STUDY OF HUMAN CHORIONIC GONADOTROPHIN LEVEL IN AMNIOTIC FLUID BY IMMUNOCHEMICAL ASSAY

by

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There is no doubt that human chorionic gonadotrophin (HCG) is produced by the placenta, the evidence for this being well summarised by Cassmer (1959) and Diczfalusy (1960). It is difficult to determine which of the cells of the placenta are responsible for the production of chorionic gonadotrophin. Present view suggests that syncytiotrophoblast is the site of production or storage of HCG. HCG can be detected in the body fluid only when chorionic tissue is present, and direct relationship has been found between the amount of circulating hormone and mass of chorionic tissue. The amnion-chorion exerts a barrier effect against the raised concentration of HCG and isolates this hormone into three physiological compartments: maternal blood, amniotic fluid and foetal blood.

Concentration of HCG has been reported by many workers (Mishell *et al*, 1963; McCarthy and Pennington, 1964; Lauritzen and Lehman, 1965; Grumback *et al*, 1968; Faiman *et al*, 1968; Berle and Schultze-Mosgau, 1968 and Crosignani *et al*, 1972) in the past both in the maternal and cord sera but very few of them measured the level of this hormone simultaneously in the amniotic fluid.

The present study includes estimation of chorionic gonadotrophin hormone by immunochemical assay, both in maternal and cord serum, along with its level in the amniotic fluid sample. Mothers included in this series are from normal pregnancy in the second and third trimesters and also from hydramnios cases at term. Simultaneously, protein fractions were characterised in these samples by agar gel immuno-diffusion and immuno-electrophoresis techniques.

Methods

Immunochemical assay of HCG in the different samples was carried out by using Pregnosticon 'All-in', manufactured by Organon, Oss, Holland. This includes one glass ampoule containing freeze dried mixture of: (a) Antigen: Sheep's erythrocytes, specially pre-treated with human chorionic gonadotrophin (HCG), which serve as carrier for the antigen. (b) Antiserum: Prepared by immunising rabbits with purified human

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Received for publication on 18-6-1974.

chorionic gonadotrophin. (c) Buffer solution.

0.1 ml of serum or concentrated amniotic fluid and 0.4 ml distilled water were added to the freeze dried content of the ampoule. The ampoule was then shaken thoroughly for one minute and placed in the test rack. The mixture was left at room temperature exactly for 2 hours without any disturbance.

If a clearly defined compact red ring appears at the bottom of the glass ampoule after 2 hours, the test is positive. In negative result, there is absence of any ring formation with well dispersed agglutinated red cells at the bottom of the ampoule even after expiry of 2 hours.

In quantitative test, the last dilution of the dilution series in which clear ring formation occurs was taken as the quantity of HCG present in the sample under test. Thus, the concentration of HCG was calculated by the formula $X = 1500\ N$ per litre, in which N is the dilution and X, the minimum quantity of chorionic gonadotrophin present. The result of this study has been expressed as concentration of HCG per ml. of serum or amniotic fluid for the sake of convenience.

The above test is based on the principle that HCG in the sample blocks the antibody in the reagent and thus the agglutination reaction with the HCG coated erythrocytes is prevented. The non-agglutinated erythrocytes thus precipitate as a compact mass of red ring at the bottom of the glass ampoule in case of positive reaction. When HCG is absent in the sample the anti-HCG antibody present in the reagent reacts directly with the antigen coated red cells producing agglutination reaction. The agglutinated cells disperse diffusely at the bottom of the ampoule without producing a compact ring.

The protein fractions in the collected samples were determined by immuno-electrophoretic technique using Coomb's serum and the immuno-globulin fractions were characterised by agar-gel immuno-diffusion test using IgG, IgM, and IgA antisera supplied by Hyland, Div., Travenol Laboratories, Inc., Costa Mesa, Calif., U.S.A.

Material

The present study was carried out in 3 groups of pregnant mothers, their amniotic fluid specimens and cord blood of the foetuses. The mothers were grouped as follows:

I.	Mothers with normal pregnancy at term ..	40 cases
I.	Mothers in second trimester (18-24 wks) of normal pregnancy ..	20 cases
III.	Mothers with hydramnios at term ..	20 cases

The mean age of the mothers in group I was 29 with a range between 19 and 39 years. Out of 40 mothers in this group only 12 were primigravidae. The mean average age of the mothers in group II was 32 years with a range between 26 and 38. All the mothers in this group were multiparae. In group III, the ages varied between 18 and 25 years with a mean of 22. Except one, all the mothers were multiparae.

4 ml. of clotted blood was collected aseptically from all the mothers at the time of labour or hysterotomy operation. Similarly, 4 ml of clotted blood was collected from the placental side of the umbilical vein after severence of the cord. The serum samples were separated aseptically and stored between 2° and

80°C using 0.1 per cent sodium azide as preservative.

About 20 ml of amniotic fluid was collected by amniocentasis from term pregnancy cases at the time of labour, while in hysterotomy cases the product of conception was taken out completely with the intact amniotic sac. The amniotic fluid was then collected from the intact sac by aspiration with a sterile syringe and needle. Samples found to be contaminated with blood were discarded. As the collected amniotic fluid samples did not reveal presence of any HCG by Pregnosticon 'All-in' test as such, the test was carried out after concentrating the fluid about 10 times its original volume. In hydramnios cases, the fluid was concentrated even further, about 20 times, as 10 times concentration failed to give any positive result. The concentration of the fluid was carried out by dialysis against saturated solution of sucrose and stored between 2° and 8°C, using 0.1 per cent sodium azide. The actual concentration of HCG in the amniotic fluid has been reported as found in the original sample.

Results

It is evident from Table I that the mean concentration of HCG in Group I cases was 91.2 I.U. per ml, in maternal serum 0.63 I.U. per ml, in amniotic fluid and 5.85 I.U. per ml in cord serum.

In group II series the mean HCG concentrations in maternal sera, amniotic fluid samples and cord sera were 307.2 I.U., 4.2 I.U. and 81.6 I.U., per ml., respectively.

In group III, the mean concentration of HCG in maternal sera was 221.2 I.U. and in cord sera the concentration was found to be 57.6 I.U. per ml. only. The

TABLE I

HCG Concentration (I.U. per ml) in Mothers' Sera, Amniotic Fluid and Cord Sera in All the Groups

	Concentrations (mean ± S.E.)		
	Maternal serum (MS)	Amniotic fluid (AF)	Cord serum (CS)
Group I ..	91.2 ± 39.87	0.63 ± 0.3132	5.85 ± 2.417
Group II ..	307.2 ± 94.06	4.2 ± 1.47	81.6 ± 22.04
Group III ..	221.2 ± 91.45	Nil	57.6 ± 26.73
	Ratios (mean ± S.E.)		
	MS/AF	MS/CS	AF/CS
Group I ..	156 ± 47.15	15.6 ± 1.744	0.11 ± 0.04
Group II ..	80 ± 32.78	3.8 ± 0.6	0.05 ± 0.01
Group III ..	—	4.2 ± 0.23	—
	Correlations		
	MS/AF	MS/CS	AF/CS
Group I ..	P 4.243	P 12.11	P 4.39
Group II ..	P 0.824	P 4.256	P 1.78
Group III ..	—	P 4.176	—

amniotic fluid samples in this group did not reveal the presence of HCG, when tested by the same method used in the previous groups, even after concentrating 20 times.

Table I also shows the ratios of mean values of HCG concentrations in maternal serum, amniotic fluid and cord serum in different groups of pregnancy. Analysis of these ratios by 't' test showed the following:

Group I: The ratios between HCG concentration of MS/AF and AF/CS were found to be significant, while that of MS/CS was highly significant.

Group II: The ratios between HCG concentration of MS/AF and AF/CS were found to be insignificant, while that of MS/CS was highly significant.

Group III: As the amniotic fluid did not reveal any presence of HCG, its concentration ratio was calculated in MS/CS, which was found to be highly significant.

cord sera showed the presence of IgM only. The amniotic fluid samples in groups I & III revealed only IgA.

Discussion

Review of literature shows that human chorionic gonadotrophin (HCG) in pregnancy, although found at higher concentration in maternal serum, is also distributed to foetus and amniotic fluid at different concentrations. Amnion-chorion may exert a barrier effect in this differential distribution. The result of the present study shows the HCG concentration of maternal sera, amniotic fluids and cord sera as 91.2, 0.63 and 5.85 I.U. per ml., respectively in normal pregnancy at term (group I). There are few previous reports of HCG concentration in amniotic fluid. McCarthy and Pennington (1964) found a mean of 0.73 I.U. per ml. by immunological method and Crosignani *et al*, (1972) noticed a concentration of 0.380 I.U. per ml. at term in normal pregnancy, after determining by radioimmunoassay. Berie and Schultze-Mosgau (1969) reported a value of 0.4—0.7 I.U. per ml. using bioassay method. These differences in HCG concentration may be due to the difference in the methods of assay. However, the results of the present study show that ratios of HCG concentra-

TABLE II

Protein Fractions of Maternal and Cord Sera and Amniotic Fluid Samples From All Groups

	Protein fractions			
	Albumin	IgG	IgM	IgA
Mother's serum	+	+	+	+
Cord serum	+	+	+	—
Amniotic fluids				
(i) Second trimester (Grade II)	+	+	—	—
(ii) At term (Grade I)	+	+	—	+
(iii) Hydramnios cases (Grade III) ..	+	+	—	+

Table II shows the important protein fractions of maternal and cord sera as well as amniotic fluid specimens from the three groups of cases. Albumin and IgG were found constantly in all the samples in 3 groups of cases. Besides these two fractions, IgM and IgA were found in all the samples of maternal sera but the

tion of MS/AF and AF/CS are significant and that of MS/CS is highly significant.

Study of HCG concentration of amniotic fluid in the second trimester of normal pregnancy (group II) is a rare one. However, Mishell *et al*, (1963) found a mean value of 2.6 I.U. per ml. between 17th and 20th weeks of gestation. In comparison, the result of the present study shows a concentration of 4.2 I.U. per ml. in the amniotic fluid in these cases (between 18-24 wks). In comparison, the maternal and cord sera levels of HCG have been found to be 307.2 and 81.6 I.U. per ml., respectively. But the ratios between HCG concentration of MS/AF and AF/CS are found to be insignificant while that of MS/CS is highly significant.

Concentration of HCG in the amniotic fluid in hydramnios has never been reported in the past. In the present study of hydramnios cases (group III) HCG could not be detected by the same immuno-chemical method even after concentrating the fluid about 20 times, near double the concentration used in groups I & II amniotic fluid samples. However, further concentration of the fluid could have revealed the presence of this hormone. The absence of HCG in these highly concentrated fluid samples can only be explained by the fact that there is more production of diluted amniotic fluid with low S.G. in these cases. Although HCG was absent in the amniotic fluid in these cases its concentration has been found to be 221.2 and 57.6 I.U. per ml. in maternal and cord sera, respectively. These figures are found to be much higher than those from normal pregnancy at term (group I).

Thus, it is clear from the above study that in normal pregnancy the concentration of HCG is higher in the maternal and cord sera and also in the amniotic fluids in second trimester than those found at term. This finding differs from those of Berle and Schultze-Mosgau (1969), who noticed a peak in the concentration of HCG in the first trimester of pregnancy and maintenance of plateau thereafter until term. This difference may be explained partly due to difference in the method of assay.

The difference in concentration of HCG in the amniotic fluid in the second trimester and full term pregnancy in this study can be explained by the fact that in the second trimester of pregnancy the placental villi are moderately reduced in size and show complete disappearance of Langhans' cells with formation of prominent syncytial giant cells. In contrast, the placental villi at term pregnancy are smaller than those of second trimester and the syncytial cells are converted into a thin membrane dotted by giant cell formation. As the HCG is produced by the syncytiotrophoblast only the variation in concentration of this hormone in maternal and cord sera and also in the amniotic fluid, may be well explained by the histological changes in the placental villi at different stages of pregnancy.

In hydramnios cases at term, the HCG concentration of maternal and cord sera is much higher than that of normal pregnancy at term but lower than the level found in second trimester. As there is an increased area of chorionic villi (since placentae are larger than normal) the total syncytial cell mass is large and there is an increased production of HCG. The absence of HCG in the amniotic fluid in these cases is due to increased production of diluted fluid.

Simultaneous study of protein fractions in maternal and cord sera along with the amniotic fluid shows that albumin and

IgG are common in all these samples. IgM is constantly absent in all the amniotic samples and IgA cannot be detected in any sample of the cord sera. It is, therefore, rational to think that HCG is associated either with albumin or IgG fraction of protein. As the albumin particles are small, it is easily diffusible through chorion-amnion partition and if HCG is associated with albumin, the hormone should have been found in same concentration in all the compartments, viz. maternal serum, amniotic fluid and cord serum. It is, therefore, likely that HCG is associated with the IgG fraction of maternal serum, which diffuses differentially in these compartments due to large particle size of this fraction. This finding is similar to that of Crosignani *et al*, (1972), who found a positive correlation between maternal serum/amniotic fluid ratio for HCG and IgG as these two substances behaved similarly in all the 3 fluid compartments, i.e. maternal serum, amniotic fluid and cord serum.

Summary

Human chorionic gonadotrophin (HCG) levels in the amniotic fluid and maternal and cord blood were estimated by immuno-chemical method (Pregnosticon All-in) in second trimester and at term in normal pregnancy and hydramnios cases.

The HCG concentration in all the samples was found to be higher in second trimester of normal pregnancy than those found at term. Although in hydramnios cases the hormone could not be traced in the amniotic fluid samples, its concentration in maternal and cord blood was found to be in between the levels mentioned in the previous groups.

This differential distribution of HCG in the 3 fluid compartments, viz. meternal, cord blood and amniotic fluid is somehow related with transference of maternal IgG particles through the amnion-chorion barrier.

Acknowledgement

We offer our sincere thanks to Principal N. Goswami, Ph.D. and Professor C. C. Mitra, Deptt. of G & O, B. S. Medical College, Bankura, for their kind permission to work in the department and publish this report. We are also grateful to Shri Biren Bannerji, Lecturer, Sammilani College, Bankura, for his kind help in statistical analysis of the results.

References

1. Berle, P. and Schultze-Mosgau, H.: Archiv fur Gynäk. **207**: 460, 1969. & Acta Endocrinol. **58**: 339, 1968.
2. Cassmer, O.: Acta Endocrinol, **58**: Suppl., 45, 1959.
3. Crosignani, P. G., Nencioni, T. and Brambati, B.: Jn. of Obst. & Gynec. Brit. Cwlth., **79**: 122, 1972.
4. Diczfalusy, E.: Acta Endocrinol. **129**: Suppl. 50, 1960.
5. Faiman, C., Ryan, R. J., Zwirek, S. J., and Rubin, M. E.: Jn. of Clin. Endocrinol. **29**: 1323, 1968.
6. Grumbach, M. M., Kaplan, S. L., and Burr, I. M.: Annals of New York Academy of Science, **148**: 501, 1968.
7. Lauritzen, C., and Lehman, W. D.: Archiv. fur Gynäk. **200**: 578, 1965.
8. McCarthy, C., and Pennington, G. W.: Am. Jn. of Obst. & Gynec. **89**: 1069, 1964.
9. Mishell, D. R., Wide, L., and Gemzell, C.: Jn. of Clin. Endocrinol. **23**: 125, 1963.