Effect of Fetal Sex on Total Levels of Maternal Serum Testosterone

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Abstract
The aim of this study was to find out the effect of fetal sex on maternal serum total testosterone level and its application for fetal sex determination.
Forty healthy pregnant (second trimester) females were recruited in the study from rural areas of district Dir lower, Khyber Pakhtunkhwa, Pakistan, having complete antenatal record. Twenty of them were carrying single male fetuses and twenty female fetuses. The inclusions criteria were age (25 to 30), second trimester, absence of serious diseases, availability of antenatal record, no drug addiction and no exposure to pesticides. Blood samples at 5 ml size were collected from each woman, serum was obtained and was assayed by Bio-check (USA) kit according to the manufacturer protocol.

In male fetus group the mean and SD was 169±27.18 ng/dl and in female fetus group the Mean± SD was 166.6±30.47 ng/dl. There was nosignificant difference (P 0.1062) between the two groups at 95% confidence level.

The results suggest that sex of the fetus has no association with maternal serum total testosterone among the study population-and should not be analyzed for sex determination. Further study with bigger sample size of different population groups in different gestational stages is needed to find the fetal effect on maternal serum testosterone because, increased level of testosterone in females can cause aggression, other behavioral changes, acnes and abrupt growth of pubic and axillary hairs.

**Keywords**: Fetus, Sex, Serum, Testosterone

1. Introduction

In men testosterone is the most important androgen, 95 % of which is synthesized by the testes and the rest by the adrenal cortex. It is very crucial for the spermatogenesis, fetal male sexual differentiation, pubertal development, maintenance of adult secondary sex characters, immunity, bones and muscles etc. (Stephen, 2003).Fetal testes produce testosterone which is important for male sexual differentiation. As soon as the testes differentiate, testosterone production started (8 week of gestation) and reaches to its peak at 10 -15 week of gestation. A decline occurs at the start of third trimester until after birth (Ellinwood et al., 1980).In Rhesus monkey and also in human, fetal testes are active steroidogenically. In human fetus the formation of testosterone by testes appeared to be independent of gonadotropin control. (Word et al., 1989). The amniotic fluids of both male and female fetuses were analyze for hormone analysisand was found that male fetuses had significantly higher level of Testosterone, while females had significantly higher level of Estrogen but non-significant correlation were found for the different androgenic hormone between levels assessed in amniotic fluid, maternal plasma, and umbilical cord serum.(Rivarola et al., 1968; Forest et al., 1971; Meulenberg and Hofman, 1991;Corneliekeet al., 2004). But in case of Asian elephant (Elephas maximus) carrying male calves, mean progesterone concentration in maternal serum is significantly greater than in those carrying female calves and are thus used for gender determination (Connie et al., 2007). Throughout the pregnancy maternal serum total testosterone concentration increases and the sources for this increase are unknownbut could be the maternal cortex and ovarian theca-interstitial cells (Nahid and Sirous, 2013).

In this study we selected forty pregnant women in their second trimester. Twenty were carrying male fetuses and twenty were carrying female fetuses. The hypothesis was that fetal sex
has effect on the total testosterone level in maternal serum, i.e., testosterone is synthesized only in male fetuses.

2. Materials and Methods

2.1 Participants

Healthy pregnant females with complete antenatal records were randomly selected from the rural areas of district Dir (Khyber pakhtunkhwa, Pakistan). Two groups were made. One group containing twenty pregnant female having single male fetuses. Other group also had twenty pregnant female but carryingsingle female fetuses. A written informed consent was signed from all the participants. This study was allowed by local community leader as well as by a registered doctor. The inclusion criteria were age ranges from 25 to 30, second trimester, absence of serious disease, availability of antenatal record, no drug addiction and no direct exposure to pesticides (excluding women working in fields).

2.2 Blood Sampling

A health technician collected 5 ml of the blood from the antecubital vein of the participants aseptically. Blood was collected in Vacutainer tubes containing no additives. Blood were sampled in their home between 9:00 AM to 10:00 AM after breakfast not before. At room temperature blood was allowed to clot and then centrifuged. The resulting serum samples were then stored at -20°C for later analysis.

2.3 Assay

For the measurement of serum total testosterone, testosterone enzyme immunoassay test kit, Bio-check (supplier, city, USA) was used according to the manufacturer’s instructions.

2.4 Statistics

The software Graph pad Prism, Demo version 6.03 (www.graphpad.com) was used for statistical analysis. Student paired t test was used for the comparison of serum total testosterone in both groups. Differences between paired values were consistent. The results were represented through Mean±SD. A value of $p<0.05$ was considered statistically significant.

3. Results

In male fetus group the Mean± SD of maternal serum total testosterone was 169±27.18ng/dl and in female fetuses group the Mean±SD was 166.6±30.47ng/dl (Table 1). The non-significant difference ($P$ 0.1062) indicates that maternal serum total testosterone level is independent of fetal sex.

Table1. Total serum Testosterone in Male fetuses group and Female fetuses group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male Fetuses group Mean±SD*</th>
<th>Female Fetuses group Mean±SD</th>
<th>95% CI of the mean difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Serum Testosterone</td>
<td>169±27.18 (ng/dl)</td>
<td>166.6±30.47 (ng/dl)</td>
<td>-5.362 to 0.5615</td>
<td>0.1062</td>
</tr>
</tbody>
</table>
4. Discussion

In the present study it was found that maternal serum total testosterone level is not significantly (P 0.1062 at 95% CI) affected by the fetal sex. This study is in agreement with the findings of Barbora et al. (2010) ; Steier et al. (2002) ; Salamalekis et al.(2006); Zondek et al.(1977) and Robertson et al., (1980), who compared the serum total testosterone and estradiol level of pregnant women’s carrying male and female fetuses and found non–significant differences, thus excluding their application for fetal sex determination. They found that plasma testosterone increases in women’s throughout pregnancy and this increase is even more significant in preeclamptic pregnancies. A fetal gender difference was not found in the maternal plasma but only in the amniotic fluids.Rodec.(1985) ;Dawood and sexena.(1977) ; Fineganet al.(1989) ; Judd et al.(1976); Nagamaniet al.(1979) and Robinson et al.(1977), analyzed maternal plasma and amniotic fluids for total testosterone. They found that in male fetal amniotic fluids testosterone concentration were higher than female fetuses but this does not predict the fetal sex reliably until pure fetal plasma is used. But still this is not the method of choice for routine fetal sexing.Jefery.(2012), analyzed umbilical blood of male and female neonates and found that total and free testosterone levels were higher in males(P<0.0001) while dehydroepiandrosterone levels were higher in females (P<0.0001).Castracane and Dela.(1990),performed experiment on rats to find the effects of number of male fetuses on maternal serum total testosterone and observed that there was no relationship between maternal serum total testosterone level and the number of male fetuses. This showing that fetal testis does not affect the maternal serum total testosterone level.According toGlass and Klein.(1981) during pregnancy the fetal testes produce testosterone. To find out whether this fetal synthesized testosterone can be used for gender determination by measuring the level of maternal testosterone. They analyzed serum testosterone (total and free) and dihydrotestosterone in pregnant women’s. The age of fetuses ranged from 4 to 20 weeks and found no any significant difference in maternal serum androgen level in women’s having male fetuses and those having female fetuses.However Steiet et al.(2002) and Nahid and Sirous.(2013), found that maternal serum testosterone and HCG(Human Chorionic Gonadotropin) were significantly elevated in preeclamptic than normotensive mothers having male fetuses. As compared to normotensive mothers, in preeclamptic mothers bearing female pregnancies maternal serum testosterone were significantly higher. But there was no significant difference in HCG concentration. Again in preeclamptic women’s bearing male pregnancies had higher serum testosterone as compared to preeclamptic women’s carrying female pregnancies but HCG were not significantly different. In uncomplicated pregnancies testosterone level did not differ significantly, however HCG level were higher in mothers carrying female pregnancies than male pregnancies.

Some studies have found that women’s carrying single male fetuses had higher testosterone level as compared to those women’s bearing single female fetuses during gestation. But most of the studies found no any direct relation between maternal serum testosterone and fetal gender. They even analyzed serum testosterone in women’s carrying two fetuses (female-female. Male-male and opposite sex) and observed no any significant difference in the maternal serum
testosterone of women’s carrying single sex or mixed sex fetuses (Cohen et al., 2005).

5. Conclusion

It is suggested that sex of the fetus has no association with maternal serum total testosterone among the study population and should not be analyzed for sex determination.

Limitations

Due to limited facilities other hormones were not analyzed. The participants were also not ready to give specific information’s regarding their health.

References


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