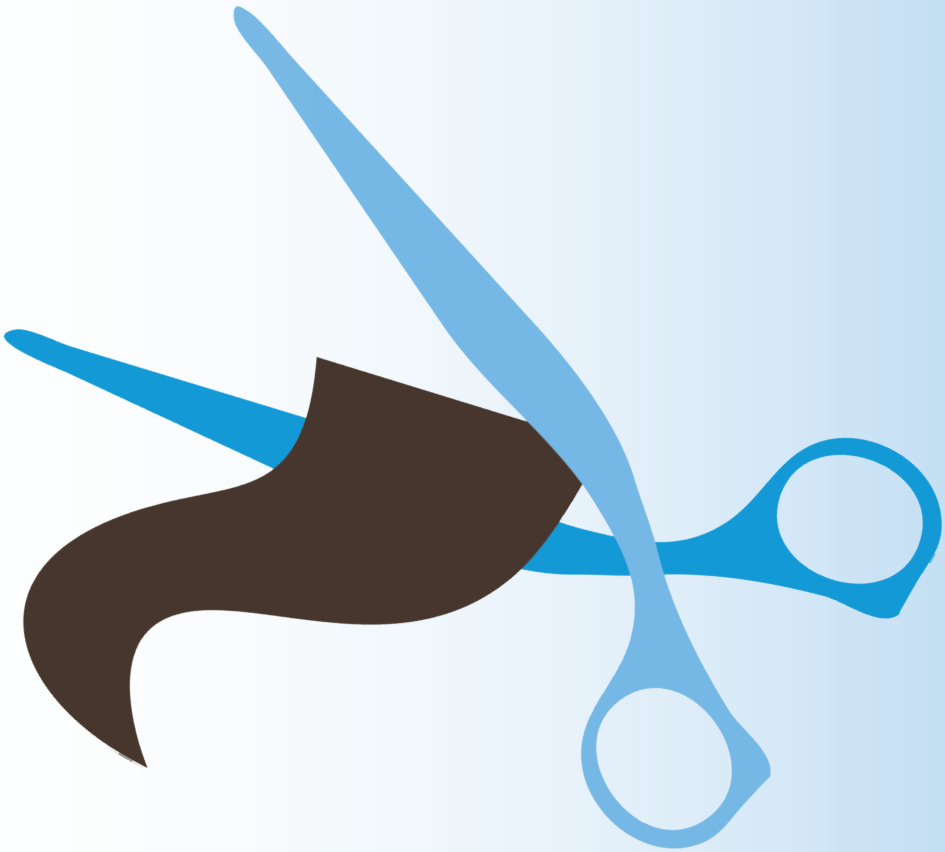
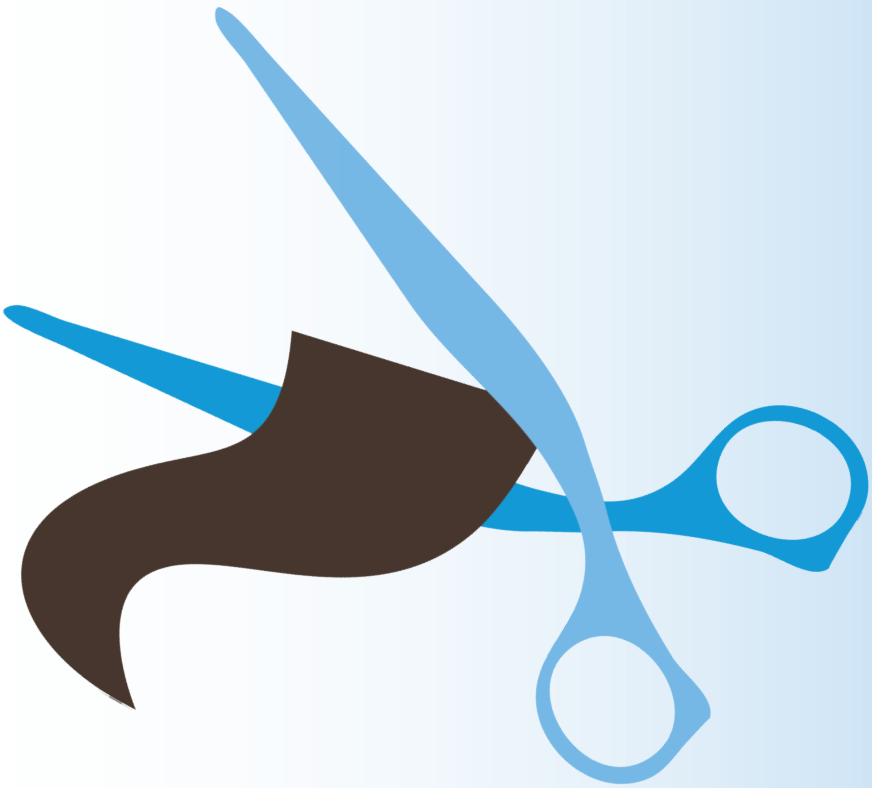


PART 2

Hair cortisol

3



LC-MS/MS-based reference intervals for hair cortisol in healthy children

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ABSTRACT

Background

Human scalp hair is a valuable matrix for determining long-term cortisol concentrations, with widespread applicability in clinical care as well as research. However, pediatric reference intervals are lacking. The aim of this cross-sectional study is to establish age-adjusted reference intervals for hair cortisol in children and to gain insight into hair growth velocity in children up to 2 years old.

Methods

A total of 625 healthy children were enrolled through recruitment in pregnancy, infant-welfare clinics, and school visits. Scalp hair cortisol levels were measured using liquid chromatography-tandem mass spectrometry. Age-adjusted reference intervals were established in children from birth to 18 years old. Hair growth velocity was determined in children 0−2 years of age by measuring hair length at 4- to 10-week intervals.

Results

Hair cortisol levels were high (162.4 pg/mg, 2.5th-97.5th percentile: 28.8–961) after birth with a sharp fall in the first 3 months of life. This is followed by lower values until age 6 and then by graduated and subtle higher values to adult concentrations are reached at the age of 18 years (3.0 pg/mg, 2.5th-97.5th percentile: 0.53–17.8). Average hair growth velocity measured in mm/month was significantly lower in infants 0–6 months of age compared to children 12–24 months (3.5 versus 9.4, $P < 0.001$).

Conclusions

This is the first study to provide age-adjusted reference intervals for hair cortisol in children from 0−18 years. Higher hair cortisol concentrations in infants might be explained by the significantly lower hair growth rate in the first year of life. The establishment of pediatric hair cortisol reference ranges broadens the potential applications of this biomarker in pediatric clinical care.

INTRODUCTION

Measurement of endogenously produced cortisol is a cornerstone for diagnosis and monitoring of a broad range of diseases. Routinely used matrices for cortisol quantification include serum, urine and saliva. However, interpretation of diagnostic tests for cortisol is complicated by the pulsatile excretion, the naturally occurring circadian rhythm and the maximum of 24 h of measurement. Over the past decade, cortisol measurement of human scalp hair as a long-term biomarker of the hypothalamus-pituitary-adrenal (HPA) axis (1,2) has become increasingly popular. Hair use offers unique benefits and characteristics, which include non-invasive sampling and a long-term retrospective reflection of cumulative hormone concentrations. These features make the use of scalp hair attractive for both clinical practice and research.

Studies have associated increased scalp hair cortisol with obesity (3,4) and, in adults, with metabolic syndrome (5) and cardiovascular disease. (6) Moreover, measurement of cortisol in hair may be an interesting tool as diagnostic test in Cushing's disease (7,8) and is a promising tool for monitoring hydrocortisone treatment in adrenal insufficiency in both adults (9) and children. (10) In addition, hair cortisol has been associated with mental health in adult literature; altered cortisol levels are found in chronic stress and anxiety disorders such as post-traumatic stress disorder. (11,12) In children, however, no significant relationship has been found between hair cortisol and anxiety or depression, and results in relation to stress and/or trauma exposure (13) have been surprisingly heterogeneous.

In adults, population-based reference ranges for hair cortisol measurement have been established in large cohort studies of healthy volunteers with liquid chromatography mass spectrometry. (14-17) Limited studies of reference intervals of cortisol concentration in children have been published (18-20); no extended studies of reference intervals using the state-of-the-art method of liquid chromatography-tandem-mass spectrometry (LC–MS/MS)(15) have been published. It is generally accepted that hair in adults grows at a fairly constant rate of approximately 10mm per month (21), meaning that the first centimeter proximal to the scalp is assumed to contain cortisol incorporated during the month prior to sampling. Studies of children's hair characteristics and hair growth patterns, needed for correct interpretation of hair cortisol concentration, are limited. (22) A study in 20 children aged 3–9 years showed a growth rate of 9.6mm per month. (23) To our knowledge, studies on hair growth rate in infants aged 0–2 years are non-existent.

In this study we assess hair growth velocity in children aged 0–2 years, establish age-adjusted reference intervals for cortisol in healthy children aged 0–18 years and determine the influence of covariates such as hair color and hair treatment.

MATERIALS AND METHODS

Subjects

In this study, data were analyzed from three groups of healthy children aged 0−18 years who were recruited in pregnancy and infantwelfare clinics, during elementary and secondary school visits and at a pediatric (outpatient) clinic.

The first group consisted of newborns who participated in the control group of a non-randomized, prospective controlled study in the OLVG West Hospital in Amsterdam, the Netherlands. (24,25) Included were infants whose mothers did not use psychotropic medication during pregnancy, who were admitted to the maternity ward or neonatal care unit and who had an expected hospital stay of at least 72 h. Most infants who fulfilled these criteria were born by caesarean section. The study was approved y the medical ethics committees of the OLVG West Hospital and VU Medical Center in Amsterdam, the Netherlands. Written informed consent was obtained from parents of all participants. Study recruitment took place between February 2012 and August 2013.

The second group consisted of six-week-old infants from the INCAS study, a multicenter observational study on maternal parenting capacity and infant development in mothers with severe mental illness. Infants of healthy mothers in the control group were recruited during pregnancy at several midwifery practices in the Netherlands. The INCAS study was approved by the Erasmus MC medical research ethics committee, and written informed consent was obtained from all participating mothers (NL42662.078012). Study recruitment took place between February 2013 and December 2014.

The third group consisted of healthy children aged 0−18 years recruited from the general population of the Netherlands for a crosssectional observational study on steroid hormone concentrations in scalp hair during childhood, adolescence and pregnancy. Participants were enrolled through visits to infant-welfare centers and primary and secondary schools in Rotterdam and Woerden and surrounding areas. In addition, two groups of children visiting an outpatient clinic were asked to participate in this study: 1) 12-week-old infants at risk for pathological developmental dysplasia of the hip who tested negative with sonographic screening and 2) healthy siblings of patients visiting the outpatient clinic. The criteria of exclusion we used were chronic disease and systemic use of glucocorticoids (GC) or other medications affecting steroid metabolism. Additional criteria of exclusion used for children aged less than 6 months were prematurity (gestational age<37 weeks) and systemic maternal use of GC. This study was approved by the Medical Research Ethics Committee of Erasmus MC and followed the Helsinki declaration. Written consent was obtained from parents and children aged 12 and above, and informed assent was given by children less than 12 years. Study recruitment took place between January 2011 and April 2018.

Determinants

For a subset of children questionnaires were filled out either by parents/caretakers or by children themselves (if older than 12 years). These covered hair care characteristics, general health and use of medication. Information was obtained about washing frequency (< 1, 1–2, 3–4, or>4 times per week), use of hair products on the day of sampling (yes/no, type of product: none, mousse, gel, wax, spray, other), frequent sweating of the scalp (yes/no), hair treatment (e.g. dyeing, bleaching, or perming) in the past three months,

topical use of GC and current tobacco smoking status (yes/no). In children who did not fill out a questionnaire, hair treatment was set to" no" for children under the age of 10 years and current smoking status was set to" no" for those under the age of 12 years. Ethnicity was determined according to country of birth of the children and their parents, derived from the completed questionnaires. Ethnicity is divided into three groups: native Dutch, Western background and non-Western background. Ethnicities are determined based on a report of Statistics Netherlands (CBS.nl). An immigrant is defined as a person one or both of whose parents were not born in the Netherlands. A Western immigrant is defined as a person one or both of whose parents were born in one of the countries in Europe (excluding Turkey), North America and Oceania, or Indonesia or Japan. A non-Western immigrant is defined as a person one or both of whose parents were born in one of the countries in the continents of Africa, Latin America and Asia (excluding Indonesia and Japan) or Turkey (26). (Statistics Netherlands, StatLine, CBS 2018).

Hair collection and preparation

Hair locks were cut with small surgical scissors from the posterior vertex, as close to the scalp as possible. The posterior vertex has the lowest variation in repeated measurement. (27,28) The hair locks were taped to a paper form with the scalp end marked and then stored in envelopes at room temperature. In the children aged 4 years and up, the proximal 3 cm of hair was used for a single analysis. The strands of hair were cut into 1-cm segments. In the newborn infants, the total length of fetal hair, typically 1 cm, was analyzed. In children up to 4 years, whose hair had not always grown to 3 cm, at least the 1 cm of hair closest to the scalp was analyzed. Subsequently, hair samples were transferred to glass tubes, weighed (mg), washed by gently shaking them in LC–MS grade isopropanol at room temperature and left to dry for at least 48 h. To determine the hair growth velocity in children up to 2 years, the place of cutting was marked and described in the logbook. A researcher, research nurse and trained student were trained to perform both the cutting and measurement. At a follow-up appointment 1–2.5 months later, the new-grown hair was measured with a measuring tape by the same person who had done the cutting.

Hair Analysis

Cortisol was extracted in 1.5 mL LC–MS grade methanol for 18 h at 25 °C in the presence of deuterated cortisol-d3, and it was subsequently cleaned using solid phase extraction. The Department of Clinical Chemistry uses an ISO 15189;2012-accredited LC–MS/MS method for hair cortisol analysis and participates in the international interlaboratory Round Robin since 2015 (29). The method is described in detail elsewhere (27) and uses a Waters Xevo TQ-S system (Waters Corporation, Milford, MA, USA). Cortisol concentrations were reported in pg per mg hair. The inter-assay coefficient of variation for cortisol was 14.8 %. The intra-assay coefficient of variation for cortisol was<11 %. The lower limit of quantification (LLoQ) of cortisol was<1 pg/mg. Measurements below LLoQ were excluded from statistical analyses.

Statistical Analysis

Statistical analyses were performed with SPSS Version 24 (IBM, New York, USA). Age-specific reference intervals for hair cortisol concentrations were estimated by analysis of 2.5 and 97.5 percentile curves via a semi-parametric model with Box-Cox transformation, which is available in the" Generalized Additive Models for Location, Scale and Shape" package. (30) To prevent overfitting, model fit was assessed using Akaike's" information criterion" and visual inspection. Hair cortisol concentrations were transformed to age-

adjusted z-scores for subsequent analyses. Associations of hair cortisol concentrations with hair characteristics, smoking, gender and ethnicity were assessed using linear regression models and applicable adjustments for gender and ethnicity were applied. As hair cortisol concentrations have a skewed distribution, log-transformation was applied to achieve a normal distribution, and hair cortisol concentrations were transformed to age-adjusted z-scores. Associations of hair growth velocity with the three age groups were assessed using one-way ANOVA.

RESULTS

Cortisol concentrations in a total of 625 children aged 0–18 years were measured to establish reference intervals. The general characteristics of participants are shown in Table 1. The three groups of healthy children were significantly different in age ($P < 0.001$) and in ethnicity ($P < 0.001$). The participants' ethnic background was mainly native Dutch (Caucasian) in the second (71 %) and third (80 %) groups and mainly immigrant with a Western origin (76 %) in the first group, in which 24 % were of Caucasian ethnicity. The groups did not significantly differ in gender ($P=0.079$).

The results of hair cortisol levels by age are shown in Fig. 1. Reference intervals are defined as median and predicted 2.5th and 97.5th percentiles, as shown in Table 2.

Newborns' scalp hair shows high levels of cortisol after birth, with a sharp fall in the first 3 months followed by further lower levels to the age of 6 years and then by graduated and subtle higher values to adult levels. The variation of cortisol levels was smaller in adolescents than in preschool-aged children.

Hair growth velocity

Fig. 2 shows the hair growth velocity in a subset (n=62) of children in the<2 years age group. The average hair growth velocity in infants 0–6 months old was 3.5mm per month (SD 2.1; n=27). The hair growth velocity in children aged 6–12 months was 6.7mm per month (SD 3.1; n=21) and in children 12–24 months, 9.4mm per month (SD 1.6; n=14). The hair growth velocity is significantly different between the three age groups ($p < 0.001$) and is higher in the older children. Due to limited hair amount we could only measure both hair cortisol and hair growth velocity in 47 of the 62 children. In these children, the lower cortisol levels during the first two years were partly explained by the increasing hair growth velocity. Log cortisol had a significant association with age ($p < 0.001$); however, when hair growth velocity was taken into account, the association was no longer significant ($p=0.252$).

Effects of determinants on cortisol measurement

Table 3 shows the effects of participant and hair related characteristics on cortisol z-scores. Hair cortisol was not associated with gender. Cortisol was lower in children of Western descent than in children of non-Western descent ($p=0.04$). All other known confounders (BMI, smoking, excessive sweating) were not associated with a difference in cortisol concentration. Hair care characteristics (washing frequency, hair product use, type of hair product and hair color) were not associated with hair cortisol level.

Table 1. Descriptive statistics of study participants (n=625)

Participants	
Age, years (n=625)	5.0 (SD 6.0)
Height,SDS (n=422)	0.11 (SD 1.04)
Body mass index, SDS (n=422)	-0.12 (SD 1.08)
Gender, girls/boys (n=625)	299/326 (48/52%)
Ethnicity (n=482)	
➢ Native Dutch (Caucasian)	349 (72.4%)
➢ Immigrant non western/western	50/83 (10.4/17.2%)
(Hair) Characteristics	
Hair color (n=237)	
➢ Black	20 (8.4%)
➢ Brown	116 (48.9%)
➢ Blond	87 (36.7%)
➢ Red	14 (5.9%)
Hair dyed yes/no (n=578)*	5/573 (1/99%)
Washing frequency (n=253)	
➢ <1x/week	26 (10.3%)
➢ 1-2x/week	81 (32.0%)
➢ 3-4x/week	108 (42.7%)
➢ >4x/week	38 (15.0%)
Excessive sweating yes/no (n=240)	29/211 (12/88%)
Use of hair product yes/no (n=255)	88/167 (35/65%)
➢ Mousse	3 (3.4%)
➢ Hairspray	6 (6.9%)
➢ Wax	16 (18.4%)
➢ Gel	23 (26.4%)
➢ Other	26 (30.0%)
➢ Multiple	13 (14.9%)
➢ Missing	7
Smoking yes/no (n=597)*	9/587 (2/98%)

Data described as mean (standard deviation) or number (percentage). Abbreviations: sd(s) standard deviation (score).

*In children who did not fill out a questionnaire, hair dyed was set to 'no' for children under the age of 10 years and current smoking status was set to 'no' under the age of 12 years.

Figure 1. Reference ranges cortisol concentrations over age.

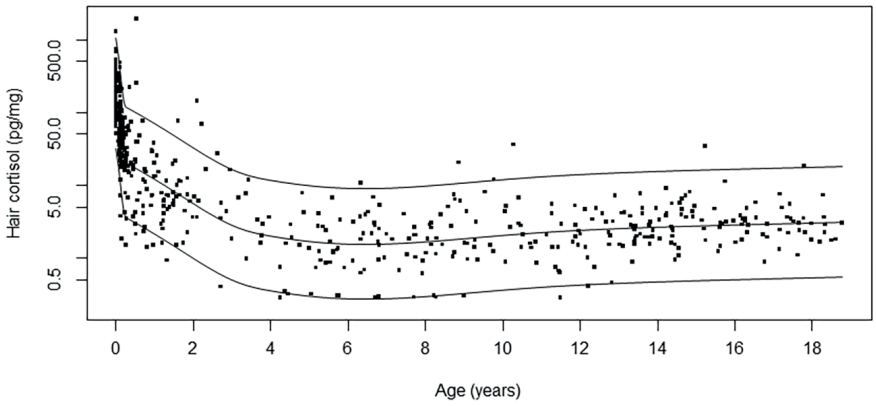


Table 2. Age adjusted reference ranges for hair cortisol (pg/mg)

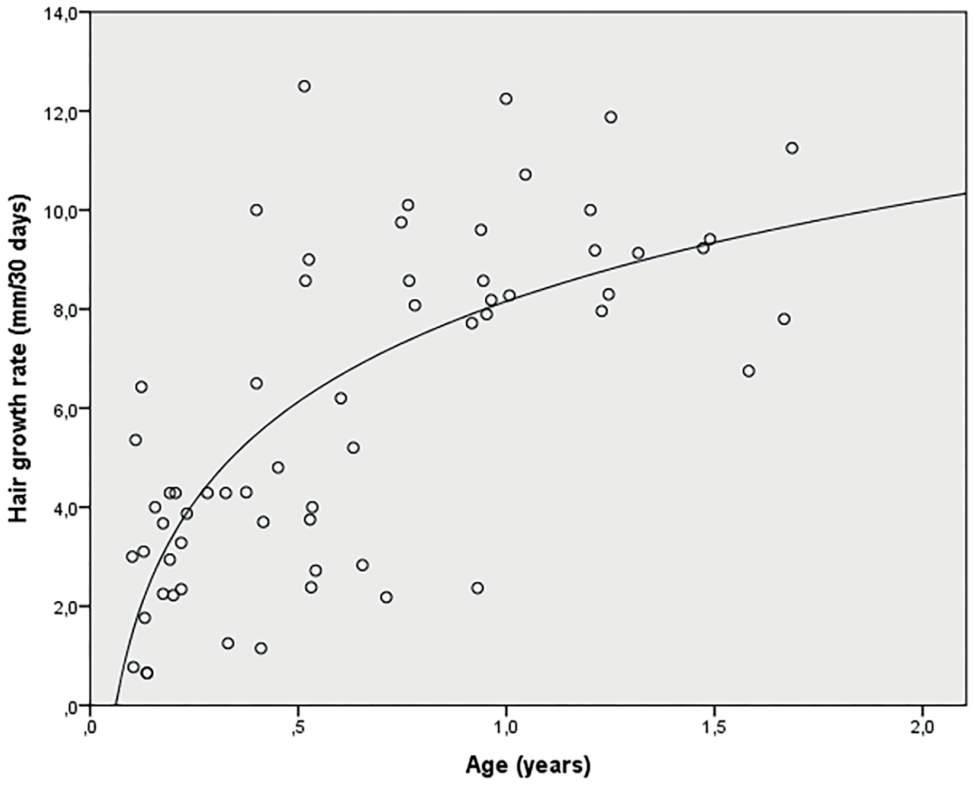
Age	Cortisol		
Percentiles	2.5th	50.0th	97.5th
at birth (n=99)	28.8	162.4	961.2
1 month (n=62)	15.7	88.8	525.7
2 months (n=71)	6.82	38.5	227.8
3 months (n=24)	3.64	20.5	121.5
6 months (n=30)	2.84	16.0	94.9
9 months (n=13)	2.42	13.7	80.9
1 year (n=15)	2.05	11.6	68.6
1.5 year (n=25)	1.45	8.20	48.6
2 year (n=14)	1.06	5.73	33.9
3 year (n=11)	0.56	2.91	17.2
4 year (n=10)	0.34	1.93	11.4
5 year (n=15)	0.29	1.65	9.79
6 year (n=20)	0.27	1.55	9.14
7 year (n=26)	0.28	1.56	9.23
8 year (n=13)	0.30	1.67	9.85
9 year (n=21)	0.32	1.83	10.8
10 year (n=10)	0.36	2.02	12.0
11 year (n=20)	0.39	2.21	13.1
12 year (n=9)	0.42	2.38	14.1
13 year (n=23)	0.45	2.51	14.9
14 year (n=25)	0.47	2.63	15.5
15 year (n=18)	0.48	2.73	16.2
16 year (n=20)	0.50	2.82	16.7
17 year (n=14)	0.52	2.91	17.2
18 year (n=17)	0.53	3.00	17.8

Table 3. Associations with hair cortisol concentrations

	Cortisol		
	Beta	SE	P
Gender			
Boys	Ref		
Girls	-0.07	0.09	0.431
Ethnicity			
Western immigrant/Caucasian	Ref		
Nonwestern immigrant	0.31	0.15	0.036
BMI sds	-0.01	0.07	0.907
Smoking: yes	-0.09	0.34	0.800
Hair color			
Brown	Ref		
Black	-0.17	0.28	0.546
Blond	0.12	0.14	0.395
Red	-0.34	0.29	0.240
Hair treatment: yes	0.11	0.24	0.658
Washing frequency			
<1x/week	0.41	0.22	0.060
1-2x/week	0.05	0.15	0.744
3-4x/week	Ref		
>4x/week	0.15	0.19	0.441
Excessive sweating: yes	0.24	0.20	0.218
Hair product use: yes	-0.04	0.13	0.778

Beta's, standard error (SE) and p-values (P) of linear regression model. All models adjusted for gender and ethnicity. Hair cortisol is provided in z-scores adjusted for age

Figure 2. Hair growth rate in relation to age



DISCUSSION

This study is, to the best of our knowledge, the first to provide both LC-MS/MS-based reference intervals for cortisol concentrations in scalp hair of healthy children from birth to 18 years of age as well as hair growth rates in infants. Hair cortisol levels are age-dependent; remarkably high levels are observed after birth then much lower values in the first months followed by further lower values to a nadir at the age of 6 years. The higher cortisol levels that were found during infancy might be explained by both the lower hair growth rates found in children aged less than 2 years and by the different hair types in this age group, with variation in the cortex and the amount of medulla. (22) This variation might have an effect on the incorporation of free cortisol into hair as this is thought to occur via passive diffusion from blood into the medulla of the hair shaft during growth. (1)

The higher hair cortisol we found in older children is consistent with previous published literature that included adult participants. In a meta-analysis that included 10,289 individuals, the respective correlation coefficient was estimated to increase by 0.09 for each 10-year increase in the standard deviation of age. (11) Consistent with this finding, we measured a median concentration of 3.0 pg/mg in 18-year-old children, while mean cortisol levels in adults range from 3.18 to 5.50 pg/mg. (14,16,28) A recent systematic review of the determinants of hair cortisol in children (13) analyzed age as a predictor of hair cortisol in children. Interestingly, in very young children, especially children under 2 years of age, cortisol was suggested to be particularly high. (18) Our study strongly confirms this finding.

Limited studies on reference intervals and potential confounders of cortisol concentration in children have been published. (18-20) Although only 25 % of laboratories use LCMS technology (13), mass spectrometry has become the preferred method for steroid analysis in high-quality clinical research. (31) The laboratory of Erasmus MC already participated in the first international interlaboratory Round Robin in 2015. (29) Results from the Round Robin showed that immunoassay concentrations are higher by approximately a factor of 3 than the concentrations measured by LC–MS/MS. Therefore, we believe that our data can be interpreted well by colleagues using immunoassays.

Rippe et al. (20) analyzed hair cortisol concentrations in a large cohort of 2484 Dutch children aged 6 years from the Generation R Study. Hair cortisol levels were associated with socioeconomic status, ethnicity, hair color and child characteristics such as birthweight, gestational age at birth, BMI, disease, allergy and medication use. The median cortisol concentrations of 1.55 pg/mg in boys and 1.38 in girls found by Rippe et al. correspond with the results found in the current study (1.55 at age 6 years). Two other studies found either comparable concentrations, Evans et al. (32) found that 10-year old Dutch children had a median concentration of 2.99 pg/mg (compared to 2.02 in our study), or fourfold higher hair cortisol values. (33) Apart from the difference in ethnicity in the Gao study (participants were Chinese), both studies were similar in that they consisted of small numbers (92 and 29 participants) and did not report on potential confounding factors such as hair characteristics and corticosteroid use. (13)

Our finding that hair cortisol, after infancy is higher in older children, corresponds with previous data on traditional cortisol measurements in serum. (34,35) Serum cortisol concentrations steadily get higher from age 4 into adolescence. Kulle et al. (36) reported age- and gender-specific reference data for serum cortisol in 905 children and reported

high cortisol levels in the group aged less than 1 year. Although the current study measures cortisol concentrations in a different matrix, the associations with age resemble those in hormone studies performed in serum.

Several studies in adults have addressed the effects of potential confounders on cortisol concentrations measured in hair. The main covariates to be considered are gender (men exhibit 21 % higher hair cortisol levels than women) (11) and ethnicity (the highest median hair cortisol in blacks, followed by hispanics and whites. (14,37) Rather weak associations are found with hair washing frequency, hair treatment and oral contraceptive use. (13,18,20,28,38) In accordance with these findings, we observed no major effects on hair cortisol concentrations due to hair characteristics but significant effects of ethnicity. Several explanations have been offered to explain this effect. (14) First, certain ethnic groups are more likely to be exposed to biopsychosocial stressors. (39) Second, there are differences in the hair textures of different ethnic groups, and some textures may lead to more incorporation of cortisol into hair. (37) Third, hair grows at different rates in different ethnic groups (40). Although the ethnic makeup of the newborn age group was different than in the other age groups, we think the difference between the very high cortisol levels after birth and the much lower values after the first months is not likely to be explained by ethnicity. In the whole cohort, the influence of ethnicity on cortisol levels was found to be significant but is subtle, within the normal range, compared to the extremely higher levels of cortisol in both western and non-western ethnic groups after birth. Finally, consistent with 11 of the 17 studies on determinants of hair cortisol in children reviewed by Gray (13), we found no significant difference in hair cortisol between girls and boys.

In our study, we noted a significantly lower growth rate of 3.5mm per month in infants up to 6 months old, compared to 9.4 mm/month in children aged 12–24 months and the generally accepted growth rate of 10,0mm per month in adults. (21) Our finding has important implications for the interpretation of hair cortisol values in very young children. There are several possible explanations for the lower growth rate. First, infant scalp hair consists of a different type of hair. In 1930, historical observations of 16 American white children using serial monthly hair samples from birth until the age of 14 years showed that the amount of medulla in newborn hair is much lower than in infant hair and increases into childhood. (41) Barth (22) further described four types of infant hair: lanugo in newborns, vellus hair, an intermediate form of hair from between 3 and 7 months up to 2 years, followed by terminal, adult-like hair. We hypothesize that these four types of infant hair have different growth characteristics. Second, toward the end of the first year, the synchronized cyclic activity of all scalp hair follicles changes to a mosaic pattern. (22,42) During fetal life and for at least the first four months post-birth, all scalp hair follicles are synchronized in the same phase of the growth cycle which consists of three phases: growth (anagen phase), cessation (catagen phase) and rest (telogen phase). This synchronization is lost near the end of the first year, resulting in differences in hair loss and in the percentage of terminal hair follicles on the scalp, which in turn leads to differences in the growth velocity of the hair present. There is considerable individual variation between ages at which the synchronization is lost, which probably explains the variation in individual growth rate from 0.7 to 12.5mm that was found in our study in the age group<12-month age group. It is important to realize that it takes time for the growing hair to reach the surface. Third, ethnicity has a small, but significant effect on the individual growth rate: African hair grows more slowly than Caucasian hair, which in turn grows more slowly than Asian hair (mean ± SD: 280 ± 50, 367 ± 56 and 411 ± 43 µm/day, respectively). (40,43) As within the groups of immigrants in our study, western and non-western, both African and

Asian hair types are represented, it is difficult to estimate the effect of ethnicity on the growth rate in our study.

Strengths of the present study include the use of a LC-MS/MS method in a representative population with multiple ethnicities. In the Netherlands 77 % of the inhabitants are native Dutch and 23 % are immigrants compared with 72.4 and 27.6 % in our study (Statistics Netherlands, StatLine, CBS 2018). Next to this we measured a broad range of potential confounders. This is the first study to examine reference intervals across all age ranges from birth to 18 years including a large number of hair samples from the difficult-to-study young age group. For the first time, hair growth velocity has been studied in infants. A limitation in the current study is that immigrant ethnicity is defined as a dichotomous variable (Western vs. non-Western), which probably underrepresents the differences between ethnicities in the non-Western group. As ethnicity in the Netherlands is strongly associated with immigration, culture, different social economic status and education level (44), it is difficult to determine how ethnicity affects cortisol levels. Another limitation is that detailed information on demographic characteristics of the cohort, such as parental education, socioeconomic status, neighbourhood level advantage/disadvantage is not available. Further reference studies in populations with different mixes of ethnicities and information on SES measures would be of additional value.

Measuring cortisol in scalp hair yields long-term cumulative cortisol concentrations unaffected by circadian rhythm or acute stress. The noninvasiveness of the procedure and easy storage of samples make this method exceptionally suitable for pediatric clinical practice. Longitudinal studies in which cortisol analysis in scalp hair are included may provide new insights into the pathophysiology of cortisol-related morbidity and leads to novel clinical applications for a range of endocrine diseases - including diagnosis and follow-up in hypercortisolism, adrenal insufficiency and congenital adrenal hyperplasia - as well as use in research and as a biomarker for stress and mental health.

This study provides knowledge about pediatric reference intervals and hair growth velocity in infants, which is essential for the implementation of hair cortisol testing and interpretation of the results in pediatric clinical practice.

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Dominique G.A. Begijn contributed to investigation, data curation and writing - review and editing.

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Supplemental table: Associations of hair cortisol and hair growth velocity

		N	Mean	Std. Deviation
Hair cortisol (pg/mg)	0-6mnd	24	39,6083	29,74392
	6-12m	11	14,1636	12,46180
	12-14m	12	9,1417	5,92245
	Total	47	25,8745	26,24392
log Hair cortisol (pg/mg)	0-6mnd	24	3,3035	1,04983
	6-12m	11	2,2127	1,04759
	12-14m	12	1,9915	,74250
	Total	47	2,7132	1,13976
Hair growth rate (mm/30 days)	0-6mnd	24	3,559	2,1289
	6-12m	11	6,945	3,6345
	12-14m	12	9,555	1,7166
	Total	47	5,882	3,5282

4

Parental stress and scalp hair cortisol in excessively crying infants; a case control study

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ABSTRACT

Background

Caring for an excessively crying infant (ECI) can be stressful for mothers and fathers and is associated with mental and bonding problems. Hair cortisol offers a unique measure for the biological reaction of the body to stress over time.

Methods

In this case-control study, scalp hair cortisol concentrations (HCC) were measured using liquid chromatography-tandem mass spectrometry (LC-MS) in 35 mothers and 23 fathers and their ECIs. The control group consisted of 64 mothers and 63 fathers of non-ECIs of similar age. Parental stress, depression, anxiety and bonding were assessed using validated questionnaires.

Results

Mean HCC were significantly lower in mothers and fathers of ECIs (2.3 pg/mg, 95%CI 1.8–2.9 and 1.6 pg/mg, 95% CI 1.3–2.0) than that in control mothers and fathers (3.2 pg/mg, 95%CI 3.0–3.7 and 2.9 pg/mg, 95% CI 2.5–3.5). In the total group of parents and within the parents of ECIs, HCC were not associated with negative feelings. In the control group, HCC showed a positive association with stress and depression ($r = 0.207$, $p = 0.020$ and $r = 0.221$, $p = 0.013$). In infants, no differences were found in mean HCC between the ECI group and the control group. No associations were found between maternal and infant HCC, paternal and infant HCC and maternal and paternal HCC.

Conclusion

Parents of ECIs showed significantly lower HCC than control parents, reflecting a diminished response of the hypothalamic-pituitary-adrenal (HPA) axis. More research is needed to examine whether this decrease in response is pre-existing or caused by excessive infant crying.

INTRODUCTION

Excessive infant crying, often referred to as infant colic, is one of the most distressing challenges for new parents. Recurrent and prolonged periods of infant crying, fussing or irritability, as reported by parents, in otherwise healthy infants under five months of age account for 10–20% of pediatric visits during the first months of life. (1,2)

Parental Distress

In these families, a vicious circle is frequently observed in which crying leads to parental stress, which negatively affects the parent–infant relationship and often leads to more crying. (3) For decades, research has focused on maternal feelings and the traditional view of mother as principal caretaker, which is remarkable since in the past decade fathers are more involved than ever in early childcare and there are good reasons to assume that paternal involvement could exert significant influence on both the developing child and the mother. Fortunately, in the last few years, studies have increasingly addressed paternal feelings and have shown that in addition to stress, excessive infant crying has been associated with mental problems such as depression and anxiety and with bonding problems in both mothers and fathers. (4-8)

HPA Axis

The neuroendocrine hypothalamic-pituitary-adrenal (HPA) axis and its main downstream effector, the glucocorticoid cortisol, is considered to be a key mediator of the link between chronic stress and mental health problems. (9,10) Limited research supports the association between salivary cortisol levels and excessive infant crying. (11) In one study, the psychological wellbeing of mothers of 24 excessively crying infants (ECIs) was predicted by the infant's morning salivary cortisol levels and crying intensity. (12) Another study, including 20 ECIs and 20 controls, showed a blunted circadian rhythm of cortisol in the ECI group. (13) This may be an important finding because, in general, flatter diurnal cortisol rhythms across the day are associated with poorer health. (14) However, salivary and blood cortisol levels represent short-term stress responses and momentary stress (15) whereas long-term cortisol measurements are needed to study the chronic stress experienced by parents coping with an ECI.

Scalp Hair Cortisol

Scalp hair cortisol concentrations (HCC) as a marker of long-term cortisol levels have been increasingly studied in the last decade. (16,17) Increased HCC were associated with chronic stress exposure, especially when the exposure was ongoing at the time of the study. (9,18) In contrast, decreased HCC have been found in anxiety disorders, such as post-traumatic stress disorder (9,19). A broad range of confounders of HCC has been determined in the past decade. (9,19)

In children, the study of HCC in relation to stress, trauma exposure and other psychosocial factors (20) showed very heterogeneous results. This may point to difficulties in defining and measuring "stress" and the fact that the HPA axis may be more sensitive to stimuli during specific developmental periods. Persistent stress, such as maternal distress, has been shown related to elevated HCC in young children. (21) Research on HCC has focused on mother-child dyads, studies in the infant group including fathers are, however, lacking. Additionally, it is interesting to investigate more objective methods to measure the severity of the stress experienced by parents and ECIs. (22)

We hypothesized that chronic stress experienced by parents and their ECI leads to increased HPA-axis activity signified by increased HCC. In the present study, our aim was to answer the following topics and questions:

- Parental HCC: Do HCC in mothers and fathers with an ECI differ from parents without an ECI (control group)?
- Parental feelings: Is parental HCC associated with experienced stress, depression, anxiety and bonding problems?
- Infant HCC: Do HCC in ECIs differ from that in control infants?
- Association between parental and infant HCC: Is there an association between parental and infant HCC and paternal and maternal HCC?

MATERIALS AND METHODS

Study Design and Setting

In this cross-sectional, case-control study, data from two groups of parents were analyzed: mothers and fathers with an ECI and control parents with a non-ECI. Infants aged up to 5 months, who presented with infant colic (1) and their parents were recruited together with their father and mother at the outpatient clinic of the Department of Pediatrics of the St. Antonius hospital in the Netherlands. For the control group, age matched infants were enrolled at infant welfare centers in the neighborhood (75%) and 25% of the controls enrolled at the outpatient clinic of the Department of Pediatrics of the St. Antonius hospital, when they received a routine ultrasound of the hip after breech delivery. Recruitment of the participants occurred between August 2015 and March 2017. Subjects were excluded for the following reasons: parents' insufficient knowledge of the Dutch language and birth at a gestational age < 36 weeks. Excessively crying infants in the control group (an affirmative answer to the question "Does your child cry excessively?") were excluded (n = 6).

As the participants were not subject to procedures and were not required to follow rules of behavior, the local medical ethical commission deemed that this study was not subject to the Dutch Medical Research Involving Human Subjects (Act WMO). Written informed consent was obtained from all participants.

Measures

Hair Cortisol

At inclusion, hair locks from the posterior vertex were collected from mothers, fathers and infants, by cutting with small surgical scissors as close to the scalp as possible. The hair locks were taped to a paper form with the scalp end marked and then stored in envelopes at room temperature. The proximal 3 cm of the hair was used for a single analysis. In infants whose hair had not grown to 3 cm, at least 1 cm of the hair closest to the scalp was analyzed. Subsequently, hair samples were transferred to glass tubes, weighed (mg), washed by gently shaking them in liquid chromatography-tandem mass spectrometry (LC-MS) grade isopropanol at room temperature and left to dry for at least 48 h. Cortisol was extracted in 1.5 mL LC-MS grade methanol for 18 h at 25˚C in the presence of deuterated cortisol-d3 and it was subsequently cleaned using solid phase extraction. The LC-MS/MS method has been described in detail previously. [23,24] Cortisol concentrations were reported in picograms per micrograms of hair. The lower limit of quantification (LLoQ) of cortisol was <1.0 pg/mg. Measurements below LLoQ were set on 1.0 pg/mg.

Parental Stress, Depression and Anxiety and Parent-Infant Bonding

Experienced parental distress was assessed using validated questionnaires as described in our previous publication (8). We assessed stress using a Dutch translation of the Perceived Stress Scale (PSS) (25) and depressive symptoms using the Dutch version of the Edinburgh Postnatal Depression Scale (EPDS). (26,27) Although this instrument was initially developed to investigate mood disturbances in women, the EPDS has proven to be a reliable and valid instrument in screening for depressive symptoms in fathers as well. (28,29) Higher scores indicate higher levels of depressive symptoms.

Symptoms of anxiety were assessed using the short form of the Spielberger State-Trait

Anxiety Inventory (STAI), (30–32) and problems in the parent-infant relationship were assessed using the Dutch version of the Postpartum Bonding Questionnaire (PBQ). (33–35) Originally the PBQ has been developed and validated for measuring mother-infant bonding. Since a questionnaire about father-infant bonding has not yet been developed, the PBQ was also used for fathers in this study. Higher sum scores in the used questionnaires indicate increased levels of parental distress and problems with bonding.

Infant Crying Behavior

Parents were asked to record the duration of infant crying in a diary for three consecutive days (36). For this study, crying episodes lasting for at least 10 min were recorded. Because crying intensity may reflect greater physiological or psychological stress than duration of crying, we additionally asked the parents to record the infant crying volume and intensity on scales ranging from 1 to 9, representing, respectively, barely audible to very loud crying and no facial expression to painful grimacing while crying. (11)

Confounders and Mediators

The following factors were obtained using standardized questions and included as potential confounders: information about demographic and hair characteristics, smoking, medication use and course, complications and experience of pregnancy and delivery. The pregnancy and delivery experience was queried using the question “How did you experience the pregnancy/delivery?” The answers were reported on a scale ranging from 1 to 5, representing very positive to very negative. Furthermore, pre-existing factors contributing to the parents’ current emotional state, such as recent stressful events, emotional problems during pregnancy and current psychiatric treatment, were assessed using standardized questions.

Statistical Analysis

Data are presented as means (±standard deviation (SD), medians (range or interquartile range), or counts (%), where appropriate. Sum scores of the four questionnaires were obtained and an average was calculated for both mothers and fathers. A maximum of 33.3% missing data within one subject was accepted for these questionnaires and corrected for by taking the participants’ mean of the completed questions.

Sociodemographic and hair characteristics were compared between the ECI and control groups with the Chi-square test/Fisher’s exact test and independent samples t-test, or the Mann–Whitney U test in case of non-parametric data. As HCC have a skewed distribution, log-transformation was applied to achieve a normal distribution. In presenting the results, the log HCC were recalculated in the real HCC and the corresponding 95% confidence intervals (CI).

Mean HCC in mothers, fathers and infants were compared between the case and control group using the independent samples t-test (unadjusted mean and 95% CI) and linear regression analysis. In these analyses, adjustments for parents (age, high education level, ethnicity and emotional/psychiatric problems during pregnancy) and adjustments for infant factors (age, male sex, medication use of infant and psychotropic medication use of mother) were applied. Potential confounders were identified by a 10% change-in-estimate criterion for estimated associations or determination as confounders in previous studies.

Multivariate linear regression analyses were also used to investigate the association

between the HCC in mothers, fathers and both parents to parental stress, depression, anxiety and bonding scores with and without adjustments for potential confounding factors and with and without splitting for case and control groups.

Additionally, we used multivariate linear regression analyses to investigate the association between the HCC in mothers, fathers and both parents to infant crying duration, volume and intensity with and without adjustments for potential confounding factors and with and without splitting for case and control groups.

The association between maternal and infant HCC, paternal and infant HCC and maternal and paternal HCC in both the case and control samples was investigated using linear regression analysis, with and without adjustments for potential confounding factors and with splitting for case and control groups. A p-value of <0.05 was considered statistically significant. All analyses were performed using IBM SPSS (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.

RESULTS

A total of 208 families (both cases and controls) were approached for participation, of whom 99 mothers, 86 fathers and 97 infants, were included in the final hair cortisol analysis (Figure 1).

The sociodemographic characteristics of the participating families are shown in Table 1. There was no difference in hair color and delivery mode between groups. In both parents, hair bleaching and dyeing occurred more often in the excessive crying group (43% vs. 20%, p = 0.017), but it was not identified as a confounder.

Table 2 shows parental distress: the reported levels of stress, depression, state anxiety and bonding behavior of both parents and crying behavior of their infant in the ECI group and control group. The mothers and fathers with ECIs reported significantly more stress, depression, anxiety, bonding problems, crying time and intensity than control mothers and fathers.

Parental HCC and infant HCC in the case group of ECIs and that in controls are shown in Table 3.

Parental HCC

Mean HCC in mothers were significantly lower in the ECI group (2.3 pg/mg, 95% CI 1.8–2.9) than in the control group (3.2 pg/mg, 95% CI 3.0–3.7). Mean HCC in fathers was also significantly lower in the ECI group (1.6 pg/mg, 95% CI 1.3–2.0) than in the control group (2.9 pg/mg, 95%CI 2.5–3.5). Adjustment for confounders did not change these results.

Parental Feelings

Multivariate linear regression analyses showed no significant association between HCC in mothers, fathers or both parents and stress, depression, anxiety and bonding sum scores. The group of parents with an ECI showed no significant associations between HCC and the sum scores of stress, depression, anxiety and bonding. However, HCC in the control group parents showed a statistically significant increase (r = 0.207, p = 0.020) with increasing PSS sum scores. A similar positive association was found between parental HCC and EPDS sum scores in the control group of control parents (r = 0.221, p = 0.013). No association was found between HCC and STAI or PBQ scores. Adjustment for confounders did not change these results.

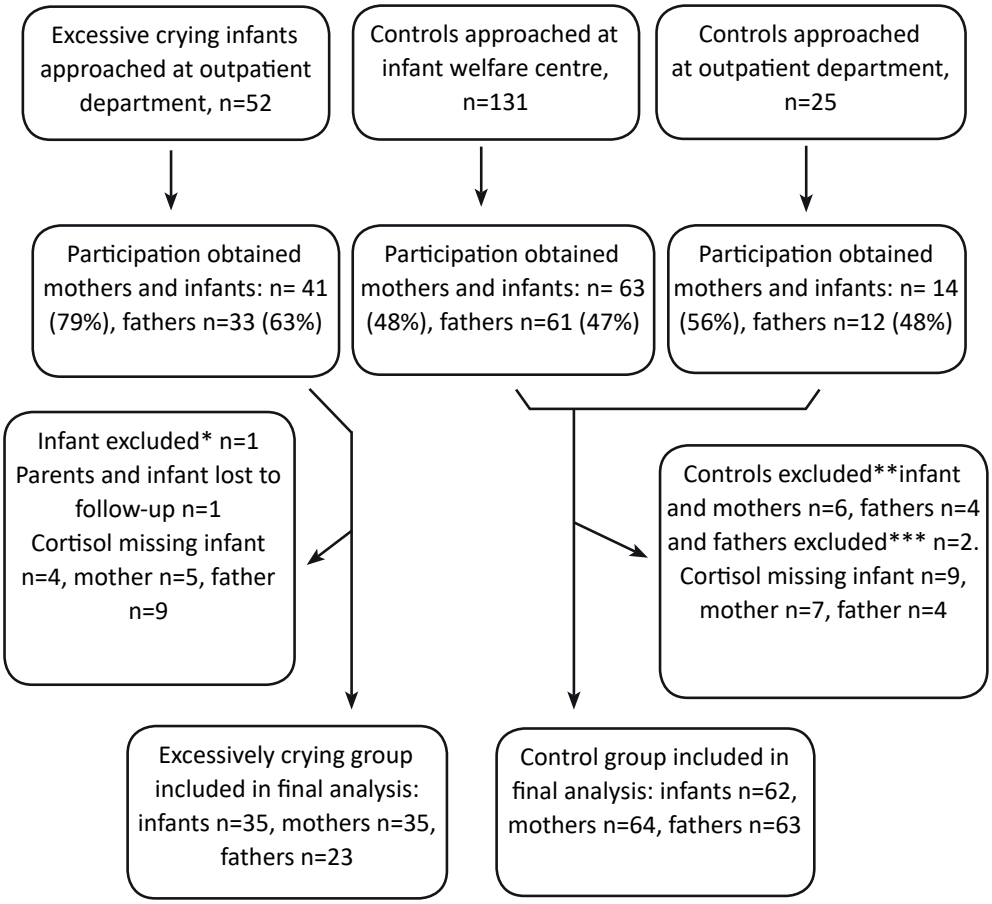
Infant HCC

No differences in HCC were found between ECIs and control infants (32 pg/mg, 95%CI 25–41 and 34 pg/mg, 95%CI 26–44).

Association between Parental and Infant HCC

The maternal HCC and infant HCC showed a non-significant association in both the case- and control groups (r = 0.25 versus r = 0.18). The correlation between paternal HCC and infant HCC in the case- and control groups (r = 0.16 versus r = -0.18) and that between maternal HCC and paternal HCC in the case- and control groups (r = -0.16 versus r = 0.05) were also not significant. Multivariate linear regression analyses showed no significant association between HCC in mothers, fathers or both parents and infant crying duration, volume and intensity.

Figure 1. Flow diagram of inclusions



* Case infant excluded because of gestational age <36 weeks, ** Controls excluded because of one of the parents experienced their infant as excessively crying based on the questionnaire, *** Control parents excluded because of oral corticosteroid use

Table 1. Sociodemographic characteristics

	Excessive crying Mean (SD) or % and (N)	**Control Mean (SD) or % and (N)**	**P-value**
Infants' age (w)	8.54 (3,37)(35)	10,19 (3.95) (62)	0.040
Male gender	57.1 (20/35)	45.2 (28/62)	0.257
Gestational age at birth (w)	38.9 (1.3) (35)	39.3 (1.4) (62)	0.150
Birthweight (g)	3312 (534) (35)	3433 (555) (62)	0.300
Feeding status			
• Exclusive breastfeeding	20.0 (7/35)	46.8 (29/62)	
• Formula only	80.0 (28/35)	40.3 (25/62)	<0.001
• Breast milk and formula	0	12.9 (8/62)	
Use of medication infant			
• Acid reflux treatment	31.4 (11/35)	0 (0/62)	<0.001
Use of medication mother			
• Psychotropic medication	14.13 (5/35)	3.1 (2/64)	0.093
Age (y)			
• Mother	31.2 (3.2) (35)	31.9 (4.6)(64)	0.354
• Father	33.4 (4.6) (23)	34.5 (4.9)(63)	
Ethnicity (Dutch-Caucasian)			
Mother	100 (35/35)	90.6 (58/64)	0.087
Father	87 (20/23)	93.7 (59/63)	0.378
Educational level of mother			
• Secondary school or less	5.7 (2/35)	4.7 (3/64)	
• Secondary vocational education	48.6 (17/35)	17.2 (11/64)	0.004
• Higher professional education	28.6 (10/35)	43.8 (28/64)	
• University	17.1 (6/35)	34.4 (22/64)	
Educational level of father			
• Secondary school or less	21.7 (5/23)	9.5 (6/63)	
• Secondary vocational education	30.4 (7/23)	33.3 (21/63)	0.298
• Higher professional education	26.1 (6/23)	30.2 (19/63)	
• University	21.7 (5/23)	27.0 (17/63)	
Current Smoking			
• Mother	14.7 (5/34)	4.8 (3/63)	0.124
• Father	17.4 (4/23)	15.9 (10/63)	1.000

Table 1. Sociodemographic characteristics (Continued)

Emotional/psychiatric problems pregnancy			
• Mother	25.7 (9/35)	10.9 (7/64)	0.056
• Father	8.7 (2/23)	1.6 (1/63)	0.173
Current psychiatric treatment			
• Mother	14.3 (5/35[1])	3.1(2/64)	0.093
• Father	8.7 (2/23)	1.6 (1/63)	0.173
Experienced stressful events			
• Mother	54.3 (19/35)	38.1 (24/63)	0.122
• Father	52.2 (12/23)	41.0 (26/63)	0.367
Negative experience of pregnancy			
• Mother	14.3 (5/35)	6.5 (4/62)	0.277
• Father	8.7 (2/23)	1.6 (1/61)	0.181
Negative experience of delivery			
• Mother	14.7 (5/34)	14.5 (9/62)	1.000
• Father	17.4 (4/23)	3.3 (2/61)	0.045

[1] two mothers > diagnoses.

Table 2. Reported levels of stress, depression, state anxiety and bonding behaviour of mother and father and crying behaviour of infant in the excessive crying and control group

Variable	Excessive crying Mean (SD) and (N)	Control Mean(SD) and (N)	P-value
Stress (PSS)			
• Mother	25.2 (8.1) (33)	14.1 (6.9) (64)	<0.001
• Father	21.0 (6.3) (20)	16.2 (6.1)(62)	0.003
Depression (EPDS)			
• Mother	8.8 (5.2) (34)	3.8 (3.2) (64)	<0.001
• Father	5.4 (4.3) (20)	2.8 (2.9) (62)	0.016
State Anxiety (STAI)			
• Mother	45.9 (12.3) (34)	31.6 (9.3) (64)	<0.001
• Father	42.1 (10.0) (21)	31.6 (7.5) (62)	<0.001
Bonding behaviour (PBQ)			
• Mother	15.2 (8.2) (16)	4.8 (4.4) (64)	<0.001
• Father	18.0 (8.4) (10)	7.9 (5.9) (62)	<0.001

Table 2. Reported levels of stress, depression, state anxiety and bonding behaviour of mother and father and crying behaviour of infant in the excessive crying and control group (Continued)

Crying duration (min)			
• during the day	112 (78) (31)	26 (24) (61)	<0.001
• during the night	74 (53) (31)	14 (15) (61)	<0.001
Intensity of crying	5.9 (1.9) (13)	2.6 (1.6) (61)	<0.001
Volume of crying	6.0 (1.8) (13)	3.5 (5.3) (61)	0.096

Table 3. Hair cortisol concentration mean (±95% CI) in cases versus controls infant, mother and father

Characteristics	***Unadjusted Mean (95% CI)***	**Log beta**	**p-value**	**Log adjusted beta**	**p-value**	**Log adjusted beta**	**p-value**
HCC infant (pg/mg)							
Control (n=62)	34.0 (26.3- 44.0)	Ref		Ref*			
Exc crying (n=35)	32.1 (25.1- 40.9)	-0.06	0.762	-0.14	0.510		
HCC mother (pg/mg)							
Control (n=64)	3.2 (3.0 - 3.7)	Ref		Ref**		Ref**	
Exc crying(n=35)	2.3 (1.8 - 2.9)	-0.35	0.009	-0.41	0.002	-0.40	0.003
HCC father (pg/mg)							
Control (n=63)	2.9 (2.5 - 3.5)	Ref		Ref**		Ref***	
Exc crying (n=23)	01.6 (1.3 - 2.0)	-0.60	<0.001	-0.62	<0.001	-0.60	0.001

* corrected for infant factors (age, male sex, medication use, psychotropic medication use of mother); ** corrected for demographic factors (age, high education level, ethnicity); *** corrected for emotional/psychiatric problems during pregnancy.

DISCUSSION

In our study, our first aim was to study parental HCC and we showed that parents with an ECI had lower HCC compared with control parents, while the HCC in control parents were in the normal range, as found in healthy adults. (37–39) Based on previous hair cortisol studies reporting increased HCC after stress exposure, we expected higher, instead of lower mean HCC in parents of ECIs, especially because the excessive crying continued at the time of the study. (18,40) One explanation for this seemingly contradictory result could be that the experience of infant crying is a different form of stress than somatic stress. Flattening of the cortisol diurnal slope or dampening of the cortisol response is a wellknown phenomenon in psychological studies (9,41) and has, for example, been associated with psychosocial stress in women with preexistent psychopathology. (42) Studies of HCC after traumatic events have shown both negative and positive associations between HCC and trauma, indicating that the strength and direction of the association are moderated not only by type and timing of trauma but also by study characteristics, like racial distribution, clinical diagnosis/or non-clinical diagnosis and features of the publication (for example the geographical region of the study). (43) The lowering of the parental HCC could not be explained by certain characteristics of the infant crying; the duration, volume and intensity of crying showed no correlation with parental HCC in our study.

In order to identify whether the type of psychological stress influenced parental HCC, our second aim was to study the association between HCC and parental feelings of stress, depression, anxiety and bonding problems. Parents of an ECI significantly experienced more stress, depression, anxiety and bonding problems than the control parents. These stressful feelings may have resulted in the downregulation of the HPA system. Still, since we did not find an association between the severity of these feelings and HCC in ECI parents, we speculate that the downregulated parental HPA system could already have been present during pregnancy. It is known that maternal anxiety during pregnancy is related to an excessive crying infant. (6) This apparent downregulation of the parental HPA system in ECI parents may result from early adverse life events inducing persistent changes in the HPA axis, which may predispose these parents to the development of mood and anxiety disorders in this period of their lives. (44)

Control parents with increasing stress and depression scores had higher HCC. In contrast, in parents of ECIs, no association was found between HCC and experienced stress, depression, anxiety and bonding problems. These findings also suggest a dampening of responsivity of the HPA axis to stress and depression in these parents.

To the best of our knowledge, this is the first study evaluating HCC in fathers of both healthy and excessive crying infants. In accordance with findings in mothers of excessive crying infants, we found the same significant lowered HCC in the exposed group and no association with infant HCC. We found no association with maternal HCC. In contrast, one cross-sectional study reported an association of HCC in 6-year-old children and their mother and father. (45) Future research should investigate if the same mechanism as in mothers, where anxiety during pregnancy is related to excessive crying (6), plays a role in fathers of infants with excessive crying and which other factors could contribute to the lowered HCC.

Our third aim was to investigate differences in HCC in ECIs and control infants. In contrast to the findings in their parents, no differences in infant HCC were found between the two groups. One potential explanation is that excessive crying is not a stressor to the infant itself. This is in line with a study looking into the correlation between neonatal hair cortisol levels and infant distress. (46) Another explanation could be that the age range of the infants plays a role. HCC levels in infants are very high at birth and decrease in the first months of life. Our recent study on normal HCC values in children (24) showed that HCC in infants are more than 10-fold higher than that in adults, have wide reference ranges and show a sharp decline in the first three months of life. Therefore, future studies on HCC in infants need to use a smaller age range (per month) and larger sample sizes per age to identify subtle concentration changes. Furthermore, there may be other factors moderating the association between stressors and physiological stress in infants, such as parental care and caregiver relationships (47), which need to be investigated in larger population cohorts.

Our last aim was to study the association between parental and infant HCC. No correlation was found between maternal and infant HCC, paternal and infant HCC and maternal and paternal HCC within both groups, in contrast to earlier studies. (48,49) However, our results are in line with a recent study, in which mother-infant dyads subjected to severe psychiatric disorders also showed no correlation between maternal and infant HCC. (50)

Strengths and Limitations

The major strength of our study is the unique sample of parents and infants experiencing the stressor “excessive crying”, in a controlled design, including the measurement and adjustment of a broad range of confounders. Existing literature concerning excessive infant crying has focused primarily on mothers’ perceptions, while feelings and caring for an excessively crying infant can be challenging for both parents. Therefore, the inclusion of fathers is another strength of this study.

This study also has several limitations. First of all, our study is limited by the crosssectional design, which prevents us from establishing causality. Secondly, the age range of the infants might have been too broad to identify a difference between HCC in ECIs and control infants. The mean infant age differed slightly between groups, for which we corrected in the analyses. Thirdly, in the control group, selection bias cannot be excluded as only half of the parents approached were willing to participate. Fourthly, the PBQ questionnaire was not designed for measuring father–infant bonding and the time frame of hair cortisol and questionnaires did not fully overlap. We investigated the association between mean HCC in 3 cm of hair—representing 3 months—while parental distress was examined at the time of the questionnaire (STAI, PBQ), during the past week (EPDS) and in the last month (PSS). Prospective studies with larger samples, smaller age ranges of ECIs, stress questionnaires during pregnancy and the first months after birth and addressing additional factors such as parental care and caregiver relationships are needed to examine whether HCC can serve as a stress marker experienced by parents caring for ECIs. Future research projects on establishing evidence-based management strategies for excessive crying/infant colic may benefit from this marker.

Conclusions

In conclusion, this study shows that HCC in the parents of ECIs is significantly lower than that in control parents. In addition to the specific stressor of excessive infant crying, the

characteristics of the parental sample, experiencing more stress, anxiety, depression and bonding problems, potentially contributed to the difference we observed. We speculate that the downregulation of the parental HPA axis already started during pregnancy, related to prenatal anxiety and may even represent the cause of excessive crying. We conclude that integrated care for both fathers and mothers should ideally start during early pregnancy to reduce feelings of stress and anxiety, which could positively contribute to preventing excessive crying in their infants.

Author Contributions

Ineke de Kruijff contributed to conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, validation, visualization, writing-original draft and writing-review and editing.

Ellen Tromp contributed to conceptualization, data curation, formal analysis, methodology, software, supervision, validation, visualization and writing-review and editing.

Mijke P. Lambregtse-van den Berg contributed to conceptualization, supervision and writing-review and editing.

Arine M. Vlieger contributed to conceptualization, supervision and writing-review and editing.

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An exploratory study of perinatal hair cortisol concentrations in mother–infant dyads with severe psychiatric disorders versus healthy controls

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ABSTRACT

Background

Maternal psychopathology during pregnancy is associated with negative outcomes in offspring. Increased placental transfer of maternal cortisol may contribute to mediate this association. Hair cortisol concentrations (HCCs) appear to be a good biomarker of long-term prenatal stress exposure. Little is known about the associations between severe maternal psychopathology and perinatal infant HCCs.

Aims

We assessed HCCs in the perinatal period in mother–infant dyads with and without severe psychiatric disorders.

Method

We examined group differences in HCCs of mother–infant dyads (n = 18) subjected to severe maternal psychiatric disorders versus healthy control dyads (n = 27). We assessed the correlation of HCCs between mother and infant within both groups, and the association between current maternal symptoms and HCCs in patient dyads.

Results

Median (interquartile range) and distribution of HCC differed in patients compared with control mothers (U = 468.5, P = 0.03). HCCs in infants of patients did not differ from control infants (U = 250.0, P = 0.67). Subsequently, we found that HCCs within healthy control dyads were correlated (n = 27, r 0.55 (0.14), P = 0.003), but were not within patient dyads (n = 18, r 0.082 (0.13), P = 0.746). HCCs in infants of patients showed a positive correlation with maternal symptoms (n = 16, r = 0.63 (0.06), P = 0.008).

Conclusions

These preliminary findings suggest that infant HCC reflect perinatal stress exposure. In infants, these early differences could influence lifetime hypothalamic–pituitary–adrenal axis functioning, which might be associated with increased susceptibility to later disease.

INTRODUCTION

Children of mothers with psychiatric disorders during pregnancy are at high risk for developing physical and psychiatric disorders later in life. (1,2) Maternal psychopathology and stress during pregnancy are among the most common intrauterine exposures associated with negative outcomes in offspring, with prevalence rates of 10–15% for depression and anxiety disorders. (3–5) Psychiatric disorders are associated with alterations in basal cortisol levels and disturbed variability of the stress response owing to dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis. (6,7) Accumulating research suggests placental transfer of maternal cortisol might play a mediating role in the effects of maternal psychopathology on the neurocognitive and physical development of the foetus (8), and increased prenatal maternal cortisol levels have been repeatedly linked to adverse child outcomes in the short and long term, such as lower birth weight, small for gestational age, intellectual disability and behavioural problems. (9–11) As most previous research has focused on less-affected individuals, the specific aim of this study is to explore the effect of severe and long-lasting psychiatric disorders during pregnancy on early hair cortisol concentrations (HCCs) in mother–infant dyads.

Hair cortisol is a reliable biomarker reflecting chronic systemic cortisol levels (12,13), as well as stress exposure. (14,15) Maternal HCC at 6 weeks' postpartum probably reliably reflects cortisol exposure in the preceding 3 months, which allows us to quantify cortisol exposure from the last 6 weeks of pregnancy to the first 6 weeks' postpartum. (16,17) In very young infants, less is known about the exact timeframe of exposure, but supposedly HCC reflects cortisol exposure in the intrauterine milieu during late pregnancy and early postpartum cortisol exposure. (18)

HPA axis and psychiatric disorders

The relationship between psychopathology and HPA axis deviations in general has not been fully elucidated. Specific psychiatric diagnoses, such as major depressive disorder, bipolar disorder and schizophrenia, have been linked to higher basal cortisol levels, whereas anxiety disorders have been associated with a combined profile of higher levels of cortisol during acute stress and lower baseline cortisol levels. (19,20) The latter finding of lower baseline cortisol levels has also been found in studies on borderline personality disorder. (21) Post-traumatic stress disorder has been generally linked to lower cortisol levels; however, cortisol appears to be elevated when the traumatic event has happened more recently, or when traumatic circumstances are still present. (22,23) It has been proposed there is a non-linear, two-stage timeline with regard to cortisol dysfunction in relation to trauma: the severity of traumatisation and more temporally distant traumatisation were related to lower HCC, whereas higher HCC was found in more recently traumatised individuals. (24) Similarly, in depression, it has been found that recurring episodes are associated with lower HCC. This evidence leads to the hypothesis that chronic overactivation of the stress response leads to blunted HPA axis activity over time, indicating that the severity and duration of stress activity might be a more important determinant of basal cortisol levels in patients with severe and long-lasting psychiatric disorders than the nature of the psychiatric diagnosis. (25,26)

Maternal HPA axis functioning and its influence on the foetus

Altered HPA axis activity in mothers who suffer from severe psychiatric disorders can influence the foetus through intrauterine programming of the HPA axis. (19,27) There

is some evidence that these early alterations in HPA axis functioning contribute to vulnerability to psychiatric disease in offspring later in life (28), by early fine-tuning of the HPA axis set point.

Under normal conditions, in the absence of psychopathology or severe stress, maternal and infant cortisol and cortisol responses appear to be positively correlated shortly after birth. (29,30) This finding is mainly based on research with saliva cortisol, but has been confirmed in animal studies on hair cortisol (31) and in studies on healthy mother–infant dyads. (17)

Studies evaluating the effects of stress and psychopathology on maternal and foetal HCC during pregnancyand beyond are rapidly emerging, but results are inconclusive. (32–38) This might be partly explained by differences in study sample (i.e. healthy versus depressed mothers) and different definitions of 'stress' (i.e. perceived stress versus psychiatric symptom scales). Inconsistent results have emerged on the association between maternal prenatal cortisol levels and self-reports of prenatal psychological distress, elevated symptoms of prenatal depression, anxiety and antidepressant use. Evidence in different studies does show that excess maternal cortisol during pregnancy is associated with decreased infant cortisol levels, as measured in infant hair, shortly after birth (33,39) and at 12 months' postpartum.34 In 2-year-old children, Bryson et al (40) found a significant association between maternal and infant HCC that was not mediated by measures of early childhood adversity. Furthermore, two studies found that elevated maternal HCC during pregnancy mediated disrupted mother–child interaction in early infancy. (26,41) These results suggest that maternal stress (i.e. psychiatric symptoms) is inconsistently related to maternal and/or infant HCC, but independently, maternal HCC seems to influence infant cortisol and mother–child interaction both in the early postpartum period and beyond.

The underlying mechanism of transmission of maternal psychopathology during pregnancy through cortisol remains unclear, as cortisol attunement between mother and foetus is composed of complex intrauterine interactions between the maternal, placental and foetal endocrine systems. The placental barrier is not completely impenetrable for transfer of cortisol, as a small proportion (10–20%) of maternal cortisol does reach the foetus. (42) However, in stressful situations, more cortisol can cross the placental barrier. (43,44) Thus, it has been proposed that in stressed mothers, the excess of maternal cortisol levels leads to downregulation of cortisol production in the foetal adrenal, (33) altering the set point of HPA axis functioning in the foetus. Because psychiatric disorders are associated with altered HPA axis activity, and this is associated with suboptimal HPA axis functioning of the infant, more insight into this process is needed in clinical and healthy dyads, to understand contributing factors and ultimately prevent adverse outcomes for offspring.

Hypotheses

In the current study, we assessed the association between severe and long-lasting psychiatric disorders and HCCs of mothers and infants. We compared HCCs of patient dyads to healthy control dyads at 6 weeks' postpartum, to further elucidate mechanisms associated with the transgenerational transmission of psychopathology. In accordance with previous studies demonstrating that psychiatric disorders are associated with differential HPA axis disturbances, we expected larger variance in cortisol concentrations in our patient group than in controls. Subsequently, we assessed the effect of the severity of current maternal symptoms on infant HCC.

Also, we expected infant HCC to be associated with maternal perinatal HCC in healthy dyads. Previous research shows that in healthy mother–infant dyads, maternal and infant HCC appear to be positively correlated. Therefore, we expected to find an attuned association of HCC in control dyads. Because maternal psychopathology is associated with both increased and decreased maternal cortisol levels, influenced by the nature, chronicity and genetic heritability of the psychiatric disorder, we expected to find a divergence of this pattern in mothers and infants who were subject to maternal severe psychiatric disorders.

METHOD

Study procedure and design

The current study was embedded in an observational study on parenting capacity of mothers with severe psychiatric disorders and their infant's cognitive and socio-emotional development (the Infant Caregiving Assessment Scales (INCAS) study). All mothers fulfilled criteria for a current severe psychiatric disorder. A common definition of severe psychiatric disorders, or 'severe mental illness', consists of having any psychiatric diagnosis with a treatment duration of 2 years or more, together with dysfunction, as indicated by lower scores on the Global Assessment of Functioning scale. (45) Common disorders that are referred to are schizophrenia, mood disorders (chronic depression, bipolar disorder), chronic anxiety and personality disorders. (46)

During pregnancy, mothers with severe psychiatric disorders were recruited from specialised psychiatry-obstetrics-paediatric secondary and tertiary out-patient clinics and other specialised mental healthcare institutions where pregnant women who suffer from psychiatric disorders are treated. Healthy control mothers, without current or a history of psychiatric symptoms, were recruited during pregnancy at midwifery practices in the central western part of the Netherlands, consisting primarily of the four largest Dutch cities and their surrounding areas.

The INCAS study was approved by the Erasmus University Medical Center Medical Research Ethics Committee (approval number NL42662.078.12); written informed consent was obtained from all mothers for their own and their infant's participation, and from fathers with legal guardianship.

Exclusion criteria

In the current study, exclusion criteria for both groups were insufficient amount of hair necessary for cortisol analysis; use of locally administered and systemic corticosteroids during or after pregnancy; use of illicit drugs or alcohol in the last trimester of pregnancy; and perinatal complications, including prematurity. Additionally, control dyads were excluded from analysis when maternal global score on the Brief Symptom Inventory (BSI) was in the clinical range or when mothers used psychotropic medication. (47)

Inclusion of clinical sample

From June 2013 to January 2016, patients and control mothers were included in the INCAS study during their third trimester of pregnancy (N = 129). A total of 64% of participating mothers (n = 83) agreed on hair donation at 46 ± 8.5 days' postpartum (range 34–84 days) for themselves, and 45% for their infant (n = 58). After exclusion based on the aforementioned exclusion criteria, HCCs were available for a total of 73 mothers (patient n = 33, control n = 40) and 47 infants (infant of patient n = 20, control infant n = 27) (see flowchart in Appendix).

Non-response analyses, comparing mothers and infants who did and did not donate hair for cortisol measurement, showed no differences with regards to maternal age, ethnicity, educational level, psychiatric symptoms, and infant birth weight or gestational age.

Measures

HCCs

Mother and child HCCs were determined from hair strands collected 6 weeks' postpartum (46 days ± 8.5, range 34–84 days). All samples were collected according to researcher protocol. In adults, scalp hair has a predictable growth rate of approximately 1 cm per month, making it possible to have an estimate of long-term exposure to cortisol. (48,49) When collected at 6 weeks' postpartum, HCC in the proximal 3 cm of maternal hair reflects the maternal HPA axis activity over the first 6 weeks after childbirth and the last 6 weeks of pregnancy. (16)

A small strand of hair was cut from the posterior vertex of the scalp, as close as possible to the scalp. Hair strands were taped to a piece of paper with the scalp end marked, and stored in an envelope at room temperature until further analysis. The proximal 3 cm of maternal hair samples were weighed and minced. For infants, the full length of the hair was analysed with a minimum of 1.25 mg, for reliable measurement. For extraction of cortisol, LC-grade methanol was used at 25°C, for 18 h, in the presence of labelled glucocorticoids as internal standard. The extraction was centrifuged and cleaned. Cortisol concentrations were quantified by liquid chromatography with tandem mass spectrometry (Waters XEVO-TQ-S system; Waters Corporation, Milford, MA, USA). Measurements were reported in picograms per milligram of hair, and log-transformed (10log) to approach normality. (50)

Psychiatric diagnosis and current symptoms

Presence and history of psychiatric diagnoses were examined with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) and Structured Clinical Interview for DSM-IV Axis II Disorders (SCID-II), by a trained interviewer. (51,52) SCID-I and SCID-II are considered to be the gold standard of semi-structured assessment instruments for clinical psychiatric disorders, with adequate to excellent validity and interrater reliability. (53)

Level of current symptoms in both the patient and control group were measured with the BSI, at 6 weeks' postpartum. (54) Severity of stress was indicated by the Global Severity Index (GSI). (55) The BSI comprises 53 items on nine symptom dimensions (somatisation, obsession–compulsion, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism). The GSI presents the mean BSI score. Normative data are available for clinical and non-clinical samples. The BSI has a high internal consistency, moderate test–retest reliability and strong convergent validity with measures of emotional functioning. (47) In our sample, Cronbach's alpha was 0.93 in patients and 0.82 in controls.

Covariates and potential confounders

Demographic data; information on smoking, alcohol and illicit drug use; and exposure to psychotropic medication during pregnancy were collected during the third trimester of pregnancy (patients) and at 6 weeks' postpartum (controls), using self-reports. Confounders were selected a priori, based on previous research. (56,57) The following confounders were controlled for the following: child gender, gestational age and birth weight; and maternal age, ethnicity, socioeconomic status, parity (primiparity versus multiparity), tobacco use and use of psychotropic medication.

Data analyses

Demographic and clinical characteristics of the control and patient sample are reported, and differences between the samples were tested with χ2-tests (for categorical variables) and t-tests or Mann–Whitney U-tests (for continuous variables). Differences in HCCs between patient and control mothers, for diagnostic subgroups in the patient group, and between infants, were tested using Mann–Whitney U-tests. For this purpose, HCCs were log-transformed.

We estimated the association between maternal and infant HCC in both the patient and control sample by regression analysis. Preliminary analyses did not show significant correlations between hair characteristics (e.g. hair treatment in the past 3 months, heavy transpiration, hair product use before hair collection) in mothers ($P > 0.201$) or infants ($P > 0.577$), or between timing of the hair sample (range 34–84 days' postpartum) and infant HCC ($P = 0.770$); accordingly, we did not control for these variables in the regression analyses. To adjust for the effects of other potential confounders, we calculated a propensity score including all available confounders as summarised in subheading 'Covariates and potential confounders', and included the propensity score as a single covariate in all analyses. (58) Differences in maternal–infant HCC associations between the patient and control samples were tested with Fisher z-scores.

We also explored whether maternal symptom severity was related to HCC in mothers and infants. Therefore, we estimated the association between perinatal symptom severity levels (based on BSI scores) and maternal and infant HCCs, using regression analysis. We conducted a sensitivity analysis, repeating the regression analysis but leaving out two outliers.

Results from the regression analyses are reported as correlation coefficient (r) and s.e.59 Q-Q plots were used to check all data for normality of the distribution. HCC data were checked for extreme outliers (defined as below quartile 1 (Q1) − 1.5 interquartile range (IQR) or above quartile 3 (Q3) + 1.5 interquartile range (IQR)), which were removed from all analyses (n = 4). Statistical analyses were performed with SPSS version 24 for Windows (IBM, New York, USA).

RESULTS

Background and clinical characteristics

A sample description is displayed in Tables 1 and 2. Mothers in the patient and control group did not differ with regard to age and ethnicity. Lower educational level and smoking were more common among patients. Expectedly, infants of patients had a significantly lower gestational age and birth weight compared with control infants. (46,60) In the patient group, depressive and anxiety disorders were most common (33.3 and 51.1%, respectively), followed by bipolar disorders (18.2%). A considerable percentage of mothers (39.4%) had two or more Axis I disorders (e.g. depressive disorder and panic disorder). Furthermore, half of the patients had a comorbid personality disorder (48.4%), mostly in Cluster C (avoidant, dependent or obsessive–compulsive personality disorder). Approximately two-thirds (67.7%) of the patient group used psychotropic medication during pregnancy, which were mostly antidepressants, followed by antipsychotics and hypnotics. A smaller group of mothers used two or more psychotropic medications (19.4%).

Differences in HCCs between patient and control dyads

HCC of patient and control dyads are displayed in Fig. 1. Median (interquartile range) and distribution ofHCCwere significantly different in patients compared with control mothers (U = 468.5, P = 0.03). Results did not differ in infants of patients (U = 250.0, P = 0.67). Correlation of HCCs within clinical and control mother–infant dyads We found a positive correlation between maternal perinatal HCC and infant HCC in the control group (n = 27, r = 0.55 (0.14), P = 0.003). The correlation between maternal perinatal HCC and infant HCC in the patient group was non-significant (n = 18, r = 0.082 (0.13), P = 0.746; see Fig. 2). The correlations between maternal perinatal HCC and infant HCC were significantly different across the patient and control group (z = −1.64, P = 0.05). The correlation analyses were repeated with the propensity score, to adjust for confounders. After adjustment, the strength of the correlation between HCCs in control mother–infant dyads increased somewhat (r = 0.65 (0.13), P = 0.001). In patient mother–infant dyads, the correlation increased greatly, but remained non-significant (r = 0.37 (0.13), P = 0.16).

Correlation of current maternal symptom severity with HCCs in patient dyads

We explored if maternal symptom severity in the perinatal period is correlated with maternal and infant HCC. Results are displayed in Fig. 3.

In mothers, symptom severity was not correlated with HCC (n = 23, r = −0.09 (0.12), P = 0.67). In infants, a positive correlation between maternal perinatal symptom severity and HCC was found (n = 16, r = 0.63 (0.06), P = 0.008). The correlation analyses were repeated with the propensity score, to adjust for confounders. After adjustment, the strength of the correlation between symptom severity and maternal perinatal HCC (r = 0.08 (0.13), P = 0.70) and infant HCC (r = 0.59 (0.18), P = 0.02) remained in a similar range. In the sensitivity analysis, leaving out the two outlier infants on the right, the strength of the correlation between maternal symptom severity and infant HCC remained in a similar range as our original result.

Table 1. Demographic characteristics of patients (n = 33), control mothers (n = 40), infants of patients (n = 20) and control infants (n = 27)

	Patient group	**Control group**	*p*-value
Demographic characteristics			
Maternal age (years, *SD*)	32.2 (6.0)	31.9 (4.4)	0.12
Maternal ethnicity (Caucasian), n (%)	27 (81.8)	32 (88.9)	0.41
Education level (low education level), n (%)	27 (84.4)	16 (45.7)	0.001
Tobacco use	11 (33.3)	3 (8.3)	0.008
Gestational age (weeks, SD)	38.3 (1.9)	39.7 (1.3)	0.002
Infant birthweight (grams, SD)	3227 (456)	3652 (608)	0.002
Infant sex (male), n (%)	13 (66.7)	18 (45.0)	0.06
Maternal symptom severity			
Brief Symptom Inventory (GSI, SD)	0.95 (0.66)[a]	0.21 (0.23)[a]	<0.001

GSI, Global Severity Index. [a]. Mean reference range was 0.93–1.32 for Dutch clinical females and 0.29–0.45 for healthy females.

Table 2. Clinical characteristics of patients (n = 33)

Maternal psychiatric characteristics	**N (%)**
Axis I Psychiatric disorder	
• Depressive disorder, n (%)	11 (33.3)
• Anxiety disorder, n (%)	17 (51.1)
• Psychotic disorder, n (%)	2 (6.1)
• Bipolar disorder, n (%)	6 (18.2)
• Comorbidity (≥2Axis I disorders)n(%)	13 (39.4)
Axis II Personality disorder	
• Cluster A, n (%)	5 (16.1)
• Cluster B, n (%)	3 (9.7)
• Cluster C, n (%)	10 (32.3)
• No personality disorder, n (%)	16 (51.6)
Psychotropic medication use[a]	
• SSRI/nSRI/TCA, n (%)	14 (45.2)
• Antipsychotics, n (%)	8 (25.8)
• Hypnotics/anxiolytics, n (%)	3 (9.1)
• ≥ 2 psychotropic medications, n (%)	6 (19.4)
• No psychotropic medication use, n (%)	10 (32.3)

SSRI, Selective Serotonin Reuptake Inhibitor; SNRI, Selective Serotonin and Noradrenalin Reuptake Inhibitor; TCA, Tricyclic Antidepressants. [a]. Any exposure during pregnancy.

Figure 1. Distribution of hair cortisol concentrations (HCCs) in patient versus control mothers, and infants of patients versus infants of controls. Median (interquartile range) and distribution of HCCs were significantly different in patients compared with control mothers (U = 468.5, P = 0.03). Results did not differ in infants of patient (U = 250.0, P = 0.67)

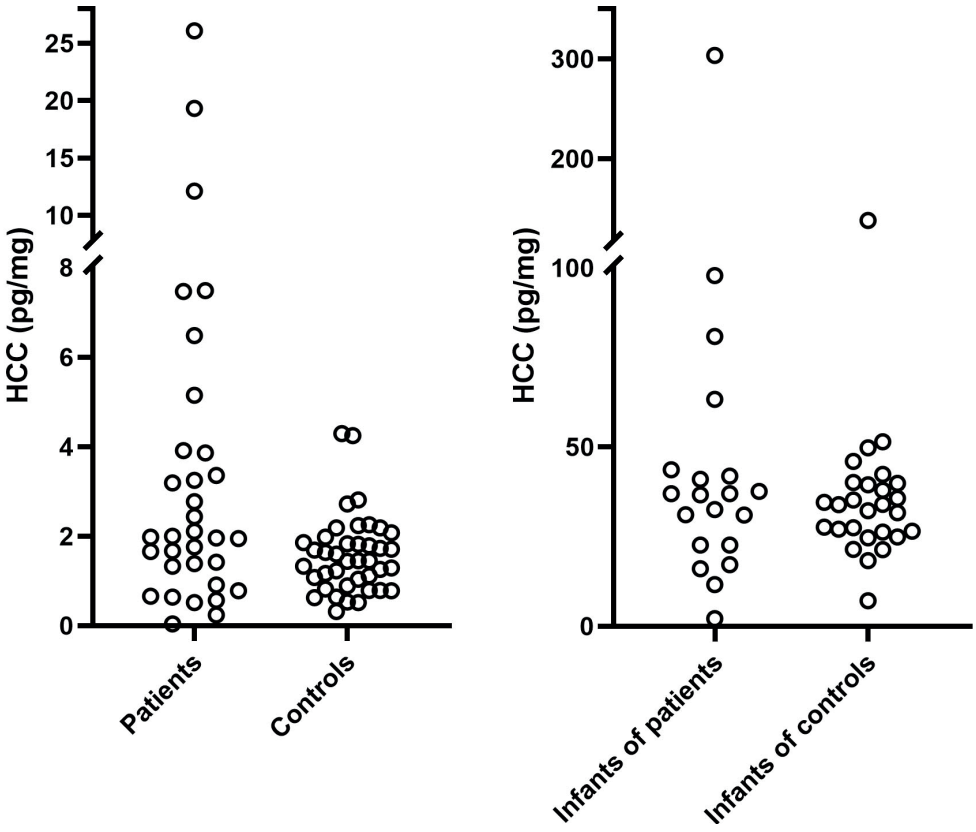


Figure 2. Correlation between mother and infant log-transformed hair cortisol concentrations (HCCs). Figure based on unadjusted results

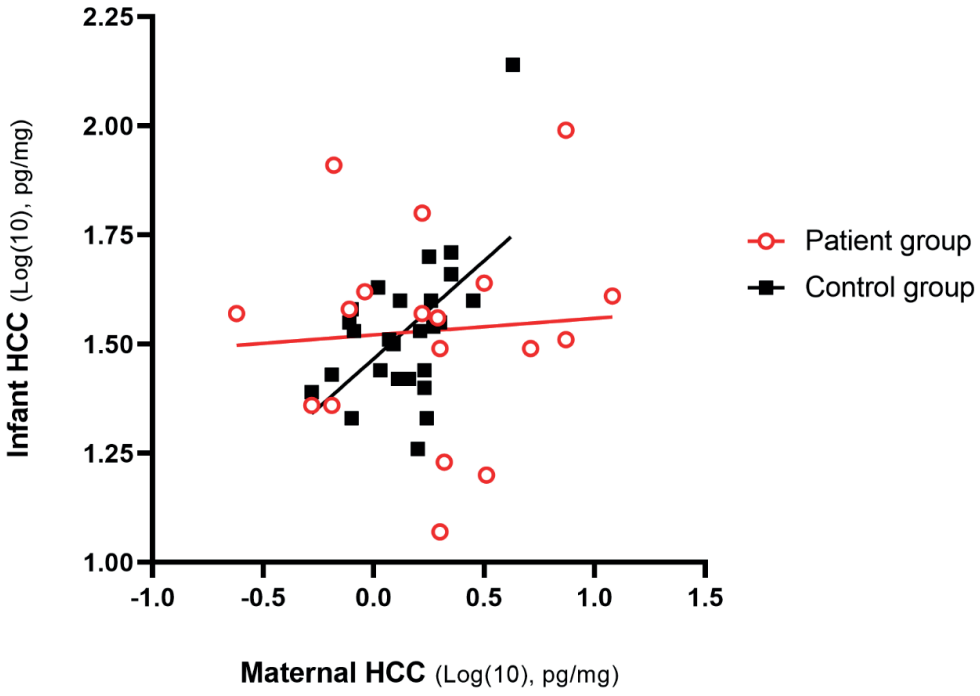
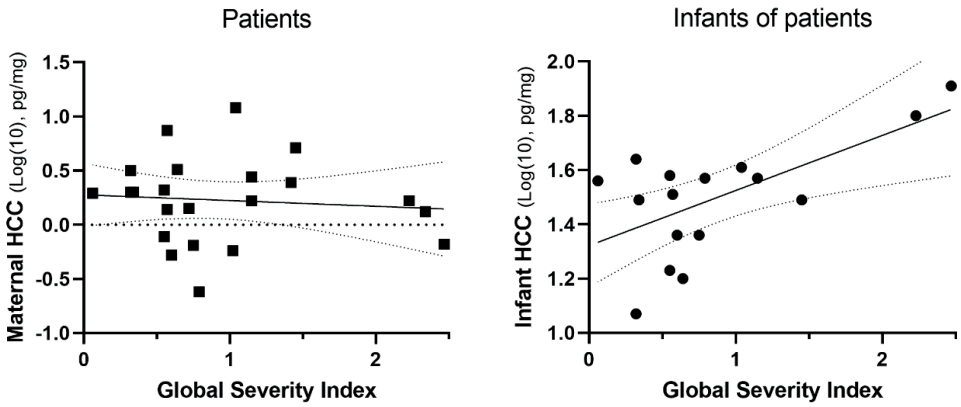


Figure 3. Association between maternal symptom severity by means of the Global Severity Index and log-transformed hair cortisol concentrations (HCCs) of patients (left) and infants of patients (right). Figure based on unadjusted results



DISCUSSION

In this study, we explored the influence of severe psychiatric disorders on HCCs of mothers and newborn infants. We found a significantly wider range of HCCs in mothers with severe psychiatric disorders compared with controls, but we did not find differences in infants. We also found that HCC of patients and infants of patients were not associated, whereas in control dyads, we found a significant positive association between mother and child HCCs. In infants of patients, HCCs were positively associated with maternal symptom severity.

HCCs in patient and control groups

In our study, a significant variation in HCC was found in mothers with psychopathology, showing both (mainly) higher and lower values than control mothers. One explanation for this finding is the nature of our clinical sample, in which women with severe and long-lasting psychiatric disorders were selected. Previous studies have shown cortisol levels in patients with mood disorders change over time. First episodes are more often associated with higher cortisol levels, whereas long-term duration and recurrence of episodes might diminish the sensitivity of the HPA axis over time. (6,61) Heterogeneity of psychiatric diagnoses and high prevalence of medication use might also be critical factors in differences of HPA axis functioning and long-term release of cortisol in affected mothers. (19,62)

Infants of patients showed no significant differences with regard to variation in HCC compared with control infants. Previous studies have shown that higher maternal perinatal HCC predicted lower HCC in newborn infants early postpartum. (33,39) We could not replicate this finding. Three factors may contribute to this inconsistence. First, the sample size of our study might not have been sufficient to uncover differences in our patient dyads. Second, the mothers in the aforementioned studies were healthy or subject to mood and anxiety disorders and only used antidepressants, whereas patients in this study had high rates of comorbidity and other medication use (including antipsychotics). Third, the absence of marked differences of HCC in infants of the patient group might reflect that foetal exposure to increased or decreased maternal cortisol concentrations during pregnancy is effectively regulated by the dynamic nature of placental 11β-hydroxysteroid dehydrogenase type II (11β-HSD-2). (63)

Prenatal synchrony of HCCs between mother and child

In line with previous studies (17,39), we found a positive association between maternal and infant HCC in healthy control dyads. This finding might reflect early physiological synchrony, which is defined as the matching of biological states between mother and child that develops via interactions among genetic predispositions, prenatal programming and postnatal behaviour. (64,65) In mother–infant dyads subject to severe psychiatric disorders, we found a divergence of this pattern. This might indicate that synchrony of the HPA axis between mother and child might be prenatally affected by the presence of a maternal psychiatric disorder. This finding should be interpreted with caution because the two groups in this study differed with regards to relevant demographic and obstetric variables (e.g. lower education level in patients, lower birth weight and gestational age in infants of patients), but it should also be noted that the findings remained the same when these factors were controlled for.

HCCs and (self-reported) symptom severity

We did not find a correlation between maternal perinatal HCC and self-reported symptom severity at 6 weeks' postpartum. Because of the previously mentioned blunted cortisol responses in long-lasting psychopathology, the absence of an association between HCC and maternal-reported stress levels might indicate reduced responsiveness of the HPA axis to stressful experiences. (7) However, the relationship between the human concept of stress and HPA axis functioning is an ongoing subject of debate. A recent meta-analysis of Kalliokoski et al (66) on hair glucocorticoids as a measure of stress suggests that self-reported assessments of stress poorly correlate with HPA axis functioning. Furthermore, symptom assessments in our study were obtained postpartum, which is an especially chaotic transition in a woman's life. It might not be accurate to relate this to cortisol levels that presumably reflect third trimester exposure of cortisol. It also cannot be ruled out that other physical factors that are important determinants of cortisol levels, such as obesity, metabolic syndrome and cardiovascular disease, were overrepresented in the patient group compared with the control group, and therefore may have influenced the outcomes. (67,68)

Interestingly, in infants of patients, we found that higher maternal symptom severity was associated with higher infant HCC, after statistically controlling for known covariates of HCC. There are several possible explanations for this finding. It has been proposed that the placental barrier function, inactivating cortisol by 11β- HSD-2, may be impaired by stress, (44,69) allowing an increased passage of maternal cortisol to the foetus. (63) Also the production of placental corticotropin-releasing hormone (CRH) might be reacting to blunting of the maternal HPA axis, leading to stimulation of the foetal adrenal. (70) Stressful circumstances during delivery and in the postpartum period might also contribute to higher infant HCC. However, this finding is subject to the same limitations (i.e. maternal self-reported stress) as in mothers, and has to be interpreted with caution.

Strengths and limitations

Our study has several strengths and limitations. The foremost strength of this study is our patient sample of mothers with various severe psychiatric disorders, representing the heterogeneity of clinical populations. Further strengths are the non-invasive measurement of chronic stress in hair performed with the state of-the-art liquid chromatography with tandem mass spectrometry method, and the availability of detailed and reliable diagnostic information, as well as the possibility to adjust for various covariates. Limitations include the limited sample size of subgroups, which only allowed for an initial exploration. Additionally, we only measured psychiatric symptom severity at 6 weeks' postpartum, and could therefore not take into account the possible variation of symptoms over time.

Conclusions and future research

In the current study, we observed differences in the association between HCCs of patients and their infants compared with healthy controls and their infants. Where in healthy control dyads there seems to be perinatal synchrony of HPA axis functioning in mother and infant, our findings suggest there is a divergence of this pattern in mother–infant dyads subjected to long-lasting and severe psychiatric disorders. In infants, these early differences might influence lifetime HPA axis functioning, as has been suggested in previous research. (28) In turn, altered HPA axis functioning may increase susceptibility to disease, both physically and mentally. Future longitudinal studies in larger clinical samples should examine how maternal and infant hair cortisol levels are intertwined perinatally, in

the early postpartum period and beyond.

Author contributions

Carlinde W. Broeks contributed to conceptualization, data curation, formal analysis, methodology, writing -original draft, writing - review and editing.

Vandhana Choenni contributed to conceptualization, data curation, funding acquisition, methodology, writing - review and editing.

Rianne Kok contributed to conceptualization, data curation, funding acquisition, methodology, writing - review and editing.

Bibian van der Voorn contributed to writing - review and editing.

Ineke de Kruijff contributed to writing - review and editing.

Erica L.T. van den Akker contributed to writing - review and editing.

Elisabeth F.C. van Rossum contributed to writing – review and editing.

Witte J.G. Hoogendijk contributed to writing - review and editing.

Manon H.J. Hillegers, contributed to writing - review and editing.

Astrid M. Kamperman contributed to conceptualization, formal analysis, methodology, writing - review and editing.

Mijke P. Lambregtse-Van den Berg contributed to conceptualization, data curation, formal analysis, funding acquisition, methodology, writing - review and editing.

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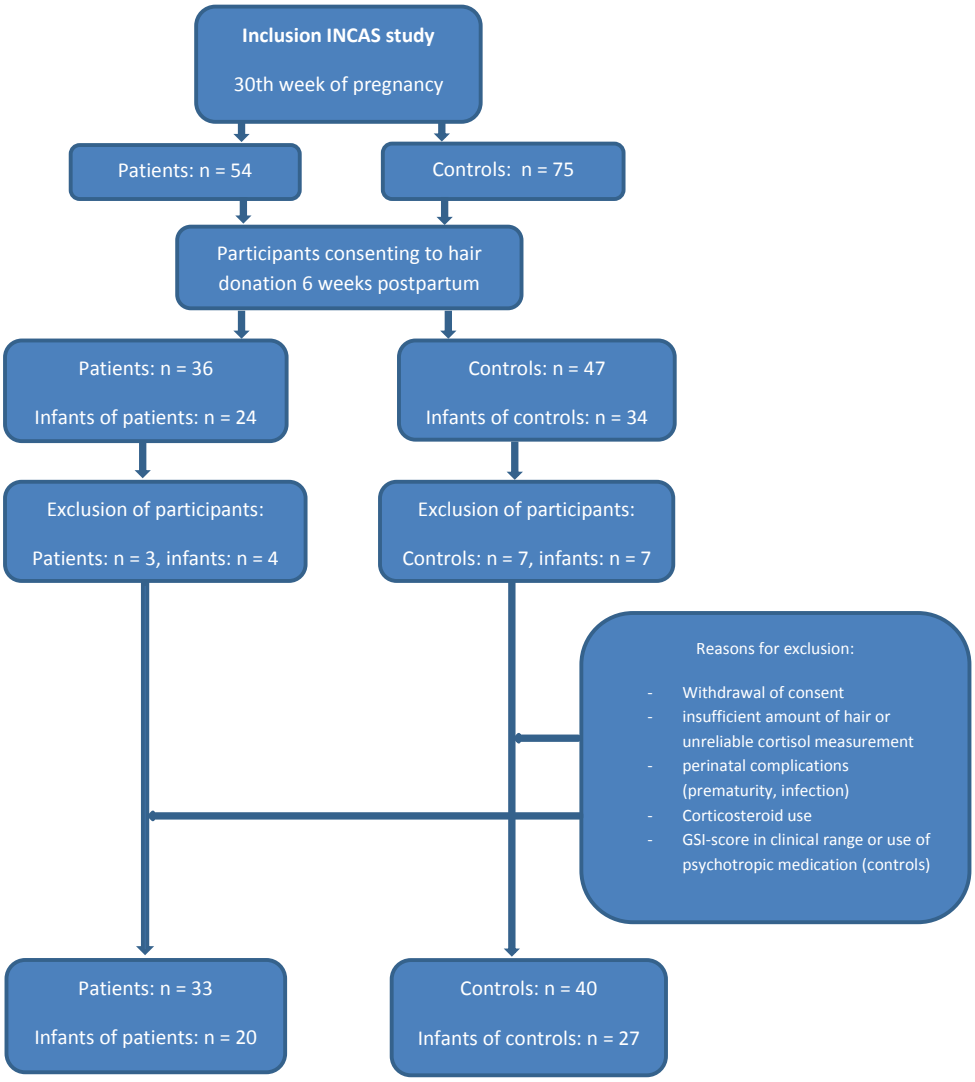
Appendix: Flow chart



SUMMARY

Excessive infant crying

New insights in the role of parental factors and long-term stress through hair cortisol analysis

Excessive infant crying, or infant colic, is one of the most distressing challenges for new parents and a common reason to visit a paediatrician. In the majority (95%) of infants presenting with excessive crying, no underlying organic disorder can be found. Therefore the focus of treatment should be on supporting the parents, through reassuring, and helping them through this challenging period. To be able to reassure and support both mother and father, information on their feelings is essential. In the first part of this thesis, three common gastrointestinal symptoms in infancy in which psychosocial stress plays an important role were investigated. The presence of these symptoms is assessed in infants of mothers with, and without a history of a psychiatric disorder (PD). Next, the feelings of the mothers and fathers of excessively crying infants visiting the paediatrician were compared to the feelings of parents of control, non-excessively crying, infants.

The pathophysiological mechanisms underlying excessive infant crying are likely multifactorial, and neuro-hormonal factors may also contribute. To gain more insight into these factors, the second part of this thesis focused on the role of the hypothalamic–pituitary–adrenal (HPA) axis by measuring the end product— the stress hormone cortisol— in the hair of children and parents. Traditionally, cortisol is measured in blood, saliva or urine which gives insight in a limited time, ranging from minutes to hours. Hair cortisol measurements enable the measurement of retrospective cumulative cortisol levels over a longer period of time. As cortisol is accumulated into hair as it grows, with an average hair growth rate of approximately 10 mm per month in adults, segments of selected length can be analysed for the mean levels of cortisol during the corresponding months. The non-invasive sampling and easy storage of the research material makes this method exceptionally suitable for both paediatric clinical care and research. We analysed hair cortisol concentrations (HCC) in healthy children, and in infants and parents dealing with excessive crying and severe maternal psychiatric disease.

PART I - Infantile gastrointestinal symptoms and parental feelings

In **Chapter 1**, the presence of excessive crying, gastro-oesophageal reflux (GER) and constipation in infants of mothers with and without a history of a PD, was assessed. Mothers in the PD group could be included if they visited a specialised Psychiatry-Obstetrics-Paediatrics (POP) outpatient clinic during pregnancy, because of their history of a PD. Mothers in the control group, without a PD, were recruited during pregnancy from various midwifery practices in the Netherlands. Infant gastrointestinal symptoms, maternal depressive symptoms, and mother-infant bonding were assessed using validated questionnaires and diagnostic criteria 1.5 months after birth. In total, 101 mothers with a history of a PD and 60 control mothers were included. There were more GER symptoms found in infants of PD mothers compared to controls, and these symptoms were associated with maternal depressive symptoms and bonding problems. No significant differences were found regarding the occurrence of excessive crying and constipation between the groups. Maternal depressive symptoms were associated with constipation,

and bonding problems were associated with excessive infant crying. Finally, the effect of the mother's depressive symptoms on infant GER and excessive crying was shown to be mediated by bonding problems.

In **Chapter 2** stress, depression, anxiety and bonding problems were assessed in a case control study with fathers and mothers with an excessively crying infant presenting at the paediatric outpatient clinic of our hospital. The controls were parents with infants who did not cry excessively, and they were recruited from the surrounding infant welfare centres. Notably, 25% of the controls were recruited at the paediatric outpatient clinic, when they received a routine ultrasound of the hip after breech birth. In total, the parents of 34 infants with excessive crying and 67 controls were included. There were significantly more symptoms of stress, depression and anxiety, and bonding problems in both the fathers and mothers of the excessively crying infants, compared to the controls. While feelings of stress and bonding problems in fathers were strongly associated with these feelings in the mothers, paternal feelings of depression and anxiety were not. We therefore speculate that integrated care, which reduces negative feelings of stress and bonding in mothers, might also have positive effects on these negative feelings in fathers. However, treatment for depression and anxiety in fathers and mothers, would probably have to be individualised.

PART II - Hair cortisol

In **Chapter 3**, to increase the applicability of hair cortisol measurements in paediatric care and research, paediatric reference intervals of the measured hair cortisol using liquid chromatography-tandem-mass spectrometry (LC–MS/MS) were determined. Additionally, hair growth rates in children up to two years of age were measured. In a cross-sectional study, it was showed that HCC do not differ between girls and boys and are age-dependent; remarkably high levels were observed after birth (162.4 pg/mg). Much lower values in the first months were followed by further lower values to a minimum at age 6 years (1.55 pg/mg). Then followed by graduated and subtle higher values, with adult concentrations reached at age 18 years (3.0 pg/mg). Average hair growth velocity measured in mm per month was significantly lower in infants aged 0–6 months compared to children aged 12–24 months (3.5 versus 9.4, $P < 0.001$). These lower hair growth rates are one of the explanations of the very high HCC which were found during infancy. Due to the lower hair growth rate, HCC in 1 cm hair measured in infants up to 6 months provided us with information about the 3 months before the sampling, in infants aged 6-12 months about the 1.5 months before sampling and in children aged 12–24 months, approximately 1 month before sampling, the same as in older children and adults.

In **Chapter 4** HCC were measured using LC-MS/MS in infants, mothers and fathers of excessively crying infants, and compared these to HCC in control families without an excessively crying infant, within the same case-control study as described in **Chapter 2.** Mean HCC were significantly lower in both the mothers ànd the fathers (2.3 pg/mg and 1.6 pg/mg, respectively) of the excessively crying infants than in the control mothers and fathers (3.2 pg/mg and 2.9 pg/mg, respectively). In the total group of parents, and within the parents of excessively crying infants, HCC were not associated with feelings of stress, depression, anxiety or bonding problems. In the control group, HCC showed a positive association with parents' stress and depression scores. As HCC also provide

insight into the months before the measurement, the lowering of HCC in parents exposed to excessive infant crying compared with the controls, may have already occurred during pregnancy. It is known that maternal anxiety during pregnancy is associated with excessive crying and anxiety disorders are associated with lowered HCC. We therefore speculate that the stressful feelings in parents of excessively crying infants may have resulted in a downregulation of their HPA system probably during pregnancy. In the infants, no differences were found in mean HCC between the groups.

In **Chapter 5** we explored the influence of severe PDs on the HCC of mothers and infants. We found a significantly wider range of HCC in mothers with severe PDs, showing both (mainly) higher and lower values than the control mothers. Long lasting PDs, the heterogeneity of psychiatric diagnoses and current psychiatric symptoms and the high prevalence of medication use might all be critical factors that could explain the large variation in HCC. No differences in HCC in infants between the groups were found.

Remarkably, we found no differences in HCC between exposed infants (to excessive crying and severe maternal PDs) and the controls. This might be explained by the small sample sizes, the age differences in the first months when HCC changes significantly, and selection bias. Another potential explanation is that the exposure to “excessive crying” or “maternal psychiatric illness” are not stressors to the infant. Finally, there may be other factors moderating the association between stressors and the physiological stress response in infants, such as the quality of parental care and their availability and parental relationships, which need to be investigated in larger population cohorts.

In conclusion, the research in this thesis can be summarized in four key messages. At first, a maternal history of PD, actual maternal depressive symptoms and postpartum bonding are important parental factors to consider for a paediatrician when dealing with infant gastro intestinal symptoms. It was shown that infants of PD mothers had more GER compared to controls, maternal depressive symptoms were associated with constipation, and bonding problems were associated with excessive infant crying. Second, both fathers and mothers with an excessively crying infant have more feelings of stress, depression, anxiety and bonding problems compared to control parents, although father’s feelings of stress and bonding problems are more strongly influenced by maternal feelings of stress and bonding problems than by excessive infant crying. Thirdly, paediatric reference intervals of hair cortisol with LC-MS/MS were determined and lower hair growth rates were found to be one of the explanations of the remarkably high HCC in infancy. Finally, HCC are significantly different in parents of excessively crying infants and mothers with a severe PD compared to controls, while HCC in their infants did not differ from the control group.

GENERAL DISCUSSION, FUTURE PERSPECTIVES AND CLINICAL RECOMMENDATIONS

"The most important thing for us was that the paediatrician really listened to us and took us seriously" – Parents of Meysa.

Many paediatricians encounter infants referred with "excessive crying", in their day-to-day practice. Although the infants are "their patients", they depend mainly on information from the parents. Besides information about the clinical condition of the infant, information on parental feelings is essential, not only to be able to supply adequate support but also to prevent the medicalization and hospitalization of the excessively crying infant.

This thesis focusses on parental factors, and the involvement of the HPA axis by measuring hair cortisol in excessive crying infants and mostly uses data form the CHIPS (Cortisol in Hair in excessive crying Infants and Parental Stress) study and describes one embedded study within the INCAS (Infant Caregiving Assessment Scales) study. The CHIPS study is a cross-sectional case-control study on parental feelings and hair cortisol in parents visiting the paediatrician with their excessively crying infant and was initiated in the St. Antonius Hospital, Nieuwegein, the Netherlands. The INCAS study is a multicentre observational study on maternal parenting capacity and infant development in mothers with severe mental illness and was initiated in the Erasmus MC, Rotterdam, the Netherlands. Finally our study on hair cortisol reference intervals additionally used data of the control group of a non-randomized, prospective controlled study in the OLVG West Hospital in Amsterdam, the Netherlands (1,2), and data of a healthy control study of children who were recruited in infant welfare clinics, during elementary and secondary school visits and at a paediatric (outpatient) clinic which was initiated in the Erasmus MC, Rotterdam, the Netherlands.

MAIN FINDINGS

The aim of this thesis was to 1) gain insight into the presence and associations with maternal symptoms of infant gastrointestinal symptoms reported by mothers with and without a history of a psychiatric disease, 2) assess parental feelings of excessively crying infants, and 3) investigate hair cortisol concentrations (HCC) in healthy children and in infant parent(s) couples dealing with excessive crying and severe maternal psychiatric disease. The message of this thesis can be summarized in four key messages: At first, a maternal history of PD, actual maternal depressive symptoms and postpartum bonding are important parental factors to consider when dealing with infant gastrointestinal symptoms. Secondly, both fathers, and mothers with an excessively crying infant have more feelings of stress, depression, anxiety and bonding problems compared to control parents, although father's feelings of stress and bonding problems are more strongly influenced by maternal feelings of stress and bonding problems than by excessive infant crying. Thirdly, paediatric reference intervals of hair cortisol with LC-MS/MS were determined and lower hair growth rates were found to be one of the explanations of the remarkably high HCC in infancy. Finally, HCC are significantly different in parents of excessively crying infants and mothers with a severe PD compared to controls, while HCC in their infants did not differ from the control group.

PART I- Infantile gastrointestinal symptoms and parental feelings

"We had no grandpa or grandma living close who could step in and help us comfort our child, we struggled for such a long time that the depression became worse."- Parents of Nienke.

In **Chapter 1**, we investigated the prevalence of excessive crying, gastro-oesophageal reflux (GER) and constipation of mothers with a history of a psychiatric disorder (PD). These mothers reported a higher mean reflux score in their infants, regardless of the presence of actual depressive symptoms, than the control mothers. In addition, maternal depressive symptoms were associated with symptoms of infant GER and constipation and these maternal depressive symptoms were mediated by maternal bonding in their association with infant reflux symptoms and excessive crying. It is well known that psychosocial stress factors play an important role in the multifactorial origin of gastrointestinal symptoms in infancy and our findings suggest that maternal history of psychiatric disease, actual depressive symptoms and bonding are important factors to consider. These factors might have a direct effect on gastrointestinal symptoms of the infant, for example by genetic predisposition. Another explanation could be that mothers with depressive symptoms misinterpret normal infant signals and infant facial expressions (3–5) leading to a suspicion of gastrointestinal symptoms and subsequently visits to the outpatient clinic. The reverse causation hypothesis is also plausible in mothers with a history of PD: infant gastrointestinal symptoms could result in the development or exacerbation of maternal psychopathology, for example through negative infant-mother interaction patterns. (6,7) More insight into the causal mechanism underlying this association and the possible intergenerational transmission of gastrointestinal illness behaviour between mothers and infants (8) could improve treatment in clinical practice. Due to underpowering and missing data, we were not able to answer the question whether excessive infant crying and constipation are reported more often by these high-risk mothers. This should be answered in larger prospective studies using the ROME criteria.

In **Chapter 2** we investigated the feelings of parents referred to the paediatrician due to experienced excessive infant crying compared to a control group. We showed significantly more negative feelings and bonding problems in both fathers and mothers compared to controls. Traditionally, the focus of paediatric research has been on maternal feelings, however in the past years an increasing number of studies has focused on paternal feelings in the postpartum period too. (9,10) Our study was the first to investigate the effect of excessively crying infants on paternal feelings in this period. We showed increased anxiety and stress in fathers of excessively crying infants compared to controls. Higher depression scores in fathers of infants experiencing crying problems, were shown before. (11,12) However, in our study, we adjusted for all maternal symptoms and showed that paternal depression, and anxiety, existed independently of maternal depression and anxiety. We confirmed the limited existing data showing that fathers of excessively crying infants experience more impaired bonding. (13) In addition, their feelings of bonding problems and stress are more negatively influenced by these feelings experienced by their wives, than by their crying infant. Our findings suggest that the intervention for mothers and fathers referred to the paediatrician with their excessively crying infant should be different. Integrated care which reduces negative feelings of stress and bonding in mothers

might also have positive effects on these feelings in fathers. However, the treatment for depression and anxiety in fathers and mothers should likely be individualized.

"For us, the most important thing was that medical causes were excluded." – Parents of Lenn.

It is important to note that our research focused on a specific group of parents. We hypothesized that this group of "referred" parents might be more concerned about organic causes of their infants crying and might perceive crying as more unpleasant. We found evidence for this hypothesis because these parents compared to controls, not only registered more crying hours with a higher volume, but also a higher crying intensity, which probably reflects greater physiological or psychological stress. (14)

In the light of our findings in Chapters 1 and 2, it might be even possible to prevent infant gastrointestinal symptoms, such as infant gastro-oesophageal reflux and excessive crying, by starting family-centered care during pregnancy focusing on parent infant bonding in high risk populations with a history of psychiatric illness. Multidisciplinary care, and even better, integrated care, with a personalized approach, is essential for these types of interventions, both prenatally and postnatally.

It is important to realize that these influences of childhood diseases on parental feelings are not restricted to infantile gastrointestinal disorders, but have been reported across the whole paediatric field of diseases, and in all age groups. For example premature babies, sleeping and feeding problems in infants and childhood chronic diseases like abdominal pain, asthma and type I diabetes have all been linked to parental stress. (15–23)

The association between paediatric illness and parental feelings are often bidirectional and influence each other. A recent meta-analysis on the association between a parental mental disorder, of which half of the studies investigated maternal perinatal mental health and 17% paternal mental disorder, and child physical health revealed significantly higher rates of paediatric accidents and injuries, such as traumatic brain injury (OR = 1.15, 95% CI 1.04–1.26), and asthma (OR = 1.26, 95% CI 1.12–1.41.(24) Our findings in Chapter 1 underline the importance of a detailed familial history in the paediatric assessment.

In summary, all these findings underline the importance for a healthcare professional to take wellbeing of both parents into account to improve outcome of childhood disease. Interventions aimed at fostering social support and decreasing perceived stress, might alleviate parents' psychological symptoms by focusing on increasing their self-efficacy which will improve the child's health. Further research is required to investigate whether referring excessively crying infants to a combined consultation of a dedicated paediatrician and psychological staff member early in the process is effective in improving outcome parameters like medication use, hospitalization rates and family stress.

PART II- Hair cortisol

In the second part of this thesis, we explored the possibilities of the use of hair cortisol in research on infants and their parents. The non-invasiveness of the procedure and easy storage of samples make this method exceptionally suitable for both paediatric clinical

practice and research. Studies in which cortisol analysis in scalp hair is included may provide new insights into the pathophysiology of cortisol-related morbidity and may lead to novel clinical applications for a range of endocrine diseases—including diagnosis and follow-up in hypercortisolism, adrenal insufficiency, and congenital adrenal hyperplasia—as well as for use in research and as a biomarker for stress and mental health. Our study provided LC-MS/MS-based reference intervals for cortisol concentrations in scalp hair of healthy children from birth to 18 years of age, determined the influence of covariates such as hair colour, and hair treatment and the hair growth rates in children 0-2 years (**Chapter 3**), data which are essential for the implementation of hair cortisol testing and the interpretation of the results in paediatric clinical practice. We showed higher HCC in older than in younger children, which is consistent with previous published literature on cortisol in hair (25, 26) and serum. (27, 28) With exception of the very young children, where we observed remarkably high HCC after birth, followed by a sharp decline in the first three months and a more gradual decline in the months and years after to a nadir at the age of six years. In the first six months we found wide reference ranges. Additionally, we noted a significantly lower growth rate of 3.5 mm, per month in infants up to six months old, compared to 9.4 mm/month in children aged 12–24 months and the generally accepted growth rate of 10.0 mm per month in adults. (29) There are several possible explanations for this lower growth rate. Firstly, by the different hair types in this age group, with a variation in the cortex and the amount of medulla. (30, 31) Secondly, by the loss of synchronization of the growth cycle of scalp hair follicles near the end of the first year, resulting in differences in hair loss and in the percentage of terminal hair follicles on the scalp, which in turn leads to differences in the growth velocity of the hair. (31, 32) It is important to note that there is a considerable individual variation between ages at which the synchronization is lost, which probably explains the variation in individual growth rate from 0.7 to 12.5 mm that was found in our study in the age group of < 12-months. Thirdly, ethnicity has a small but significant effect on the individual growth rate. (33, 34) In our study the ethnic makeup of the newborn age group was different than in the other age groups. Due to the categorization in two groups of ethnically mixed samples, with different hair types, it is difficult to estimate the effect of ethnicity on the growth rate. However, the previously found effect of ethnicity on growth rate is too small to explain our study results.

Our findings have important implications for the interpretation of hair cortisol values and the use of hair cortisol in research in very young children. Due to the lower hair growth rate, HCC measured in infants up to 6 months provides us with information about the 3 months before the sampling was done, in infants 6-12 months about the 1.5 months before sampling and in children aged 12–24 months, approximately one month, the same as in older children and adults. Additional research on hair growth velocity using smaller age ranges (per month) in the age group up to 1 year would provide more insight regarding the precise period the cortisol values represent. In addition, future studies on HCC in infants should focus on smaller age ranges (per month), different mixes of ethnicities and larger sample sizes per age to identify subtle concentration changes.

In light of the increase in HCC research in children, it might be useful to examine the validation process with regard to the weight of inlay and functional sensitivity of the LC-MS/MS method again. The functional sensitivity (also referred to as the lower limit of quantification, LLoQ) is considered as the lowest concentration of analytes that can be reproducibly measured with an precision coefficient of variation (CV) of ≤20%.

In the original validation manuscript of Noppe (35) the LLoQ of cortisol was determined

in hair extracts of male and female adults, by serial dilution of hair extract, beginning with 40 mg/ml to 0.3125 mg/ml was <1.3 and the weight of the hair inlay was set on 10 mg. In our research we set the LLoQ to 1.0 as unpublished data have shown that this is valid and still has a CV of ≤20%. In addition, it is difficult to collect a hair strand in young children of 10 mg, because very young children have high HCC lower weights up to 2.5 mg might also gave good results, and a lower cut off point of hair inlay may also be adequate. Future paediatric hair cortisol studies would benefit from an improvement of this technique and international agreements on methodological issues such as those mentioned.

In accordance with previous research, we observed no major effects on hair cortisol concentrations due to hair characteristics but significant effects of ethnicity. (36, 37) Different factors contribute to this effect; more exposure to biopsychosocial stressors in specific ethnic groups (38), differences in the hair textures of different ethnic groups leading to more incorporation of cortisol into the hair (37) and differences in hair growths rates. (33) In contrast to adult studies and consistent with 11 of the 17 studies on determinants of hair cortisol in children (26), we found no significant difference in hair cortisol between girls and boys.

The pathophysiology of excessive infant crying is considered complex and many factors play a role which interact. Within the possible associated neurohormonal factors, cortisol is suggested to play a role, and our exploratory research presented in **Chapter 4 and 5**, indeed showed differences in the HCC of mothers with exposure to their excessively crying infant and to severe psychiatric illness, compared to the control mothers.

In our first study on HCC (**Chapter 4**), we found lower HCC in mothers with an excessively crying infant compared with control mothers. As these mothers experience significantly more stress, depression, anxiety, and bonding problems than controls (Chapter 1), we speculate that the type of experienced psychological stress downregulated their HCC. Flattening of the cortisol diurnal slope or dampening of the cortisol response are well-known phenomena in psychological studies (25,39) and have, for example, been associated with psychosocial stress in women with pre-existent psychopathology. (40) As HCC also provides insight into the months before the measurement, the lowering of HCC in mothers exposed to excessive infant crying compared with controls in our study likely originated during pregnancy. It is known that maternal anxiety during pregnancy is related to an excessively crying infant (4), and this might have downregulated the maternal HPA system. Control parents with increasing stress and depression scores had higher HCC. In contrast, in parents of excessively crying infants, no association was found between HCC and experienced stress, depression, anxiety, or bonding problems. These findings also suggest a dampening of responsivity of the HPA axis to stress and depression in these parents.

In our second study (**Chapter 5**) we assessed HCCs in the perinatal period in mother–infant dyads with and without severe psychiatric disorders. We found significantly different median and distribution of HCC in patients compared with control mothers and no differences in HCC of the infants. The wider range of HCC in mothers with severe psychiatric disorders we found (mainly) higher and lower values than control mothers. Explanations for this finding of variation could be due to the nature of our clinical sample, in which women were selected with severe and long-lasting psychiatric disorders. Previous studies have shown that cortisol levels in patients with mood disorders change over time. The first episodes are more often associated with higher cortisol levels, whereas long-

term duration and the recurrence of episodes might diminish the sensitivity of the HPA axis over time. (41, 42) Heterogeneity of psychiatric diagnoses, the variation of psychiatric symptoms over time and the high prevalence of medication use might all be critical factors in differences of the HPA axis functioning and the long-term release of cortisol in affected mothers. (43, 44)

We found no correlation between maternal and infant HCC in both the patient groups with either maternal psychopathology or excessive crying. Interestingly, we found contradictory results in the control groups of our study; a positive association (Chapter 5) and no association (Chapter 4). Although we can speculate on the found positive association as reflecting early physiological synchrony of mother and child (45), it remains uncertain what the exact cause of our findings is.

In addition to measurement in mothers and infants, we investigated HCC in fathers of excessively crying infants compared to controls and found the same significant lowered HCC in the exposed group, as we found in mothers (chapter 5). In addition to a cross-sectional association study in an older age group (46), and the published study protocol (47), this is the first study on HCC in fathers of young infants. Future research should reveal whether the same mechanism as in mothers, where anxiety during pregnancy is related to an excessively crying infant (4), plays a role in fathers and which other factors contribute to the lowered HCC.

Remarkably, we showed no differences in HCC between exposed infants and controls. This might be due to our small sample sizes, the broad age range of HCC in these infants and selection bias. Another potential explanation is that the exposure to “maternal psychiatric illness” or “excessive crying” are not stressors to the infant itself. This is inconsistent with previous studies on maternal stress during pregnancy, which have shown that higher maternal perinatal HCC predicted lower HCC in newborn infants early postpartum. (48, 49) However, this is in line with a study investigating the correlation between neonatal hair cortisol levels and infant distress. (2) Furthermore, there may be other factors moderating the association between stressors and physiological stress in infants, such as parental care and caregiver relationships (50), which need to be investigated in larger population cohorts. The absence of marked differences in HCC in the infants of the patient group might also reflect the hopeful hypothesis that fetal exposure to increased or decreased maternal cortisol concentrations during pregnancy is effectively regulated by the dynamic nature of placental 11β-hydroxysteroid dehydrogenase type II (11β-HSD-2).(51)

The question of HCC can be used as markers correlating with the amount of experienced parental distress when caring for an excessively crying infant should be answered by performing large prospective controlled studies including variables such as experienced distress during pregnancy, parental care and caregiver relationships.

CLINICAL RECOMMENDATIONS

- Screening on psychopathology in both fathers and mothers visiting the paediatric outpatient clinic with an excessively crying infant.
- Health care professionals working with excessively crying infants should actively involve fathers in the care of their infant.
- Prevention by starting family-centered care during pregnancy focusing on education of normal baby behaviour and parent infant bonding in high-risk populations with a history of psychiatric illness or actual psychiatric symptoms.
- Children's healthcare centres should provide information about their work/role, possibilities to help ("what if), and normal baby behaviour with regard to feeding, sleeping, and crying to all pregnant women at the time of the pertussis vaccination which they coordinate in the 22nd week of pregnancy.
- Children's healthcare centres should refer as soon as possible to a paediatrician specialized in excessive infant crying, in the case of parental concerns of crying behaviour.
- The waiting list for referred infants with excessive crying to a paediatric outpatient clinic should be not more than 3 days.
- We recommend providing reliable information on excessive crying on the internet and linking sites to each other with this information (for example, www.thuisarts.nl, www.opvoeden.nl and www.cyberpoli.nl).
- The NCJ multidisciplinary guideline for excessive crying (2005) should be rewritten and actualized by participating disciplines including disciplines such as gynaecology, psychiatry, and infant mental health specialists.
- Always take parents seriously in their concerns about their crying baby.

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ADDENDUM

NEDERLANDSE SAMENVATTING

Excessief huilen van zuigelingen

Nieuwe inzichten in de rol van ouderfactoren en chronische stress door haarcortisol analyses

Excessief huilen van zuigelingen of “infant colic” vormt één van de grootste uitdagingen voor nieuwe ouders en is een veel voorkomende reden om een kinderarts te bezoeken. Bij de meerderheid (95%) van de “huilbaby’s” kan geen onderliggende lichamelijke aandoening worden gevonden. De behandeling zal derhalve gericht zijn op het ondersteunen van de ouders, hen gerust te stellen en hen door deze moeilijke periode heen te helpen. Om zowel moeder als vader gerust te kunnen stellen en te kunnen ondersteunen, is informatie over hun gevoelens essentieel. In het eerste deel van dit proefschrift, werden drie frequent voorkomende gastro-intestinale symptomen bij zuigelingen waarbij psychosociale stress een belangrijke rol speelt onderzocht. De aanwezigheid van deze symptomen is bekeken bij zuigelingen van moeders met, en zonder een voorgeschiedenis van een psychiatrische ziekte (PZ). Daarnaast werden de gevoelens van moeders én vaders van excessief huilende zuigelingen die de kinderarts bezochten vergeleken met de gevoelens van ouders van controle, niet excessief huilende, zuigelingen.

De pathofysiologische mechanismen die ten grondslag liggen aan excessief huilen zijn waarschijnlijk multifactorieel, en ook neuro-hormonale factoren zouden mogelijk kunnen bijdragen. Om meer inzicht te krijgen in deze factoren, richt het tweede deel van dit proefschrift zich op de rol van de hypothalamus-hypofyse-bijnier (HPA) as door het eindproduct te meten—het stresshormoon cortisol— in het haar van kinderen en ouders. Van oudsher wordt cortisol gemeten in bloed, speeksel of urine, dit geeft inzicht in een beperkte tijd, variërend van minuten tot uren. Haarcortisolmetingen bieden de mogelijkheid om over langere tijd retrospectieve cumulatieve cortisolspiegels te meten. Aangezien cortisol zich in het haar ophoopt terwijl het groeit, met een gemiddelde haargroeisnelheid van ongeveer 10 mm per maand bij volwassenen, kunnen segmenten van geselecteerde lengte worden geanalyseerd voor de gemiddelde cortisolspiegels gedurende de overeenkomstige maanden. De niet-invasieve techniek en eenvoudige opslag van het onderzoeksmateriaal maken deze methode uitermate geschikt voor zowel kindergeneeskundige zorg als onderzoek. We analyseerden haar cortisol concentraties (HCC) bij gezonde kinderen, en bij zuigelingen en ouders die te maken hadden met excessief huilen en ernstige maternale psychiatrische aandoeningen.

DEEL 1 - gastro-intestinale symptomen bij zuigelingen en gevoelens van ouders

In **Hoofdstuk 1** werd de aanwezigheid van excessief huilen, gastro-oesofageale reflux (GOR) en obstipatie onderzocht in zuigelingen van moeders met, en zonder een voorgeschiedenis van een PZ. Moeders in de PZ-groep konden worden geïncludeerd wanneer ze tijdens de zwangerschap een gespecialiseerde Psychiatrie-Obstetrie-Kindergeneeskunde (POP) polikliniek bezochten, vanwege hun voorgeschiedenis van een PZ. Moeders in de controlegroep, zonder een PZ, werden tijdens de zwangerschap geworven bij verschillende verloskundigenpraktijken in Nederland. Gastro-intestinale symptomen bij de zuigelingen, depressieve symptomen bij de moeders en moeder-kind binding werden beoordeeld met behulp van gevalideerde vragenlijsten en diagnostische criteria 1,5 maand na de geboorte. In totaal, werden 101 moeders met

een voorgeschiedenis van een PZ en 60 controlemoeders geïncludeerd. Er werden meer GOR-symptomen bij zuigelingen van PZ-moeders gevonden in vergelijking met controles, en deze symptomen waren geassocieerd met depressieve symptomen van de moeder en bindingsproblemen. Er werden geen significante verschillen gevonden in de aanwezigheid van excessief huilen en obstipatie tussen beide groepen. Depressieve symptomen bij de moeder waren geassocieerd met obstipatie, en bindingsproblemen met excessief huilen. Ten slotte bleek het effect dat depressieve symptomen van de moeder hadden op GOR en het excessief huilen van de zuigeling, te worden gemedieerd door bindingsproblemen.

In **Hoofdstuk 2** zijn stress, depressie, angst en bindingsproblemen onderzocht in een case-controle studie bij vaders en moeders met een excessief huilende zuigeling, die zich op de kindergeneeskundige polikliniek van ons ziekenhuis presenteerden. De controles waren ouders met zuigelingen die niet excessief huilden, welke werden geworven bij consultatiebureaus in de regio. Vijfentwintig procent van de controles werden geworven op de polikliniek kindergeneeskunde, waar ze een routine-echo van de heup kregen na een stuitbevalling. In totaal werden ouders van 34 zuigelingen met excessief huilen en 67 controles geïncludeerd. Er waren significant meer gevoelens van stress, depressie en angst, en bindingsproblemen bij zowel de vaders als moeders van de excessief huilende zuigelingen in vergelijking met de controles. De ervaren gevoelens van stress en bindingsproblemen bij vaders, waren sterk geassocieerd met deze gevoelens bij moeders, de gevoelens van depressie en angst bij vaders niet. We veronderstellen daarom dat geïntegreerde zorg, die negatieve gevoelens van stress en binding bij moeders vermindert, ook positieve effecten zou kunnen hebben op deze negatieve gevoelens bij vaders. De behandeling van depressie en angst bij vaders en moeders zal waarschijnlijk geïndividualiseerd moeten worden.

DEEL 2 - Haarcortisol

In **Hoofdstuk 3** werden, om de toepasbaarheid van haar cortisol metingen in de kindergeneeskundige zorg en het onderzoek te vergroten, pediatrische referentie-intervallen van haarcortisol bepaald met behulp van vloeistofchromatografie-tandem-massaspectrometrie (LC-MS/MS). Hiernaast werden haargroeisnelheden bij kinderen tot twee jaar gemeten. In een cross-sectionele studie werd aangetoond dat HCC niet verschillen tussen meisjes en jongens, en leeftijdsafhankelijk zijn; opmerkelijk hoge waarden worden gezien na de geboorte (162,4 pg/mg). Daarna veel lagere waarden in de eerste maanden, gevolgd door verder lagere waarden tot een dieptepunt op de leeftijd van 6 jaar (1.55 pg/mg). Hierna gevolgd door geleidelijk hogere waarden tot volwassen concentraties worden bereikt op de leeftijd van 18 jaar (3,0 pg/mg). De gemiddelde haargroeisnelheid gemeten in mm per maand was significant lager bij zuigelingen van 0-6 maanden in vergelijking met kinderen van 12-24 maanden (3,5 versus 9,4, P <0,001). Deze lagere haargroeisnelheid is een van de mogelijke verklaringen voor de zeer hoge HCC die gevonden werd bij zuigelingen. Vanwege de lagere haargroeisnelheid geven HCC in het haar van 1 cm gemeten bij zuigelingen tot 6 maanden ons informatie over de 3 maanden voordat de monstername werd gedaan, bij zuigelingen 6-12 maanden over de 1,5 maand vóór de monstername en bij kinderen in de leeftijd 12-24 maanden, ongeveer 1 maand, hetzelfde als bij oudere kinderen en volwassenen.

In **Hoofdstuk 4** werden HCC gemeten met behulp van LC-MS/MS bij zuigelingen, moeders en vaders van excessief huilende zuigelingen, en deze vergeleken met HCC in controlegezinnen zonder een excessief huilende zuigeling, binnen dezelfde case-

control studie als beschreven in **Hoofdstuk 2**. Gemiddelde HCC waren significant lager bij zowel moeders àls vaders van excessief huilende zuigelingen (2,3 pg/mg en 1,6 pg/mg) dan die bij controle moeders en vaders (3,2 pg/mg en 2,9 pg/mg). In de totale groep ouders en binnen de ouders van excessief huilende zuigelingen, waren HCC niet geassocieerd met gevoelens van stress, depressie, angst of bindingsproblemen. In de controlegroep vertoonden HCC een positieve associatie met stress en depressie scores. Omdat HCC ook inzicht geven in de maanden voorafgaand aan de meting, is de verlaging van HCC bij ouders die zijn blootgesteld aan excessief huilen van zuigelingen in vergelijking met controles, mogelijk al ontstaan tijdens de zwangerschap. Het is bekend dat maternale angst tijdens de zwangerschap geassocieerd is met excessief huilen, en dat angststoornissen geassocieerd zijn met verlaagde HCC. We veronderstellen daarom dat de stressvolle gevoelens bij ouders van excessief huilende zuigelingen mogelijk al tijdens de zwangerschap hebben geleid tot een downregulatie van hun HPA-systeem. Bij zuigelingen werden geen verschillen gevonden in gemiddelde HCC tussen de groepen.

In **Hoofdstuk 5** onderzochten we de invloed van ernstige PZs op HCC van moeders en zuigelingen. We vonden een significant grotere range aan HCC bij moeders met ernstige PZs, met zowel (voornamelijk) hogere als lagere waarden dan controlemoeders. De lange duur van de PZs, de heterogeniteit van psychiatrische diagnoses en actuele psychiatrische symptomen, en de hoge prevalentie van medicatiegebruik zouden allemaal kritische factoren kunnen zijn die de gevonden grote variatie in HCC zouden kunnen verklaren. Er werden geen verschillen gevonden in HCC bij de zuigelingen tussen de groepen.

Opmerkelijk was dat we geen verschillen in HCC vonden tussen blootgestelde zuigelingen (aan excessief huilen en moeders met een ernstige PZ) en controles. Dit zou verklaard kunnen worden door de kleine steekproefomvang, de leeftijdsverschillen in de eerste maanden waarin HCC significant veranderen, en selectiebias. Een andere mogelijke verklaring is dat de blootstelling aan “excessief huilen” of “psychiatrische aandoeningen van de moeder” geen stressfactoren zijn voor het kind zelf. Ten slotte kunnen er andere factoren zijn die de associatie tussen stressoren en de fysiologische stressrespons bij zuigelingen modereren, zoals de kwaliteit van de zorg van ouders, hun beschikbaarheid, en relaties tussen ouders, die in grotere populatiecohorten moeten worden onderzocht.

Concluderend kan het onderzoek in dit proefschrift worden samengevat in vier kernboodschappen. Ten eerste, zijn een voorgeschiedenis van PZ bij moeder, actuele depressieve symptomen bij moeder en postpartum binding belangrijke ouderlijke factoren om rekening mee te houden als kinderarts bij het omgaan met zuigelingen met gastro-intestinale symptomen. Er werd aangetoond dat zuigelingen van PD-moeders meer GOR hadden in vergelijking met controles, depressieve symptomen van de moeder geassocieerd waren met obstipatie en bindingsproblemen geassocieerd waren met excessief huilen van zuigelingen. Ten tweede, hebben zowel vaders als moeders met een excessief huilende zuigeling meer gevoelens van stress, depressie, angst en bindingsproblemen in vergelijking met controle ouders, alhoewel de gevoelens van stress en bindingsproblemen bij vader sterker worden beïnvloed door de gevoelens van stress en bindingsproblemen bij moeder als door het excessief huilen van de zuigeling. Ten derde, werden pediatrische referentie-intervallen van haarcortisol met LC-MS/MS bepaald en bleken lagere haargroeisnelheden één van de verklaringen te zijn voor de opvallend hoge HCC op de zuigelingen leeftijd. Ten slotte, zijn HCC significant verschillend bij ouders van excessief huilende zuigelingen en bij moeders met een ernstige PZ in vergelijking met controles, terwijl HCC bij hun zuigelingen niet verschilden van de controle groep.

Invloed huilbaby op welzijn van beide ouders

Achtergrond

Overmatig huilen bij baby's is ingrijpend voor ouders en leidt regelmatig tot een consult bij de kinderarts of zelfs ziekenhuisopname. Bij < 5% van de baby's blijkt er een medische oorzaak voor het huilen te zijn. Daarom is uitleg, geruststelling en inzicht in de gevoelens van ouders van groot belang. Het is bekend dat moeders van een huilbaby meer angst en depressieve gevoelens ervaren. De gevoelens bij vaders zijn niet eerder onderzocht.

Centrale vraag

Hebben ouders van een huilbaby meer last van stress en negatieve gevoelens? En wat is het verschil in reactie tussen moeders en vaders?

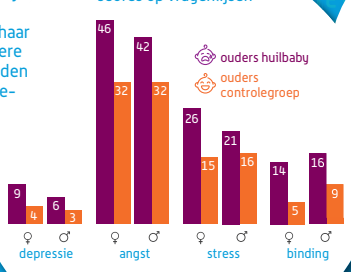
Interventies

Vaders en moeders van een huilbaby en uit controlefamilies vulden vragenlijsten in over hun gemoedstoestand (gevoelens van depressie, angst en stress) en de ervaren binding met hun baby. Met een haarsample (haarlok) is de concentratie van het stress-hormoon cortisol gemeten.

Resultaat

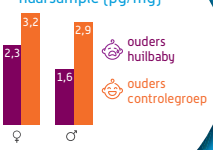
Vaders en moeders ervaren significant meer negatieve gevoelens en verminderde binding bij de zorg voor een huilbaby. Vaders ervaren deze gevoelens minder dan moeders [fig 1]. Ervaren stress en verminderde binding bij vaders blijkt sterk beïnvloed te worden door deze gevoelens bij moeder. Na correctie voor haar gevoelens verdwijnt het significante verschil. In het haar werd bij ouders van een huilbaby een lagere gemiddelde cortisolconcentratie gevonden [fig 2]. De oorzaak hiervan is niet duidelijk, mogelijk speelt angst, wat de cortisol aanmaak kan onderdrukken, hierbij een rol.

Gemiddelde scores op vragenlijsten



Figuur 1

Gemiddelde cortisolconcentratie haarsample (pg/mg)



Figuur 2

Onderzoeksteam

Ineke de Kruijff, (hoofd-) onderzoeker en kinderarts; Moniek Veldhuis, onderzoeker en ANIOS kindergeneeskunde; Ellen Tromp, epidemioloog; allen St. Antonius Ziekenhuis

Wie deden mee aan het onderzoek?

35 huilbaby's <5 maanden uit het St. Antonius Ziekenhuis en hun ouders

62 controle baby's <5 maanden en hun ouders: **14** uit het St. Antonius Ziekenhuis en **48** benaderd bij consultatiebureaus in omgeving Utrecht/Woerden.

Conclusie

Gezien de uitkomst is het belangrijk om in de zorg en begeleiding van een huilbaby zowel moeder als vader te betrekken en hun psychologische welzijn te bespreken en waar nodig te begeleiden.

Publicaties

-Distress in fathers of babies with infant colic. Acta Paediatrica. 2021;00:1–7.
-Parental stress and scalp hair cortisol in excessively crying infants. Submitted.

Invloed op de zorg

Beide ouders van opgenomen huilbaby's krijgen nu standaard een gesprek met een psychiatrisch verpleegkundige. Vanaf 2022 sluit ook bij elk polibezoek van een huilbaby aan de kinderarts, een medisch pedagogisch zorgverlener aan.

Samen zorgen voor kwaliteit van leven

Dit onderzoek is een voorbeeld van hoe het St. Antonius Ziekenhuis zijn ambitie *Samen zorgen voor kwaliteit van leven* in de praktijk brengt.

RESEARCH & DEVELOPMENT
ST ANTONIUS

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PUBLICATIONS

This thesis

de Kruijff I, Choenni V, Groeneweg JT, Vlieger AM, Benninga MA, Kok R, Kamperman AM, Lambregtse-van den Berg MP. Gastrointestinal symptoms in infants of mothers with a psychiatric history and the role of depression and bonding. J Pediatr Gastroenterol Nutr. 2019;69:662-667.

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de Kruijff I, Tromp E, Lambregtse-van den Berg MP, Vlieger AM, Benninga MA, de Rijke YB, van den Akker EL. Parental Stress and Scalp Hair Cortisol in Excessively Crying Infants: A Case Control Study. Children (Basel). 2021;30;8:662.

Broeks CW, Choenni V, Kok R, van der Voorn B, de Kruijff I, van den Akker ELT, van Rossum EFC, Hoogendijk WJG, Hillegers MHJ, Kamperman AM, Lambregtse-Van den Berg MP. An exploratory study of perinatal hair cortisol concentrations in mother-infant dyads with severe psychiatric disorders versus healthy controls. BJPsych Open. 2021;7:e28.

Other

Nguyen LA, Pouwer F, Winterdijk P, Hartman E, Nuboer R, Sas T, de Kruijff I, Bakker-Van Waarde W, Aanstoot HJ, Nefs G. Prevalence and course of mood and anxiety disorders, and correlates of symptom severity in adolescents with type 1 diabetes: Results from diabetes LEAP. Pediatr Diabetes. 2020;22:638-648.

Wessels M, Velthuis A, van Lochem E, Duijndam E, Hoorweg-Nijman G, de Kruijff I, Wolters V, Berghout E, Meijer J, Bokma JA, Mul D, van der Velden J, Roovers L, Mearin ML, van Setten P. Raising the Cut-Off Level of Anti-Tissue Transglutaminase Antibodies to Detect Celiac Disease Reduces the Number of Small Bowel Biopsies in Children with Type 1 Diabetes: A Retrospective Study. J Pediatr. 2020;223:87-92.e1.

Nefs G, Nguyen L, Winterdijk P5, Hartman E Sas T, Nuboer R, De Kruijff I, Bakker-van Waarde W, Aanstoot HJ, Pouwer F.Study protocol of Diabetes LEAP: a longitudinal study examining emotional problems in adolescents with type 1 diabetes and their parents/ caregivers. BMC Pediatr. 2019;19:377.

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PhD PORTFOLIO

Name:	Ineke de Kruijff
PhD period:	04-06-2013 – 01-10-2021
PhD supervisor:	Prof. dr. M.A. Benninga
Co-supervisors:	Dr. M.P. Lambregtse-van den Berg Dr. E.L.T. van den Akker

1. PhD training

	Year	Workload (ECTs)
General courses		
Writing in English for Publication	2014	1.5
BROK	2014	1,5
BROK registration prolongation	2018	0,3
CAT search in Pubmed	2014	0.1
Courseref works	2015	0.1
Basic course SPSS	2016	0.8
Basic course Redcap	2020	0.1
Seminars, workshops and master classes		
• IMH course RINO Amsterdam psychological factors and psychopathology in children aged 0-6 years	2013	2,5
• Endocrine meeting Amsterdam University Medical Center, two monthly	2013-2021	4
Oral Presentations		
Crying and bonding: How maternal psychiatric disorders psychiatry may influence the first year of her infants life. • Symposium “Samen nog Beter”, Zeist, The Netherlands	2015	0.5
Pregnancy and psychotropic drugs: intrauterine exposure of psychotropic drugs, effects on the infant. • Altrecht pharmacotherapeutic meeting, Zeist, The Netherlands	2015	0.5

Intrauterine Psychotropic Drug Exposure, Effects on the infant and Breastfeeding • Paediatric post graduate week Wilhelmina Childrens Hospital , Utrecht, The Netherlands	2015	0.5
Vulnerable unborn children in the picture: the multidisciplinary approach at the POP outpatient clinic • NVK (Dutch Association of Pediatrics) congress, Veldhoven, The Netherlands	2015	0.5
The vulnerable pregnant woman and mother: which care, whose care's? A case based on the Screening instrument "Mind2Care" • Metropolitan perinatal health 3. Rotterdam, The Netherlands	2015	0.5
Basics Psychiatrics and Pregnancy: Mind 2 Care • Basic course LKPZ (national centre for psychiatry and pregnancy), Hilversum, The Netherlands	2018	0.5
Help, why is my Child crying? • Symposium L. Wafelman, Hoorn, The Netherlands	2018	0.5
Mind2Care, an innovative screen- and-advice model for psychopathology, psychosocial problems and substance use, during pregnancy • NASPOG (North American Society for Psychosocial Obstetrics and Gynecology), Philadelphia, USA	2018	0.5
Interdisciplinary approach for vulnerable pregnant women • ISPOG (International Society of Psychosomatic Obstetrics & Gynaecology), Paris, France	2019	0.5
Poster presentations		
Should we screen for maternal mental illness in the pediatric clinic? • WAIMH (world association for infant mental health) Edinburgh, Scotland.	2014	0.5
Prenatal caring for our pregnant women with a psychiatric illness or vulnerability and their future babies: experiences of a pop outpatient clinic in The Netherlands. • WAIMH (world association for infant mental health) Edinburgh, Scotland	2014	0.5
Common gastrointestinal symptoms in infants of mothers with a history of psychiatric disorder • NASPOG (North American Society for Psychosocial Obstetrics and Gynecology), Philadelphia, USA	2018	0.5

(Inter)national conferences		
• WAIMH (world association for infant mental health) Edinburgh, Scotland	2014	1.5
• Symposium “Samen nog Beter”, Zeist, The Netherlands	2015	0,5
• Altrecht pharmacotherapeutic meeting, Zeist, The Netherlands	2015	0,2
• NVK (Dutch Association of Pediatrics) congress, Veldhoven, The Netherlands	2015	0,3
• Metropolitan perinatal health 3. Rotterdam, The Netherlands	2015	0,3
• Basic course LKPZ (national centre for psychiatry and pregnancy), Hilversum, The Netherlands	2018	0,3
• NASPOG (North American Society for Psychosocial Obstetrics and Gynecology), Philadelphia, USA	2018	1
• ISPOG (International Society of Psychosomatic Obstetrics & Gynaecology), Paris, France	2019	1
2. Teaching		
Lecturing		
• Teaching session on excessive crying for general practitioners	2015	0,5
• Teaching session on IMH for paediatricians cluster Utrecht	2018	0,5
• Withdrawal symptoms in the newborn and the use of the finnegan score system for maternity nurses	2018	0,5
Supervision research internships		
• Jasja T. Groeneweg, master Medicine; Gastroesophageal reflux and excessive Crying in the POP outpatient clinic	2016	1,5
• Moniek S.Veldhuis; master in Medicine; parental stress in excessive crying infants: different reactions between fathers and mothers?	2017	1,5
• Dominique G.A. Begijn; master in Medicine; Hair Cortisol in infants	2018	1,5

DANKWOORD

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De leden van de leescommissie: Prof. dr. R.J.L. Lindauer , Prof. dr. E.M. van de Putte, Prof. dr. M.H.J. Hillegers, Prof. dr. A.S.P. van Trotsenburg, Dr. A.H. Teeuw en Dr. H.M.A. de Bie wil ik bedanken voor het kritisch lezen van dit proefschrift.

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CURRICULUM VITAE

Ineke de Kruijff was born on the 15th of October 1970 in Vianen. She grew up with her parents and younger brother in Houten. After secondary education at the Bonifatius College in Utrecht, she stayed in Utrecht for her medical study, which started in 1989. During her medical study her research project in the paediatric rheumatology centre and her paediatric internship in the Diaconessen Hospital in Utrecht further developed her interest in paediatrics. After her graduation in 1996, she started working as a resident in paediatric medicine in the St. Antonius Hospital, Nieuwegein and in 1997 she started in the Wilhelmina Children's Hospital. In 1998 she started with her paediatric training in the Juliana Children's Hospital in Den Hague and during these years she developed interest in both diabetes and research and performed a multicentre study on the use of a Color-Coded Slide Rule in Children With Diabetes. After graduation she started working in the Zuwe Hofpoort Hospital in Woerden in 2002 and further developed her interest in paediatric endocrinology one day a week in the VU Medical Centre in Amsterdam. In 2005 the Regional Psychiatric Centre Woerden (RPCW) was the second in the Netherlands to open its Mother Baby Unit (MBU) and due its location in the hospital a collaboration between psychiatrists, obstetrics and paediatricians was born. In 2011 this lead in to the start of the multidisciplinairy "POP"outpatient clinic and in that year Ineke joined the board of the Landelijk Kenniscentrum Psychiatrie en Zwangerschap (LKPZ). In order to nationally implement screening on psychopathology, psychosocial problems and substance use during pregnancy the Stichting Mind2 care was founded in 2016 and Ineke joined the board. During her years in paediatric practice multiple important questions were raised on the topic of "excessive infant crying" of which answers were essential to optimally guide families through the first months of life. This research project emerged out of these questions and this thesis is the result.

Ineke lives together with her husband Guus Hermsen and has 4 daughters: Maartje, Sietske✡, Floor and Nelleke.

Scan for a 5 minute explanation
on the topic by Ineke and Nelleke