

ABSTRACT

CONTEXT: Elevated hypothalamic CRH has been implicated in melancholic major depression in non-pregnant individuals, but the role of placental CRH in maternal prenatal depression is largely unexplored.

OBJECTIVE: The objective of the study was to examine the association of maternal mid-pregnancy plasma CRH levels with prenatal depression and the possible correlation between mid-pregnancy depression and the way of delivery (caesarian section or vaginal delivery).

MATERIAL AND METHODS

-SUBJECTS: The patients of our study were collected during the last 2 years from the outpatient department of Obstetrics and Gynecology of the Hospitals G.H.Nikaia Pireaus-“Ag.Panteleimon”, G.H.N.Ionia-“Konstantopoulio” and G.H.Kalamatas. We included 65 pregnant women at the gestational age of 26-29 weeks. The gestational age was assessed by determination of menstrual dates, and from the ultrasound scan at the 1st and 2nd trimester of pregnancy.

Exclusion criteria were ,multiply pregnancy, preeclampsia, pre-existing hypertension, diabetes mellitus Type I, drug abuse, chronic diseases, premature rupture of fetal membranes, women who received tocolytic and cortisone therapy, previous preterm labour, congenital fetal anomalies, hydramnio ,oligohydramnio ,intrauterine growth restriction, women with clinical sings of infection and age under 18 years.

The 65 pregnant women who fulfilled the criteria of admission for participation were delivered either with normal vaginal delivery(1st group) or with elective caesarean section after 37 weeks of pregnancy(2nd group). All the vaginal deliveries were without pain relief, and the caesarean section was with epidural analgesia.

-PROTOCOL: It was conducted in two phases. During the first phase, pregnant women between 26-29 weeks of pregnancy participated and they were called to response in 3 questionnaire:

- ***EPDS (Edinburg Postnatal Depression Scale)*** The EPDS has been validated as a screening instrument for postnatal depression in numerous communities over the last 20 years. Cox and colleagues developed the EPDS in 1987 (Cox et al, 1987). This screening instrument is a 10-item, easy to complete, self-rating scale that was constructed especially for postnatal women. Items on vegetative symptoms were not included since these can also be attributed to

the physiological changes during puerperium (Cox and Holden,2003). The cut-off point of the original scale validated in a British population and is 12/13. However, Leonardou, Zervas and et al validated a new cut-off point for this scale in a Greek population which is 11/12. (44)

- **Athens Insomnia Scale** The AIS is a self-assessment psychometric instrument designed for quantifying sleep difficulty based on the ICD-10 criteria. It consists of eight items: the first five pertain to sleep induction, awakenings during the night, final awakening, total sleep duration, and sleep quality; while the last three refer to well-being, functioning capacity, and sleepiness during the day. The cut-off point of this scale is validated in number 6. Score over this cut-off means that there is problem of insomnia. (24)
- **Rosenberg Self-Esteem Scale (RSES)** The purpose of the 10 item RSE scale is to measure self-esteem. Originally the measure was designed to measure the self-esteem of high school students. However, since its development, the scale has been used with a variety of groups including adults, with norms available for many of those groups. To score the items, assign a value to each of the 10 items as follows:
 - For items 1,2,4,6,7: Strongly Agree=3, Agree=2, Disagree=1, and Strongly Disagree=0.
 - For items 3,5,8,9,10 (which are reversed in valence): Strongly Agree=0, Agree=1, Disagree=2, and Strongly Disagree=3.The cut-off we used is 15, which means score lower than this, has to do with low self esteem of the pregnant woman.

A blood collection for hormones determination (ACTH-CRH-CORTISOLE) was accomplished simultaneously with the completion of the questionnaire.

METHODS: The pregnant women (gestational age of 26-29 week) were recruited for the monthly follow-up at the outpatient departments of Obstetrics and Gynecology of the Hospitals. A written and spoken informed consent was obtained from all the patients. A general questionnaire with regard personal informations (parity, age,family condition, week of pregnancy, L.M.D, u/s of 2nd trimester , drugs administration ,or administration of cortisone, no previous history of mental or systemic disorders, educational level, consumption of alcohol or cigarettes) was given for completion to the pregnant women who fulfilled the above mentioned criteria ensuring the secrecy of these data. Following this they completed 3 questionnaires, a screening scale of depression, one for the qualitative of the night sleep and the Rosenberg Self Esteem Scale. A blood collection for hormones determination (ACTH-CRH-CORTISOLE) was also accomplished. All participants completed the EPDS scale, again, when they came to the clinic to deliver their baby.

COLLECTION AND PROCEDURES OF BLOOD SAMPLES.

We measured CRH, CORT, and ACTH levels in an antecubital venous blood sample from the mother collected using a Vacutainer system (Becton Dickinson, Meylan, France) into two chilled tubes of 3 ml with EDTA monovette (Sartedt, Nombrecht, Germany) and another blood sample in a chilled tube of 4 ml without anticoagulant, but with gel of segregation. One tube with EDTA was centrifuged at 3000 rpm for 10 minutes in refrigerated centrifuge (4°C), and the collected plasma was kept in eppendorf tubes at -80°C. The blood samples of the second tube were transferred to a centrifuge tube containing aprotinin (0.6 TIU/ml blood) to inactivated proteases. The samples were centrifuged at 1600 rpm for 15 min at 4°C. The plasma was transferred into test tubes, diluted with 2 ml buffer A (1% trifluoroacetic acid, TFA), and centrifuged for 5 min at 1200rpm at 4°C. Plasma samples were stored at -80°C. Before the determination, was added 500 µl of second buffer (60% acetonitrile in 1% TFA) for the isolation of the CRH.

DETERMINATION OF CRH, CORTISOL ACTH

The samples were stored at -80°C. The concentration of CRH was measured in the second tube, using the radioimmunoassay method (RIA) of DRG Instruments GmbH (Marburg, Germany). The assay is based upon the competition of ¹²⁵I peptide and peptide (either standard or unknown) binding to the limited quantity of antibodies specific for peptide in each reaction mixture. ACTH and CORTISOL were measured by using a commercial enzyme immunoassay (DPC Biermann GmbH, Bad Nauheim, Germany) on an automated hormone analyzer <Immulite 2000>.

RESULTS: No correlation between mid-pregnancy depression (score EPDS > 12) and the way of delivery (caesarian section or vaginal delivery) (**p-value 0,357 > 0,05**), neither with the maternal mid-pregnancy plasma CRH levels (**p-value 0,663 > 0,05**), was found in our study. Also, the result of EPDS was independent from the results of the Athens Insomnia Scale (**p-value 0,2013 > 0,05**) and Rosenberg Self-Esteem Scale (**p-value 0,334 > 0,05**). However, mid-pregnancy levels of ACTH (**p-value 0,023 < 0,05**) and Cortisole (**p-value 0,029 < 0,05**) are associated with EPDS > 12. Moreover, it seems that women who complete their pregnancy with vaginal delivery have higher mid-pregnancy levels of ACTH (**p-value 0,001 < 0,005**), Cortisole (**p-value 0,011 < 0,05**) and CRH (**p-value 0,001 < 0,005**) than those who deliver by elective cesarean section. Finally, it is remarkable that **54%** (35/65) of women have had mid-pregnancy score of EPDS > 12 (range 13-17) while before delivery **94%** (61/65). As the result of the above it seems that prenatal depression is a phenomenon which concerns many pregnant women and has a strong correlation with stress biochemical markers.

ΠΕΡΙΛΗΨΗ

ΣΚΟΠΟΣ: Σκοπός της εργασίας είναι η διερεύνηση της σχέσης των επιπέδων της CRH στη μέση της εγκυμοσύνης με την κατάθλιψη της εγκυμοσύνης, καθώς και την πιθανή συσχέτιση μεταξύ της τελευταίας και του τρόπου περάτωσης τοκετού.

ΥΛΙΚΟ ΚΑΙ ΜΕΘΟΔΟΣ: Το δείγμα γυναικών που συμπεριλαμβάνει η παρούσα μελέτη προήλθε από τα Τακτικά Εξωτερικά Ιατρεία των δημόσιων μαιευτικών κλινικών των Γ.Ν.Νίκαιας-Πειραιά, Γ.Ν.Ν.Ιωνίας-Κωνσταντοπούλειο και Γ.Ν.Καλαμάτας τα τελευταία δύο χρόνια. Το δείγμα αποτελούν 65 γυναίκες που διανύουν την 26η με 29η εβδομάδα κύησης. Κριτήρια αποκλεισμού αποτελούν η χρήση φαρμάκων, χρόνια νοσήματα, κύηση υψηλού κινδύνου, λήψη τοκολυτικών σκευασμάτων ή κορτιζόνης και η ηλικία της μητέρας κάτω των 18 ετών. Συνολικά 35 εξ' αυτών γέννησαν με φυσιολογικό τοκετό (1η ομάδα) χωρίς αναλγησία ή ψυχοπροφύλαξη και 30 με προγραμματισμένη καισαρική τομή υπό επισκληρίδιο αναλγησία (2η ομάδα). Η έγγραφη συγκατάθεση όλων των συμμετεχόντων ελήφθει κατόπιν ενημέρωσης τους σχετικά με το αντικείμενο της έρευνας καθώς και την ακολουθούμενη μεθοδολογία. Κάθε έγκυος συμπλήρωσε τα ερωτηματολόγια τριών ψυχομετρικών κλιμάκων :

- **EPDS (Edinburg Postnatal Depression Scale)**
- **Athens Insomnia Scale**
- **Rosenberg Self-Esteem Scale (RSES)**

Επίσης, υπεβλήθει σε αιμοληψία προκειμένου να προσδιοριστούν τα επίπεδα τριών ορμονών του stress (ACTH-CRH-CORTISOLE). Κάθε γυναίκα συμπλήρωσε εκ νέου το ερωτηματολόγιο της EPDS κλίμακας προσερχόμενη στο μαιευτήριο για να γεννήσει.

ΑΠΟΤΕΛΕΣΜΑΤΑ: Καμία συσχέτιση δεν αποκαλύφθηκε μεταξύ κατάθλιψης εγκυμοσύνης ($EPDS > 12$) και του είδους τοκετού ($p\text{-value } 0,357 > 0,05$) ούτε και με τη συγκέντρωση της CRH στην κυκλοφορία της μητέρας στη μέση της εγκυμοσύνης ($p\text{-value } 0,663 > 0,05$). Επιπλέον, το αποτέλεσμα της EPDS είναι ανεξάρτητο από τα αποτελέσματα των Athens Insomnia Scale ($p\text{-value } 0,2013 > 0,05$) και Rosenberg Self-Esteem Scale ($p\text{-value } 0,334 > 0,05$). Αντιθέτως, υψηλά επίπεδα των ACTH ($p\text{-value } 0,023 < 0,05$) και Κορτιζόλης ($p\text{-value } 0,029 < 0,05$) ορού συσχετίζονται με υψηλή βαθμολογία στην EPDS (> 12). Επίσης, φάνηκε ότι οι γυναίκες που ολοκλήρωσαν την εγκυμοσύνη τους με φυσιολογικό τοκετό έχουν υψηλότερα επίπεδα των ACTH ($p\text{-value } 0,001 < 0,005$), CRH ($p\text{-value } 0,001 < 0,005$), Κορτιζόλη ($p\text{-value } 0,011 < 0,05$) ορού στη μέση της κύησης σε σχέση με εκείνες που γεννούν με προγραμματισμένη καισαρική τομή. Τέλος, αξίζει να σημειωθεί ότι το **54%** των γυναικών είχαν βαθμολογία της EPDS > 12 (range 13-17) στη μέση της εγκυμοσύνης, ενώ στο τέλος αυτής, αντίστοιχη βαθμολογία που παραπέμπει σαφώς σε καταθλιπτική συμπτωματολογία, είχαν το **94%**. Από τα ανωτέρω συμπεραίνουμε ότι η κατάθλιψη κύησης είναι ένα φαινόμενο που αφορά ένα μεγάλο μέρος των εγκύων, ενώ εμφανίζει ισχυρή συσχέτιση με τους βιοχημικούς δείκτες του stress.