Estimation of Cystine Aminopeptidase in Pregnancy Induced Hypertension

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ABSTRACT

Introduction: During pregnancy aminopeptidase degrade oxytocin is present in the maternal serum. The present study was under taken to determine the levels of cystine aminopeptidase in Pregnant mothers who were normotensive and pregnancy complicated by Gestational Hypertension.

Material and methods: Total number of cases studied are 60 pregnant women were selected from Antenatal clinic, 30 women were controls and other 30 cases. Data evaluated from 30 Patients suffering from PIH defined as Blood Pressure Levels in excess of 140/90 MM of Hg. We excluded Diabetes Mellitus, Patients who developed PIH. cystine or cystyl-aminopeptidase activities is determined spectrophotometrically by Tuppy and Nesvadba.

Results: 13 patients had Blood Pressure of 140/90 - 160/100 between these ranges and 3 patients had proteinurea and 3 patients had Oedema. Again, gestational HTN Group 10 Patients had BP ranging from 166/100 to 170/110and among these cases 5 patients had protenuria and 7 patients had Oedema. 7 Patients had EP ranging from more than 180/110 that is severe with 7 Patients having Proteinuria and Oedema in 5 Patients. The mean values of CAP activity 40U/L and standard deviation in the Normal Pregnant Patient from 28-40 Weeks of Gestation It was noticed that level of CAP in mild Toxaemias was near to normal, Where as in severe gestational HTN the level of CAP was below the level of Normal Control.

Conclusions: Study suggest that the enzyme is useful as an index of placental function, particularly in moderate and severe pre clampsia, postmaturity and in cases of IUGR.

Keywords: Gestational Hypertension, Cystine aminopeptidase, Proteinuria.

INTRODUCTION

There is an evidence that the serum of pregnant women inactivates oxytocin and the inactivating enzyme was named oxytocinase and is also referred to as cystine or cystyl-aminopeptidase.[1] During pregnancy changes in serum CAP activity was highest at day 13 of pregnancy and lowest at day 17. The activity in the male serum was significantly low. Although the CAP level was high in the maternal placenta, levels were also high in the fetal placenta, uterus and liver as compared with the serum, suggesting CAP synthesis in these tissues.[2]

The confirmation of clinical suspicion of placental insufficiency by reliable lab techniques would enable the obstetrician to decide the best time for delivery in a number of abnormal conditions. For this purpose the measurement of Urinary Oxytriol excretion has been used but the value of this method is limited because of the range of normal values and great fluctuations often found in serial estimations. Hence it is desirable therefore those alternative laboratory indices of placental function should be investigated. Study of the placental enzymes in maternal serum, appears to be one of the more promising method. The placenta contains a complex system of enzymes the majority of which are present in the maternal serum.

Enzyme studies in Maternal serum during pregnancy indicate that the levels of Glutamic and cycloacetic transaminase, lactic dehydrogenase, Alkaline Phosphatase show a progressive rise as Pregnancy advances. while the levels of other enzymes such as cholinestease and tributyrinase decrease during Pregnancy. Since many of these enzymes are produced by tissues other than the Placenta function.[3] In contrast an enzyme produced solely and largely by the Placenta might provide a useful measure of Placental function Cystine aminopeptidase "Oxitocinase" is such an enzyme. It is higher levels have been found in patients approaching term than in patients during early pregnancy shows the placental function

The present study was under taken to determine the levels of cystine aminapeptidase in pregnant mothers who were normotensive and pregnancy complicated by Gestational Hypertension.

MATERIAL AND METHODS

Total number of cases studied were 60 pregnant women, selected from Antenatal clinic, in-patient wards of Princess Nilofer Hospital, G.M.H. and Sultan Bazar, Hospital attached to Osmania Medical College, from which 30 women were controls and other 30 were cases.

Controls: B.P. Less than 140/90; No proteinuria, No family history of H.T.N. (Hypertension) No disparity in height of the Utrerus and period of Amenorrhoea.

Cases: BP greater than 140/90 with or without proteinuria

Data evaluated from 30 Patients suffering from PIH defined as Blood Pressure Levels in excess of 140/90 MM of Hg. We excluded Diabetes Mellitus, Patients who developed PIH. Cystine or cystyl-aminopeptidase activities was determined spectrophotometrically by Tuppy and Nesvadba. For collection of sample we used only Haemoglobin free pregnant serum (24th week to 40th week of Pregnancy).

STATISTICAL ANALYSIS

Tables and graphs were made with the help of Microsoft office 2007. Descriptive statistics like mean and percentages were

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used to infer results.

RESULTS

Mean values ie activity of CAP increase as gestational age increases To avoid confusion with overlapping lines, the CAP activity of the Normal Pregnant women is shown in the graph. In controls there was progressive increase in the CAP activity with occasional transient decreases. Occasional decrease in CAP levels were associated with Braxton Hicks Contractions. However this was a inconsistent correlation.

The mean values of CAP activity 40U/L and standard deviation in the Normal Pregnant Patient from 28-40 Weeks of Gestation is given in Figure-1. There was the progressive rise in CAP levels with advancing period of Gestation. Mean values of CAP Levels in IU/L and standard deviation in gestational HTN complicated pregnancy. It was seen the mean serum CAP values in gestational HTN of pregnancy were compared to the mean values of Normal pregnant to the corresponding Gestational periods. Occasional high level of CAP were noted in 2 patients which could be false negative. IUGR was noted in few patients with very low levels of CAP. 13 patients had Blood Pressure of 140/90 - 160/100 between these ranges and 3 patients had proteinurea and 3 patients had Oedema.

Again, gestational HTN Group 10 Patients had BP ranging from 166/100 to 170/110 and among these cases 5 patients had protenuria and 7 patients had Oedema. 7 Patients had EP ranging from more than 180/110 that was severe with 7 Patients having Proteinuria and Oedema in 5 Patients. It was noticed that level of CAP in mild toxaemias was near to normal, where as in severe gestational HTN the level of CAP was below the level of Normal Control.

DISCUSSION

The spectroflorometric method for the determination of cystine aminopeptidase is based on a zero-order reaction and the progress curve is linear over the required range of measurements. For this reason and because the method is simpler, quicker and much sensitive than other methods. We have adopted it for routine assay of the enzyme. It is possible for one technician to perform 50 duplicate assays comfortably during a working day. Only a small amount of serum is required and daily assays are feasible without difficulty either to the patient or to the laboratory. We are also attempting to develop an automated method on the other land. The total oestrogen method requires a 24 hours specimen of urine, with all the attendant inaccuracies. A "quick" method, suitable for a general laboratory providing a routine services to a large maternity unit, undoubtedly sacrifices some accuracy to speed.

The cystine aminopeptidase curves which we have obtained, BP in normal Pregnancy by spectrofluorimetric determination show a progressive rise, particularly in the last trimester. The shape of the curves found by this method are in agreement with those found by other workers using biological, chemical and spectrophotometric methods. In contrast, the total oestrogen curves fluctuate considerably in spite of the fact that these were normal patients.[4] These fluctuations make interpretation during pregnancy difficult and can be misleading.

An ideal biochemical test not only reflects the clinical state but is one in which specimen collection is easy and reliable, and the assay procedure is simple, quick and reproducible. The assay of serum cystine aminopeptidase fulfill these criteria.

Significant measurable serum oxytocinase activity after 3 months of storage at 4°C (refrigerator temperature) has been demonstrated. The present study could not find any statistically significant difference in the mean oxytocinase levels after 18

Gestation in Weeks	Number of Controls	Number of Cases
28-30	2	3
30-32	5	2
32-34	3	5
33-34	9	12
35-36	5	3
37-36	2	2
39-40	3	2
>40	1	1
Total	30	30

Table-1: Details of patients in study.

Cases with HTN	Range of BP	Proteinuria	Odema	IUGR
13	140/90-160/100	3	3	0
10	160/100-170/110	5	7	0
7	more than 180/110	7	5	1

Table-2: Range of Blood pressure and its associated clinical signs

Weeks of Gestation	Lower Limit of CAP (U/L)	Upper limit of CAP (U/L)	Mean CAP (U/L)
Controls			
28-30	13.173	44.099	22.049
30-32	-	-	23.088
32-34	13.926	111.408	47.348
33-34	88.198	90.713	89.455
35-36	23.21	208.89	85.942
37-38	17.568	206.89	85.064
39-40	17.444	98.444	95.161
>40	13.116	40.756	84.045
Cases			
28-30	11.852	34.815	24.839
30-32	10.223	13.099	11.946
32-34	12.703	62.667	27.428
33-34	15.630	150.865	31.011
35-36	13.161	102.124	42.974
37-38	14.556	89.999	45.087
39-40	18.344	31.223	34.444
40	20.889	30.173	11.852

Table-3: Range of CAP activity and Mean values

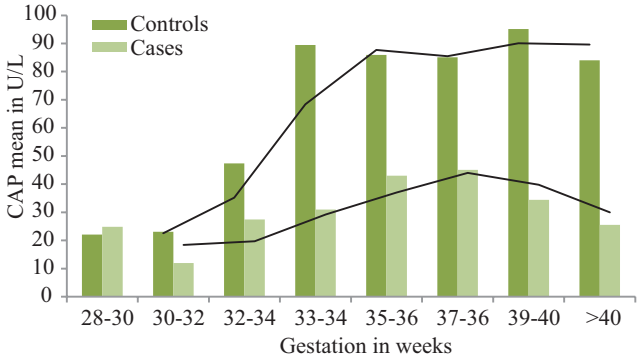

Figure-1: CAP mean values in controls and cases

days of storage between room temperature, 4°C and - 2°C. Serum oxytocinase, being very stable, therefore has good potential for use in areas where there is no constant electricity supply and such areas utilise a centralised laboratory service.

This study did not show any remarkable fluctuations in serial oxytocinase over 150 min in early, late second and third trimesters of pregnancies. It should have desirable to estimate the oxytocin levels simultaneously along with serial oxytocinase measurements. The lack of radioimmunoassay facility in this centre was the major constraint in this direction. Nevertheless', there is the suggestion that serum oxytocinase levels do not fluctuate dramatically over a short time interval and indeed is appreciably steady in the same subject within the study period. Furthermore, the mean coefficient of variation of only 3.6% implies a steady release of oxytocinase into the circulation rather than the occasional "Spurt", "episode" or "Pustule' release noted with oxytocin.[5] The steady oxytocinase level may owe as much to its slow removal from the circulation (half-life of 72h)[8] as to an even rate of input.

A gradual rise in Oxytocinase levels from early pregnancy towards term have been observed. However, no obvious relationships have been found between Oxytocinase aloe uterine contractions.[7,8,13] CAP values rise steadily during normal pregnancy. In normal pregnancy the correlation between CAP and birth weight at term is statistically significant (r=0.46. P 0.01). In an unselected group of complicated pregnancies there is no correlation between the final CAP result and birth weight. Beck F and James R et al suggested that most of the CAP in the maternal serum comes from the syncytiotrophoblast.[6] The relationship between the activity of the syncytiotrophoblast and placental function as a whole is not clear. Thompson et al, in reviewing the relationship of placental weight to birth weight, concluded that placental weight is a poor indicator of placental adequacy, and that placental insufficiency from small placental size is probably rare.

In most twin pregnancies CAP values are increased. The small-for-dates baby may be the result of poor placental function or genetic inheritance. In the present series there were two groups of small-for-dates pregnancies. One group with CAP values static or rising, and the other with CAP values falling. In the former group pregnancy was usually uncomplicated. In the later group it was always complicated by hypertension', which can cause placental insufficiency.[7]

Three of the four patients with intrauterine fetal deaths had hypertension. All three had falling CAP values. CAP values which continue to rise suggest normal placental function. Falling CAP values suggest placental failure with an increased risk to the fetus.

Placental Insufficiency is a clinical syndrome characterized by impaired fetal growth, resulting in infants whose birth weights, lengths, and head circumferences may be below average for a given well-documented gestational age. The term "insufficiency" implies that the placenta is unable to synthesize, or transport adequately the nutritional constituents necessary for normal growth of the fetus. The placenta is thought to be the source of elevated CAP activity in pregnancy, and histochemical studies locate the highest CAP activity in the syncytiotrophoblast.[8]

Serum cystine aminopetidase is present in small traces in blood of the non-pregnant female from other sources like but the concentration rises appreciably and progressively during pregnancy. The placenta is certainly the source of cystine aminopeptidase which appears in the blood of pregnant woman, and this enzyme originates from the syncytiotrophoblast. It is possible therefore that serum cystine aminopeptidase levels reflect placental function.[9,10]

The results presented in this study suggest that the enzyme is useful as an index of placental function, particularly in moderate and severe pre clamptic toxemia, postmaturity and in cases of IUGR.

Pregnancy serum oxytocinase activity is apparently due to a cystine aminopeptidase which can be measured by spectrophotometry. Serum cystine aminopeptidase activity was investigated in normal and toxaemic pregnancy.

The enzyme levels show a progressive rise throughout normal pregnancy reaching maximum levels in the later weeks. Decline is noticed with the onset of labour. Sera from pre-eclamptic patients have significantly lower enzyme levels.

Further work is necessary, however, to establish the clinical application of the method in determining the optimum time for delivery of the foetus, associated with placental insufficiency needed to be studied more closely.

CONCLUSION

Determination of Serum CAP levels is an easy, quick and accurate method for monitoring the well being of specially in high risk pregnancies. Serum CAP levels show a progressive rise with advance of Gestation in normal patient reaching maximum levels near term. Subnormal values of Serum CAP levels seen especially in last four weeks. The levels for severe pre-eclampsia at week 39 and 40 were lower than in normal pregnancy. With sub-normal but steadily increasing CAP levels the fetus is not at risk. Continuously decreasing Serum CAP Value/Sudden falls in level suggest immense risk/IUD of fetus.

REFERENCES

1. Nada Majkić-Singh, Ana Vuković, Slavica Spasić, Aleksander Ruzic, Marina Stojanov, Ivan Berkeś, Oxytocinase (CAP) activity in serum during normal pregnancy, Clinical Biochemistry. 1982;15:152-55.
2. T. Uete, H. Motokura, Y. Kitano, C. Fukutani, N. Uenishi, N. Ando, Comparison of Substrates for Measuring Cystyl-Amino Peptidase. Activity in Serum of Pregnancy and Hepatic Disease and in Various Tissues, Clinical Chemistry and Laboratory Medicine. 1981;19:3-6.
3. L. Chapman, R. Burrows-Peakin, V. P. Rege, E. Silk, Serum Cystine Aminopeptidase And The Normal Weight Baby In Normotensive And Hypertensive Pregnancy, BJOG: An International Journal of Obstetrics and Gynaecology. 1975;82:278-281.
4. Tsujimoto M, Mizutani S, Adachi H, Kimura M, Nakazato H, Tomoda Y. Identification of human placental leucine aminopeptidase as oxytocinase. Archives of Biochemistry and Biophysics.1992;292:388–392.
5. Mizutani S, Naruse K, Hattori A, Tsujimoto M, Kobayashi H. Physiological and pathophysiological roles of placental aminopeptidase in maternal sera: possible relation to preeclampsia and preterm delivery. Expert Opinion on Medical Diagnostics. 2009;3:479–49.
6. Beck F, Erler T, Russell A, James R. Expression of Cdx-2 in the mouse embryo and placenta: possible role in patterning of the extra-embryonic membranes. Dev Dyn.

1995;204:219–227.
7. H. Kleiner, M. Brouet-Yager, G. Graff, Serum L-Leucyl-Beta-Naphthylamide Hydrolase Activity In Normal Single And Twin Human Pregnancies And In Pregnancies Associated With Fetal Dysmaturity, BJOG: An International Journal of Obstetrics and Gynaecology. 1969;76:127-130.
8. Davies J, Glasser SR. Histological and fine structural observations on the placenta of the rat. Acta Anat Basel. 1968;69:542–608.
9. Davies J, Glasser SR. Histological and fine structural observations on the placenta of the rat. Acta Anat Basel. 1968;69:542–608.
10. Razia Sultana, Kamal Raj Singh, Vrunda Joshi. Study of oxidative stress in pregnancy and its association with pregnancy induced hypertension. International Journal of Contemporary Medical Research. 2016;3:946-948.

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