BRAIN DERIVED NEUROTROPHIC FACTOR IS ALTERED IN HUMAN PREGNANCY

Deborah R. Kim, Juan M. Gonzalez, Mary D. Sammel, Samuel Parry, C. Neill Epperson

Abstract

Objective: Brain derived neurotrophic factor (BDNF) is a neuroprotective peptide crucial to the development and function of the nervous system that is important for both a healthy mood and pregnancy. The aim of this study was to definitively characterize serum BDNF levels in 3rd trimester pregnant women as compared to follicular phase, non-pregnant women who had been carefully screened for the absence of psychiatric illness.

Method: Twenty healthy pregnant women ≥ 28 weeks gestational age with a healthy, singleton pregnancy and 20 non-pregnant, healthy women in the follicular phase of their menstrual cycle were consecutively recruited from a general obstetrics clinic at the University of Pennsylvania from March 2010 through October 2010. Peripheral serum BDNF levels were evaluated in 20 pregnant women and 20 non-pregnant women in the follicular phase of their menstrual cycle.

Results: Median serum BDNF in the pregnant group was 11781.1 pg/ml (IQR 9435.2, 15936.4) and in the non-pregnant group was 23212.5 pg/ml (IQR 14644.0, 26287.9). Serum BDNF was significantly lower in the pregnant group compared to the non-pregnant group ($p = .0029$).

Conclusions: This study confirms that serum BDNF is significantly lower in pregnant women compared to non-pregnant, follicular phase women even in the absence of psychiatric illness. In a subset of vulnerable women, low BDNF levels may precipitate an environment that puts women and their babies at increased risk for affective or neurodevelopmental disturbances.

Key words: women's mental health, brain derived neurotrophic factor (BDNF), pregnancy, human, neuropeptides, mood disorders

Declaration of interest: none

Deborah R Kim (1), Juan M Gonzalez (2), Mary D. Sammel (3), Samuel Parry (4), C. Neill Epperson(1)

(1) Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania
(2) Department of Maternal-Fetal Medicine, Wayne State University
(3) Department of Biostatistics and Epidemiology, Perelman School of Medicine at the University of Pennsylvania
(4) Department of Maternal-Fetal Medicine, Perelman School of Medicine at the University of Pennsylvania

Corresponding author
Deborah R Kim
Department of Psychiatry,
Perelman School of Medicine at the University of Pennsylvania
E-Mail: drkim@upenn.edu

Introduction

Peripheral serum levels of brain derived neurotrophic factor (BDNF), a neuroprotective peptide crucial to the development and function of the nervous system, are decreased in depression (Islam et al. 2009, Huang and Reichardt 2001, Lewin and Barde 1996). After treatment with antidepressants, these levels increase, suggesting that peripheral BDNF levels may be a signal of central BDNF dysregulation in depression (Schmidt and Duman 2010). A separate line of evidence shows that BDNF is important during pregnancy for healthy embryo implantation and fetal maturation (Kawamura et al. 2009, Kawamura et al. 2007, Dissen et al. 1995). In animals, fetuses unable to produce BDNF are not viable (Kodomari et al. 2009). Altering perinatal environments, such as giving high fat diets or inducing pregestational stress, leads to decreased BDNF in the hippocampus of rodent offspring (Huang et al. 2010, Tozuka et al. 2010). Since normal BDNF levels are important for both a healthy mood and pregnancy, it is a peptide of interest to researchers attempting to understand the biological causes and effects of antenatal depression.

Only one previous study evaluated peripheral BDNF levels in pregnant women compared to non-pregnant

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women (Lommatzsch et al. 2006). Counterintuitively, serum BDNF was significantly lower during pregnancy despite studies showing that BDNF levels rise under conditions of high estrogen (Lommatzsch et al. 2005, Pluchino et al. 2009, Begliuomini et al. 2007). In addition, some of the pregnant subjects were depressed at the time of the blood draw which may have resulted in spuriously decreased BDNF levels. Alternatively, if peripheral BDNF is truly lower during pregnancy this could put some women at risk for developing antenatal depression. The aim of this study was to definitively characterize serum BDNF levels in 3rd trimester pregnant women as compared to follicular phase, non-pregnant women who had been carefully screened for the absence of psychiatric illness.

Methods

Twenty healthy pregnant women ≥ 28 weeks gestational age with a healthy, singleton pregnancy and 20 non-pregnant, healthy women in the follicular phase of their menstrual cycle were consecutively recruited from a general obstetrics clinic at the University of Pennsylvania from March 2010 through October 2010. All subjects were ≥ 18 years of age and able to give informed consent. All procedures were approved by the University of Pennsylvania's Institutional Review Board. Subjects were given the Structured Clinical Interview for Diagnosis (SCID) (First et al. 1995) to rule out lifetime or current psychiatric illnesses. Subjects were given the Hamilton Depression Rating Scale-17 (HDRS-17) (Hamilton 1967), the Beck Depression Inventory (BDI) (Beck et al. 1961), Beck Anxiety Inventory (BAI) (Beck et al. 1988) and the Edinburgh Postnatal Depression Scale (EPDS) (pregnant subjects only) (Cox et al. 1996). The following was required to proceed to the blood draw: HDRS and BDI < 8, BAI < 10 and additionally for the pregnant women an EPDS < 10. An EPDS score ≥ 10 is considered an indication of major depression. In addition, while initially developed for use postpartum, the EPDS has been found to have high sensitivity and specificity in pregnant populations (Bunevicius et al. 2009). Menstrual cycle phase was determined by asking the subject the date of the last menstrual period. Follicular phase was considered to be from day 1 – day 13 of the menstrual cycle. Approximately 15 ml of blood was drawn from the antecubital vein of subjects. Blood was allowed to clot for at least 30 minutes and then centrifuged for 10 minutes at 13,000 revolutions per minute. Supernatants were aliquoted and stored at -80°C until measured. BDNF serum concentrations were measured using an enzyme-linked immunosorbent assays (ELISA) according to the manufacture's protocol (Quantikine, R & D Systems, Minneapolis, MN).

The minimal detectable BDNF level was less than 20 pg/ml. Samples were diluted by 20 fold into calibrator diluents as per the manufacturer's protocol. The absorbance at 450 nm was read on a microplate reader. Mean concentrations were determined in duplicate based on a standard curve. The standard curve was prepared using recombinant human BDNF as per the manufacturer's protocol. The Quantikine Human BDNF Immunoassay has been validated to measure human BDNF in serum. Descriptive statistics were used to describe the sample. Serum BDNF levels were compared between the groups using a Wilcoxon-Rank Sum test as levels were not normally distributed. Power calculations assumed a two-sided type one, alpha, error of 5% along with 90% power. We determined that 20 subjects per group would be sufficient to detect a difference of 0.75 standard deviation units in BDNF levels between the groups.

Results

Sample Characteristics: 40 women completed the study (**table 1**). The mean age of the pregnant group was 30 years (SD 6.6) and mean gestational age was 34.5 weeks (SD 3.7). The mean age of the non-pregnant group was 30 years (SD 6.3). There were no significant differences between groups for age, race and rating scales ($p > .05$) except for the HDRS. Although the HDRS score was significantly higher in the pregnant group, it did not indicate this group was depressed. This was expected given that HDRS scores indicating clinically relevant depressive symptoms was an exclusion criterion. HDRS scores were not significantly

Table 1.

	Non-Pregnant (N=18)	Pregnant (N=18)	P-values
Age in years (Mean, SD)	30.4 (6.3)	30.3 (6.6)	0.96
Race % (N) Caucasian African Am Other	 50.0% (9) 38.9% (7) 11.1% (2)	 38.9% (7) 33.3% (6) 27.8% (5)	0.45
OCP use % (N)	27.8% (5)	N/A	
HDRS (Mean, SD)	0.72 (1.23)	2.94 (2.1)	0.0005
BDI (Mean, SD)	1.89 (2.1)	2.78 (2.3)	0.24
BAI (Mean, SD)	2.06 (2.7)	2.17 (1.9)	0.89

OCP = oral contraceptive pill; HDRS = Hamilton Depression Rating Scale; BDI = Beck Depression Inventory;
BAI = Beck Anxiety Inventory

associated with BDNF levels (p=.48). BDNF samples with a coefficient of variation (CV) < 20 were included, leaving 18 subjects in each group for analysis. Samples were run in duplicate and a CV > 20 (greater than 20%) indicates the variation between duplicate samples is too large to reliably include the data.

BDNF Level Comparisons: Median serum BDNF in the pregnant group was 11781.1 pg/ml (IQR 9435.2, 15936.4) and in the non-pregnant group was 23212.5 pg/ml (IQR 14644.0, 26287.9) (**figure 1**). Serum BDNF was significantly lower in the pregnant group compared to the non-pregnant group (p = .0029).

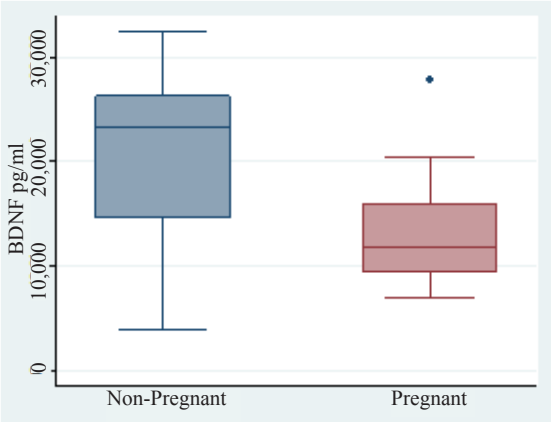

Figure 1. *Median serum BDNF in the pregnant group was 11781.1 pg/ml (IQR 9435.2,15936.4) and in the non-pregnant group was 23212.5 pg/ml (IQR 14644.0, 26287.9). Serum BDNF was significantly lower in the pregnant group compared to the non-pregnant group (p = .003)*

Discussion

This study confirms that serum BDNF is significantly lower in pregnant women compared to non-pregnant, follicular phase women even in the absence of psychiatric illness. Given the importance of BDNF for a healthy mood and pregnancy, this was an unexpected finding. A prior study compared interindividual serum BDNF during pregnancy to a non-pregnant cohort and found levels to be decreased in pregnancy and postpartum suggesting an underlying vulnerability to the development of perinatal mood disorders (Lommatzsch et al. 2006). While this study had a single measure of depression, no studies have carefully characterized subjects to rule out a concurrent depression diagnosis and to control for menstrual cycle phase in the non-pregnant cohort.

The mechanism by which peripheral BDNF levels are decreased during pregnancy is unclear. Possibilities include fetal sequestration, hemodilution, changes in platelet number or activity and the effect of sex steroids on BDNF levels. Some molecules important to fetal health, such as iron and folic acid, are efficiently transported from mother to fetus (even at the risk of depleting the mother's stores) suggesting that these molecules are sequestered by the fetus (Gambling et al. 2009, Milman et al. 1999, Guerra-Shinohara et al. 2002). For the fetus to sequester BDNF, it would have to pass from mother to fetus. In mice, BDNF crosses the utero-placental barrier such that when BDNF is given peripherally to mothers, a dose dependent increase is seen in the fetuses' brains (Kodomari et al. 2009). However, umbilical cord levels of BDNF have been shown to be lower than maternal levels making fetal sequestration questionable (Nikolaou et al. 2006).

Hemodilution of pregnancy is another possible mechanism that would lower BDNF levels during pregnancy. Hypervolemia associated with normal pregnancy averages about 40 to 45 percent after 32 to 34 weeks when compared to the non-pregnant state (Whittaker et al. 1996, Pritchard 1965). In mice, maternal serum BNDF does not change with gestational age (Kodomari et al. 2009) but in human amniotic fluid, there was a modest, negative correlation between BDNF levels and gestational age (Marx et al. 1999). A more recent study found that in 20 women intraindividual serum BDNF levels were lower at 32-36 weeks gestation compared to 10-12 weeks (Vega et al. 2011). The postpartum data suggests that BDNF may continue to be decreased until 10 weeks postpartum (Lommatzsch et al. 2006, Vega et al. 2011) which contradicts hemodilution as a mechanism. Hemodilution can have important clinical effects during pregnancy. For example, pregnancy can affect the pharmacokinetics of anti-epileptic drugs at all levels, absorption, distribution, metabolism and elimination, resulting in declining plasma concentrations and hence decreased clinical effect (Wegner et al. 2010, Sabers and Tomson 2009).

Platelet counts can be decreased in pregnancy due to hemodilution, increased platelet consumption and increased platelet aggregation. However, only 7-8% of pregnant women have significant thrombocytopenia during pregnancy (Burrows and Kelton 1990). Given that almost all serum BDNF originates from platelets (Karege et al 2005, Fujimura et al. 2002), decreased platelet counts may have accounted for the results of this study. This was not the case for the Lommatsch study who found that platelets were not significantly correlated with BDNF levels (Lommatzsch et al. 2006). In future studies, platelet counts should be obtained.

Menstrual cycle phase does affect serum BDNF levels. The 3 studies that have examined this have all found that follicular phase levels are lower than luteal phase levels suggesting an effect of hormone fluctuation on BDNF (Lommatzsch et al. 2005, Pluchino et al.

2009, Begliuomini et al. 2007). None of these studies looked at serum levels, all looked at plasma or platelet levels. Given that we looked at follicular phase subjects, our differences may have been greater than if we had looked at luteal phase subjects. We chose the follicular phase because its hormone profile is most different from the pregnant hormone profile. Even so, the large difference we found between pregnant and non-pregnant women is likely significant.

Limitations of our study include the small sample size and the lack of hormonal validation of menstrual cycle phase. In addition, we were unable to control for demographic differences such as psychosocial supports that may impact psychological well-being and have an effect on BDNF levels. However, since the study was powered to detect only large differences between groups, the difference in BDNF levels between groups is likely a finding that can be replicated. The significance of low serum BDNF during pregnancy should be investigated. It may be for many women and their fetuses that a low BDNF state is compatible with normal mood or development. But in a subset of vulnerable women, it may set up an environment that puts women and their babies at increased risk for affective or neurodevelopmental disturbances. In addition, BDNF could be a candidate biomarker for adverse obstetrical outcomes given the studies which demonstrate that BDNF plays an essential role during pregnancy for healthy embryo implantation and fetal maturation. BDNF serum levels are highly variable depending on the study used given that different collection and detection methods are used. It is important that our data replicates data that already exists but further longitudinal study of pregnant and postpartum women would be beneficial to the field.

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