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Research Paper

Effect of *Lactobacillus rhamnosus* HN001 in Pregnancy on Postpartum Symptoms of Depression and Anxiety: A Randomised Double-blind Placebo-controlled Trial

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ABSTRACT

Background: Probiotics may help to prevent symptoms of anxiety and depression through several putative mechanisms.
Objective: The aim of this study was to evaluate the effect of *Lactobacillus rhamnosus* HN001 (HN001) given in pregnancy and postpartum on symptoms of maternal depression and anxiety in the postpartum period. This was a secondary outcome, the primary outcome being eczema in the offspring at 12 months of age.
Design, Setting, Participants: A randomised, double-blind, placebo-controlled trial of the effect of HN001 on postnatal mood was conducted in 423 women in Auckland and Wellington, New Zealand. Women were recruited at 14–16 weeks gestation.
Intervention: Women were randomised to receive either placebo or HN001 daily from enrolment until 6 months postpartum if breastfeeding.
Outcome Measures: Modified versions of the Edinburgh Postnatal Depression Scale and State Trait Anxiety Inventory were used to assess symptoms of depression and anxiety postpartum.
Trial Registration: Australia NZ Clinical Trials Registry: ACTRN12612000196842.
Findings: 423 women were recruited between December 2012 and November 2014. 212 women were randomised to HN001 and 211 to placebo. 380 women (89.8%) completed the questionnaire on psychological outcomes, 193 (91.0%) in the treatment group and 187 (88.6%) in the placebo group. Mothers in the probiotic treatment group reported significantly lower depression scores (HN001 mean = 7·7 (SD = 5·4), placebo 9·0 (6·0); effect size −1·2, (95% CI -2·3, −0·1), $p = 0·037$) and anxiety scores (HN001 12·0 (4·0), placebo 13·0 (4·0); effect size −1·0 (−1·9, −0·2), $p = 0·014$) than those in the placebo group. Rates of clinically relevant anxiety on screening (score > 15) were significantly lower in the HN001 treated mothers (OR = 0·44 (0·26, 0·73), p = 0·002).
Interpretation: Women who received HN001 had significantly lower depression and anxiety scores in the postpartum period. This probiotic may be useful for the prevention or treatment of symptoms of depression and anxiety postpartum.
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1. Introduction

Major depression in pregnancy and after birth occurs in 10–15% of women in New Zealand, a rate comparable to other western countries (Abbott and Williams, 2006). Postnatal depression (PND) is associated with persistent depression, and even, in a few cases each year, death from suicide (PMMRC, 2014). This disorder may affect a mother's ability to care for and bond with her new infant, as well as her quality of life and daily functioning (Da Costa et al., 2006). In addition, maternal depression can produce long-lasting effects on children's cognitive, social-emotional and health outcomes (Tronick and Reck, 2009; Grace

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et al., 2003). In addition to depressed mood, PND is associated with hopelessness, excessive fatigue, psychomotor agitation, appetite and sleep disturbances, guilt, or feelings of inadequacy, particularly regarding one's ability to care for the newborn and co-morbid anxiety. Anxiety often coexists with depression and like PND, the prevalence varies widely depending on the timing and the type of disorder (generalised anxiety disorder (GAD) vs obsessive compulsive disorder (OCD)) and the way it is measured (self-report vs structured interview). Despite this, most women with PND are either not recognised as being depressed, are unable to access psychological therapy or are reluctant to take antidepressant medication in pregnancy or while breastfeeding. Furthermore it takes several weeks for the therapeutic effect of antidepressants to appear and there is a 15–30% discontinuation rate (Gartlehner et al., 2005). Safe and effective therapies to prevent and treat PND are needed (Battle et al., 2003).

A "healthy" diet comprising higher intakes of fruit and vegetables, whole grains and lean meat and fish is associated with a reduced likelihood of depression (Lai et al., 2013). These observational studies are supported by a recent randomised controlled trial (RCT) showing that a dietary improvement intervention was effective as an adjuvant therapy in patients with moderate to severe depression who were being treated with psychotherapy or antidepressants (Jacka et al., 2017). Furthermore, it has been suggested that fermented foods alter dietary items before they are ingested, resulting in phytochemical transformation into bioactive chemicals which reduce oxidative stress and inflammation (Selhub et al., 2014).

There is a growing literature linking the gut microbiota to brain chemistry and behaviour via multiple bi-directional pathways (the microbiome-gut-brain axis), including the immune system, neuroendocrine, hypothalamic pituitary adrenal axis (HPA axis), short chain fatty acids or tryptophan and sympathetic and parasympathetic arms of the autonomic nervous system including the enteric nervous system, the vagus nerve, and the gut microbiota (Wang and Kasper, 2014; Dinan and Cryan, 2015). Microbial dysbiosis is associated with many health problems including neuropsychiatric disorders, such as autism spectrum disorder, depression and anxiety, and are associated with elevated levels of pro-inflammatory cytokines, increased oxidative stress, altered gastrointestinal (GI) function, and lowered micronutrient and omega-3 fatty acid status (Dawson et al., 2016). Intriguingly, alterations in the pattern of gut microbial composition in healthy adults influences mood (Li et al., 2016).

Probiotics are live microorganisms that when consumed in adequate amounts provide health benefits to the host (Hill et al., 2014). In 2005 it was first suggested that probiotics might be an adjuvant therapy for major depression (Logan and Katzman, 2005) and others have also suggested that probiotic enhancement of gut microbiota may improve mood outcomes (Dinan and Cryan, 2016). Pre-clinical studies have demonstrated that the anxiety phenotype of mice can be changed with fecal transplantation and that the changes in microbiota are accompanied by changes in brain chemistry (Collins et al., 2013). Furthermore, probiotic treatment has also been shown to have a positive effect on anxiety-like and depressive-like behaviour in animal studies (Bravo et al., 2011; Desbonnet et al., 2010), with mediating mechanisms including GABA receptor expression in specific locations of the central nervous system (Bravo et al., 2011), the HPA axis (Desbonnet et al., 2010) and the vagus nerve which transmits information from the gut luminal environment to the CNS (Carabotti et al., 2015). Interestingly, probiotic supplementation in adults has not been found to substantially alter their gut microbiota as sampled by fecal samples (Kristensen et al., 2016), although this does not exclude their potential effect higher up in the small intestine or on the adherent mucosal gut microbiome.

Clinical trials of probiotic treatment have yielded mixed results, and systematic reviews of human trials concluded that the evidence for beneficial effects of probiotics on mood may not be as strong as some recent narrative studies purport (Romijn and Rucklidge, 2015). A recent systematic review identified 10 clinical trials of the effect of probiotics on symptoms of depression (Wallace and Milev, 2017). Seven studies were in healthy subjects, 2 in chronic fatigue syndrome and one in depression. Three of 5 studies reported improved mood with probiotics, and 5 of 7 studies reported improvements in stress and anxiety. A recent study that was published after these reviews reported that obese women treated with a weight-reduction programme and probiotic had reduced symptoms of depression compared with the comparison group, but this effect was not seen in men (Sanchez et al., 2017). There was no effect on anxiety. Both reviews suggested further RCTs were needed. To date probiotic effects on postnatal depression have not been studied in a clinical trial.

Our aim was to evaluate whether probiotic supplementation with *Lactobacillus rhamnosus* HN001 (HN001) had a beneficial effect on postnatal symptoms of depression and anxiety in a group of healthy women. This was a secondary outcome, the primary outcome being eczema in the offspring at 12 months of age.

2. Methods and Materials

2.1. Study Design

The Probiotics in Pregnancy Study (PIP Study) is a two-centre (Wellington and Auckland, New Zealand) randomised double-blind placebo-controlled trial testing the effect of the probiotic HN001 on the development of eczema and atopic sensitization in offspring (the primary outcome) and pregnancy outcomes (secondary outcomes) in women. The full protocol is published (Barthow et al., 2016).

2.2. Study Population

The selection of participants, randomisation process and quality control measures have been described in detail previously (Barthow et al., 2016). In brief, 423 women were recruited at 14–16 weeks gestation between December 2012 and November 2014. 212 women were randomised to HN001 and 211 to placebo. Women were considered eligible if they were English-speaking, planning to breastfeed, and if either they or the unborn child's biological father had a history of asthma, hayfever or eczema requiring medication. Women were excluded from the study if aged <16 years, planning to move outside the study centres during study duration, planning on taking probiotics, or if they had serious medical or health problems related to the pregnancy.

2.3. The Intervention

Women were randomised to receive either HN001 at a dose of 6×10^9 colony-forming units (cfu) or placebo (corn-derived maltodextrin), to be taken daily from enrolment until birth and, from birth up till six months post-birth whilst breastfeeding. The capsules were indistinguishable. Both researchers and participants were blinded to treatment assignation of participants. To assess adherence, capsule bottles were collected at regular intervals and counts of remaining capsules were completed by an independent person.

2.4. Randomisation

Randomisation was managed by Fonterra Co-operative Group Ltd. and concealed from all study staff and participants. Randomisation was stratified by study centre and performed in blocks of random lengths according to a computer-generated randomisation list. Research staff screened and enrolled eligible participants, and provided enrolled participants with the next available sequentially-numbered capsule container.

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