EVALUATION OF CORTICOTROPIN RELEASING HORMONE (CRH) IN PREECLAMPSIA

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ABSTRACT

Background: CRH level is increased during normal pregnancy as it is released from the placenta. This implies a definite role of CRH in pregnancy or labor. CRH is a hormone of stress and as stress has been implicated in the basic pathophysiology of preeclampsia, a greater rise of CRH is expected in this condition.

Objectives: To evaluate the level of CRH in normal pregnancy and compare it with pregnancies complicated with preeclampsia.

Study design: This case controlled prospective study was done in the department of physiology and clinical lab of Isra University Hyderabad. Forty three diagnosed cases of preeclampsia were selected in their 31-34 weeks of gestation. Forty three age and gestational matched controls were included who fulfilled the inclusion criteria. Blood sample was taken and level of CRH was determined by Elisa method by specific kit.

Results: Mean CRH value in cases of preeclampsia at 31-34 weeks of gestation was 60.33 + 10.47 ng/ml. The mean CRH value in age and gestational age matched controls was 50.03 + 09.33 ng/ml. The mean CRH value was significantly higher in preeclampsia patients as compared to the controls ($p < .001$).

Conclusion: It is concluded that CRH levels are significantly raised in preeclampsia as compared to the normal pregnancy. This indicates a possible role of stress in preeclampsia.

Keywords: Preeclampsia, CRH, ELIZA

INTRODUCTION

Corticotropin-releasing hormone (CRH) is a peptide with 41 amino acid residues that has been localized to paraventricular nucleus of the hypothalamus. In primates, CRH is also produced by placenta. It is secreted in both fetal and maternal circulations. In the second half of pregnancy, there is exponential rise in maternal CRH blood level. Unlike hypothalamic CRH, which is inhibited by cortisol, rising levels of cortisol secreted by fetal adrenal glands stimulate placental CRH[1].

In normal men and women, the concentration of CRH in peripheral blood is very low and barely detectable by immunoassay. During human pregnancy, placental CRH expression increases as much as 100 times in the final 6-8 weeks of gestation[2].

The most consistent fact about CRH detected in pregnant women is its link to both potential maternal-fetal distress, and greater metabolic and physiological demands. CRH and cortisol are increased following bacterial infectious diseases, pre-eclampsia, diabetes mellitus, growth retarded fetal development, multiple gestation, and psychological stress[3].

In the mammalians adaptive response to stress, the hypothalamus plays a central role, predominantly via CRH, which regulates the secretion of ACTH from pituitary gland[4].

Pre-eclampsia is a multi-system disorder specific to pregnant women. It is major cause of fetal and maternal mortality. The pathogenesis of this condition is not fully understood but much evidence suggests that one of the major underlying pathological changes occur in the placental bed. There is failure of or incomplete trophoblastic invasion of spiral arteries resulting in placental ischemia[5].

Significantly elevated maternal CRH concentrations were demonstrated in subjects suffering from preeclampsia in the third trimester of pregnancy. This elevation of maternal CRH concentrations could be detected as early as in the mid-trimester before clinical signs of the disease have appeared. When used

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for prediction of pre-eclampsia, CRH concentrations alone in the mid-trimester has no strong predictive value[5].

Various theories regarding pathophysiology of preeclampsia proposed an imbalance between;

1. Oxidant & antioxidant capacity [6,7, 8]
2. Vasoconstrictors & vasodilators[7, 9,10]
3. Coagulants & anticoagulants [7, 8]

Since no such study has been reported from our country and preeclampsia is common, so we designed this study. The objectives were to estimate the level of CRH in pregnant women so as to have base line values in our society. Then compare these values with the pregnant women having preeclampsia, to support the stress hypothesis for preeclampsia pathophysiology.

MATERIALS AND METHODS

This study was conducted in the department of physiology, Isra University Hyderabad clinical laboratory in collaboration with Liaquat University of Medical and Health Sciences Hospital Hyderabad, and Countess of Dufferin Fund hospital Hyderabad. The duration of study was six months.

Eighty six subjects, who fulfilled the inclusion criteria, were selected for the study. The subjects were classified into two groups:

Group A (patients):

Forty three pregnant women, diagnosed cases of Preeclampsia were recruited for this study in their 3rd trimester. The criteria for diagnosis of Preeclampsia were:

1. Hypertension (systolic blood pressure greater than 140mm Hg, together with either a single diastolic blood pressure reading of at least 110mm Hg or consecutive readings of at least 90mm Hg on more than one occasion at least 4h apart).

2. Proteinuria greater than 0.3g/day or 2+ on dipstick testing in two clean-catch mid-stream urine specimens collected at least 6 hour apart after 20th week of pregnancy.

Group B (controls):

Forty three healthy women, with apparent normal pregnancy and no preexisting medical disease or antenatal complications were recruited from the antenatal clinics during the 3rd trimester.

Inclusion Criteria

Pregnant women in their 3rd trimester with gestational age of about 31-34 weeks

Pregnant women diagnosed as cases of Preeclampsia having gestational age of about 31-33 weeks. Women having singleton intrauterine pregnancy. All ages inclusive.

Exclusion Criteria

Women with following conditions/ diseases will be excluded from this study;

Twin or multiple pregnancies, chronic hypertension, chronic heart disease, having renal disease, endocrine disorders, using steroids, severe mental health problems, history of fetal congenital or chromosomal anomalies, smokers, and abnormalities of uterus and cervix.

Method of data collection:

Personal information of the subjects:

Personal information of every individual was recorded on a specifically designed questionnaire after obtaining the informed consent.

A detailed history of the subjects was taken with the help of a standard questionnaire developed for that purpose. This was followed by through clinical examination.

Gestational age was determined by;

1. Physical examination
2. Date of last menstrual period
3. Ultrasound data.

Laboratory data:

Laboratory investigations carried out included:

Plasma CRH level

Collection of blood sample and preparation of plasma:

5ml of blood was withdrawn from the antecubital vein under aseptic condition.

After collection of sample, blood was transferred to a bottle containing EDTA (Anticoagulant) in a concentration of 1.5 mg per

ml. blood and mixed gently with the anticoagulant.

Plasma was obtained by centrifugation at 3000 rpm for 5 minutes.

Plasma was then frozen at - 200C for batch wise analysis of CRH. Each sample was labeled with specific identification number of subjects.

Estimation of plasma CRH level:

Level of plasma CRH was estimated by enzyme immunoassay by commercially available kit EIA-1631 manufactured by DRG International Inc., USA and provided by Global Marketing Services Rawalpindi. It was performed on Diamet ELIZA plate reader machine.

Principle of enzyme immunoassay with this kit:

Wells were coated with secondary antibody.

Secondary antibody can bind with Fc fragment of primary antibody (Anti-CRH-antibody)

Fab part of primary antibody binds with two chemicals; Biotinylated peptide and CRH peptide (sample antigen)

The biotinylated peptide is able to interact with streptavidin-horseradish peroxidase (SA-HRP)-

SA-HRP catalyzes the substrate solution composed of 3, 3′, 5, 5′- tetramethylbenzidine (TMB) and hydrogen peroxidase to produce a blue colored solution.

The enzyme-substrate reaction is stopped by hydrogen chloride (HCL) and the solution turns to yellow.

The intensity of the yellow is directly proportional to the amount of biotinylated peptide-SA-HRP complex but inversely proportional to the amount of the peptide in standard solutions or samples. This is due to the competitive binding of the biotinylated peptide and the peptide in standard solutions or samples to the peptide antibody (primary antibody) - i.e. if more biotinylated peptide binds with primary antibody, more enzyme activated, more reaction, more color. When biotinylated peptide binds more with primary antibody, sample antibody will have fewer sites to bind.

Procedure:

20X assay buffer- diluted with 950 ml of distilled water. Used to reconstitute all of the other compounds in this kit and the extract of plasma sample.

From standard peptide- Standard solutions with strengths of 100ng/ml-10ng/ml-1ng/ml-0.1ng/ml and 0.01ng/ml were made.

Primary antiserum, biotinylated peptide, and positive control rehydrated and prepared

Immunoplate taken and wells were filled.

1. Wells A-1 and A-2 - left empty as Blank.

2.1X assay buffer-added into wills B-1 and B-2 as Total Binding.

3.50 µl of the prepared peptide standards-added into the wells from C-1 to C-1 to G-1 to G-2 respectively.

4.50 µl of rehydrated positive control-added into wells H-1 and H-1.

5.50 µl of prepared samples - added into their designated wells.

6. 25 µl rehydrated primary antiserum - added into each well except the Blank well.

7.25 µl rehydrated biotinylated peptide-added into each well except the Blank well.

Immunoplate sealed and incubated-contents of wells discarded –wells washed with IX assay buffer-blot dried.

100 µl SA-HRP solutions- added into each well.

Immunoplate sealed and incubated-contents of wells discarded –wells washed with IX assay buffer-blot dried.

100 µl of TMB substrate solution-added into each well.

Incubated for 1 hour

100 µl 2N HCI -added into each well to stop the reaction.

The color in the well changed from blue to yellow.

Immunoplate was loaded onto a Microtiter Plate Reader.

Absorbance O.D. was read at 450 nm.

Statistical analysis:

The data was analyzed using SPSS version 16.0. Numerical parameters are presented as Mean + Standard Deviation and student t test was applied to compare the means (2 tailed) among the groups (Pre-eclampsia and control).

A P value <0.05 was considered as statistically significant level for all comparisons.

RESULTS

Results are summarized in the table.

The mean age of preeclampsia group was 23.84 + 3.87 years and that of control group was 23.88 + 4.01 years. There was no significant difference in the mean age of two groups.

The mean gravida of preeclampsia group was 2.09 + 0.92 and that of control group was 2.00 + 0.90. There was no significant difference in the mean gravida of two groups.

Comparison of mean blood pressure and CRH values between controls and preeclampsia patients is summarized in table-1. The mean systolic pressure of eclampsia group was 150.12 + 7.67 mmHg and that of control group was 122.79 + 9.08 mmHg. There was significant difference between the values of two groups.

The mean diastolic pressure of eclampsia group was 98.49 + 7.03 mmHg and that of control group was 80.23 + 6.23 mmHg. There was significant difference between the values of two groups.

The mean plasma CRH value in preeclampsia group was 60.33 + 10.47 ng/ml and that in control group was 50.03 + 9.33 ng/ml. There was significant difference between the values of two groups.

DISCUSSION:

CRH is a peptide hormone released from hypothalamus during stress. Its levels are undetectable in men and non-pregnant women. It levels increase tremendously during pregnancy, especially in the last trimester[2].

This signifies that it has a definite role in pregnancy/ labor.

Preeclampsia is a disorder of pregnancy characterized by hypertension and proteinuria[11]. Its exact mechanism is not known but all theories regarding its pathogenesis finally conclude that the basic abnormality is stress that in turn leads to other processes[6-9,12,13]

So, we combined these two together and evaluated the CRH values in cases of preeclampsia and compared these with those of normal pregnancy. We are not able to find any such study in our country.

In the present study we measured the level of plasma CRH at 31-33 weeks of gestation in normal pregnant women and pregnant women having preeclampsia.

In the present study CRH values were found to be significantly higher in patients with preeclampsia as compared to normal pregnant women. These findings are in agreement with several other studies[5, 14-17].

Preeclampsia being a condition of stress is associated with high CRH levels.

In the mammalians adaptive response to stress, the hypothalamus plays a central role, predominantly via CRH[4].

In the presence of hypo perfusion of the feto-placental unit as in the case of preeclampsia, the elevation of CRH concentrations could be a protective response to placental ischemia[5].

Therefore despite of difference in socio-economic conditions, nutritional status, types of stressess, genetic and environmental factors of our women from those of western countries, results of present study are in agreement with most of the studies done abroad.

CONCLUSION

Plasma CRH is increased during pregnancy as normally it is not detectable. This rise is significantly higher in pregnant women having preeclampsia. This signifies stress as one of the postulated mechanism for pathophysiology of preeclampsia.

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Table-1 Comparison of mean blood pressure and mean plasma CRH values between group A and B n = 86

Variable	**Patients n = 43**	**Control n = 43**	**P value**
Blood pressure (systolic) mm/Hg	150.12 $\pm$ 7.67	122.79 $\pm$ 9.08	< 0.001†
Blood pressure (Diastolic) mm/Hg	98.49 $\pm$ 7.03	80.23 $\pm$ 6.23	< 0.001†
Corticotropin-Releasing Hormone (CRH) ng/ml	60.33 $\pm$ 10.47	50.03 $\pm$ 9.33	< 0.001†

Results are presented as Mean $\pm$ Standard Deviation

† *P* value is statistically highly significant