AMNIOTIC FLUID TESTOSTERONE (T) IN MIDPREGNANCY IN DETERMINATION OF FETAL SEX

by

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Abstract

Amniotic fluid Testosterone (AF-T) levels were measured by R.I.A. on samples obtained between 15 to 18 weeks of pregnancy from 32 cases with male fetuses and 26 cases with female fetuses. The mean ± S.D. AF-T concentration for the male fetus pregnancies was (517.8 ± 483.5 pg/ml) which was significantly higher ($P < 0.001$) than that in pregnancies with female fetuses (173.8 ± 84.9 pg/ml).

Introduction

Normal development demands adequate hormonal action for some morphogenetic events such as sex differentiation and for ontogenesis or for full maturation of physiological function. Hormones have a multifarous role in a developing organ and participate actively in the characteristics of a developing system. For several species, convincing evidence has been presented that fetal testicular androgen and estrogen have important function in the normal differentiation of the reproductive ducts.

Little search has been made on the amniotic fluid (AF) Testosterone (T) concentration during pregnancy. Recent reports suggest its clinical importance in prenatal sex determination.

Younglai (1972) was the first to demonstrate that total 17 Betahydroxyandrogen, both conjugated and unconjugated in amniotic fluid at term are significantly higher in pregnancies carrying female fetuses, though he was not able to differentiate the sex with confidence in the majority of cases. Giles *et al* (1974) and Judd *et al* (1976) reported that AF-T levels are significantly higher in pregnancies with male fetuses than in those with female fetuses between 15 to 19 weeks of gestation.

Dawood and Saxena (1977) reported AF-T concentration in midtrimester pregnancies with significantly different values between male and female fetuses. They found AF-T concentration of 165.2 ± 154.0 pg/ml in case of male fetuses, whereas it was 27.6 ± 26 pg/ml in case of female fetuses ($p < 0.001$) with some overlap between the male and female fetus values. They found similar concentration range in AF-T in late pregnancies as compared to those found in early pregnancies.

However overlapping values were more in late pregnancies.

We report our work undertaken to establish the AF-T levels in normal preg-

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nant Indian Women and also to further investigate the corresponding reports as regards the utility of AF-T determination in predicting fetal sex. To the best of our knowledge, there has been no report from India on amniotic fluid testosterone.

Material and Methods

Fifty eight amniotic fluid samples were collected by transabdominal amniocentesis from women undergoing prenatal sex determination (P.S.D.) at P.S.D. Clinic of Sir H.N. Hospital in midtrimester of pregnancy.

The duration of pregnancy was calculated from the first date of last menstrual period and clinical examination.

Samples contaminated with blood were excluded from the study. All samples obtained were centrifuged and supernate preserved in deep freeze at 20°C until analysed. The cell pellet used for nuclear sexing by Barr body and Y-chromatin fluorescence count.

The sex of the fetuses were confirmed after termination of pregnancies or after full term delivery.

Amniotic fluid testosterone levels were estimated by R.I.A. using the double antibody method supplied by BIODATA (Switzerland). In this procedure the radioactive antigen-antibody complex is precipitated with the addition of antigammaglobulin serum at the end of incubation and is collected by centrifugation. The sensitivity of this method is of about 100 pg/ml.

Pure testosterone standard of 40 ng/ml was used with 5 serial 1:2 dilutions with assay buffer.

I^{125} labelled testosterone 3-TME used as the labelled hormone was prepared by labelling the Testosterone-3-0-carboximethyloxime-Tyrosine methyl ester with the Chloramine-T method.

Results

Fig. 1 shows the distribution of AF-T in 58 samples and their relationship to sex

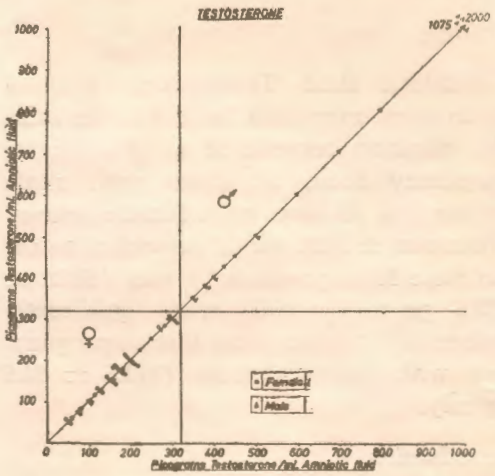

Fig. 1.

of the fetus. The mean ± S.D. in early pregnancy with male fetuses was 517.8 ± 483.5 pg/ml (range 60 to 2,000 pg/ml, N = 32). This was significantly higher (P <0.001) than the mean ± S.D. of 173.8 ± 84.9 pg/ml in early pregnancy with female fetuses (range 50 to 375 pg/ml, N = 26). Some overlap was found between upper end of range and lower end of male range—

TABLE I

Range and Mean ± S.D. of AF-7 (pg/ml) at 16 to 18 Weeks of Pregnancy With Male and Female Fetuses

Male		Female		
Mean ± S.D.	Range	Mean ± S.D.	Range	P Value
517.8 ± 483.5	60-2,000	173.8 ± 84.9	50-375	Significant P <0.001

Discusssion

The most reliable method of fetal sex determination at present is karyotype analysis. However, this is expensive and time consuming. Next important method is of Barr body and Y chromatin fluorecent count of uncultured amniotic fluid cells. Thus second is more rapid and practical. However, occasionaly the number of cells obtained is too small for accurate sex determination. In such cases it would be worth having hormone assay by R.I.A. as an adjunct so that the necesscity of second amniocentesis may be avoided. Midtrimester hormone analysis as a potential means for prenatal sex determination has been explored by several workers (Giles *et al*, 1974, Dawood and Saxena, 1977).

We have found significantly higher levels of AF-T in male than in female fetus carrying pregnancies ($P < 0.001$). With some overlap of values between the sexes. Our values of AF-T in female fetuses were higher than those reported by other workers. Whether this difference is ethnic or methodological needs further investigation. We have used I^{125} labelled Testosterone-3 TME with sensitivity 100 pg/ml, while others have used H^3 labelled testosterone with sensitivity 4 pg/ml to 16 pg/ml. AF-T levels more than 320 pg/ml were compatible with male fetuses in 94.2 per cent of the cases, whereas AF-T levels less than 320 pg/ml were compatible with female fetuses in 64.1 per cent of cases, only.

In our study, AF-T levels were determined at 15-17 weeks of pregnancy. However, form reported work we can conclude that there is no significant difference in AF-T concentration in any one week of pregnancy compared to another for both sexes. (Judd *et al*, 1976).

Thus the practical application of Testosterone analysis for the determination of fetal sex should be considered. Results of the T measurement can be obtained within two days.

Therefore, it seems that hormone analysis of amniotic fluid may be used to complement one of the cytological methods used for the determination of fetal sex.

Predictive value of this test for prenatal sex determination appears to be poor as compared to other methods, especially in case of female fetuses. These findings are in contradiction to reports of Serge *et al*, (1977) but are in agreement to reports of Dawood and Saxena (1977). However, when amniocentesis does not yield sufficient number of cells for Barr-body counts and Y-chromatin flouresence count, this alternative method may prove to be of some predictive value.

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