Early Diagnosis of Fetal Sex and its Relationship to Some Biochemical and Physiological Variables in Pregnant Women in Salah Al-Din Governorate

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Abstract: Genes for sex determination are widely analyzed and used to predict fetal sex during different trimesters of pregnancy. However, their correlation with sex prediction based on ultrasound results, as well as with testosterone and estrogen levels in the first trimester, is quite limited. Therefore, the present study aimed to assess the degree of agreement between several sex-determining genes (SRY, DYS14, and DAZ) analyzed in the first trimester and Doppler-predicted sex, and to evaluate their correlation with hormone levels. Whole blood samples (5 mL) were collected from 110 Iraqi pregnant women between the 1st and 12th weeks of pregnancy. DNA was extracted from all samples, and the presence of the selected genes was confirmed using real-time PCR. The detection of amplification was considered a positive result for each gene, indicating male sex. The concentrations of testosterone and estrogen were measured using the ELISA technique. All pregnancies were monitored to confirm the Doppler results postnatally. The results of the present study showed no significant correlations between sex predicted by the SRY, DYS14, and DAZ genes and sex predicted by Doppler. Furthermore, the levels of both hormones did not significantly correlate with sex predicted by either molecular or Doppler methods. In conclusion, sex prediction in the first trimester of pregnancy frequently yields inaccurate results.

1 INTRODUCTION

The process of determining the sex of the fetus in the early stages of pregnancy is of great importance for the diagnosis of genetic disorders associated with the X chromosome, as more than 100 genetic diseases associated with the X chromosome have been discovered in humans, including muscular dystrophy, fragile X syndrome, and hemophilia [1].

Early determination of the sex of the fetus is precisely through targeted invasive procedures either by chorionic villus samples in the first trimester or by amniocentesis early in the second trimester pregnancy [2]. However, these procedures are associated with the risk of miscarriage of the fetus and are also expensive, making them less acceptable [3]. Therefore, studies have resorted to determining the sex of the fetus using 2D ultrasound technology, which provides 100% accurate results after 20 weeks of pregnancy [4]. Despite the high accuracy of ultrasound technology, its accuracy depends on gestational age and decreases to 70% in the first 11 weeks of pregnancy [5]. Therefore, the discovery of low-cost, non-invasive diagnostic methods with more accurate results in determining the sex of the fetus is especially important in the first trimester.

Testing for fetal DNA in maternal body fluids is one of the most reliable procedures for determining fetal sex in the first trimester [6]. and its presence can be demonstrated through non-targeted methods of peripheral blood of pregnant women as cell-free fetal DNA (cffDNA) or as free fetal DNA in the blood of pregnant women [7], or through targeted procedures through chorionic puncture or in chorionic villus samples [8], where the presence of fetal nucleated red blood cells (fNRBCs) is a circulating cell in the peripheral blood of pregnant women and contains the whole genome of the fetus, which is a source for determining the sex of the fetus [9].

The availability and level of fetal DNA is one of the necessary requirements to obtain accurate results, and the use of non-targeted methods results in obtaining low concentrations of DNA, which

requires the use of highly sensitive and specific techniques to determine the sex of the fetus [10], and many previous studies targeted the genes of the sex chromosome Y to determine the sex of the fetus using different genetic sequences of the Y chromosome In particular, single-copy sex determination region Y (SRY) [11], However, SRY is a single copy and is therefore not sensitive enough in the process of determining the sex of the fetus, so using a combination of sequences in the Y chromosome with multiple copies such as the deleted gene in Azoospermia (DAZ) and the testicular protein gene linked to the Y Testis Specific Protein Y-linked 1 (TSPY1) also known as (DYS14) gives more accurate and more sensitive results in determining the sex of the fetus in the early stages and more accurately through the use of advanced techniques such as Real time Polymerase chain reaction (qPCR).

At the beginning of pregnancy, most of the hormones secreted by chorioblasts are the pregnancy hormone human chorionic gonadotrophin (hCG), which stimulates corpus luteum to produce and secrete progesterone [12], the results of previous studies showed the relationship of fetal sex with some levels of hormones such as testosterone and progesterone, while the results of some studies such as a study [13] show low progesterone levels in pregnant women with female fetuses but more recent studies have shown no significant coefficient relationships between hormone level and fetal sex [14], 15], [12].

Through the results of previous studies, it is noted that the relationship of the fetal sex with hormone levels in the first trimester of pregnancy is not clear and the results of previous studies on the use of PCR techniques in determining the sex of the fetus are inconsistencies and there are no previous study correlates expected fetus gender with the results of doppler, the current study assumes that by relying on measuring the levels of sex-specific genes and linking them to hormone levels, it can give results with greater dependence and give clearer results for the relationship of hormones with the sex of the fetus, so the current study aimed to assess the degree of agreement in expectation fetus gender in first trimester of pregnancy between molecular technique and doppler results as will in correlation of concentrations of hormones.

2 MATERIAL AND METHODS

2.1 Subjects and Samples

Blood samples (5 ml) were collected from 110 of Iraqi women pregnancy during first trimester (from the 1st week to the 12th week), all women were approved for inclusion in the present study, and the study design was ethically approved by Ministry of Health / Salah El-Din Health Department.

2.2 DNA Extraction and Real-Time Reaction

Maternal DNA from blood was extracted using protocol recommended by commercial Blood mini kit DNA extraction (Qiagen, USA), and primers used of genes of this study (SRY, DYS14, and DAZ) are listed in Table 1 and qPCR reaction were done using ready 2×Ultra Sybr qPCR Mix (Tinzyme/China) that contain SYBR green, Taq DNA Polymerase, PCR Buffer, and dNTPs and final additions were 25µl of 2×Ultra Sybr qPCR Mix, 1µl for each forward and reverse primer, 2µl of template DNA, 50µl ddH_2O, and the reaction steps and amplification cycle are listed in Table 2 and designed according to [16].

Table 1: Primers used for sex determining genes.

Gene	Primer Sequence ('3 → '5)		Ref
SRY	Forward	AGTATCGACCTCGT CGGAAG	Khorshid *et al.*, 2013
	Reverse	TCTTGAGTGTGTGG CTTTCG	
DYS14	Forward	AGCCCTGATCACTG ACGAAG	
	Reverse	TGCAGAGATGAACA GGATGC	
DAZ	Forward	TACCTCCAAAGCAC CAGAGC	
	Reverse	AATCTACCCATTCC CGAACC	

Table 2: Reaction of qPCR.

Step	Temperature	Time	Cycles
Pre-denaturation	95˚ C	10 min	1
Denaturation	95˚ C	15 s	35
Annealing and Extension	60˚ C	1 min	
Melting curve	60˚C-95˚C	15 s	1

2.3 Statistical Analysis

Chi-square test and Kappa values were used to assess the degree of agreement between the expected gender by qPCR and Doppler expected results (a kappa value below 0 considered as poor agreement, between 0.00 and 0,20 is considered slight agreement, between 0.21 and 0.40 is considered fair agreement, between 0.41 and 0.60 considered moderate agreement, nearly 1 considered perfect agreement), then independent-sample T-test were used to show the correlation between levels of hormones and expected gender by both qPCR and doppler.

3 RESULTS AND DISCUSSION

Statistical analysis of the present study shows non-significant correlation between the expectation of fetal gender molecularly by assess presence of amplification of SRY, DYS14, and DAZ genes by real-time PCR, as the Table 3 show that the all maternal DNA were positive for SRY compared to doppler expected gender where only 67 of total pregnancy were bearing male gender while other were female gender, this was the different slightly for both DYS14 and DAZ, were show 106 and 108 positive amplification respectively from total 110 samples.

The degree of agreement between the two techniques was analyzed statistically using the Kappa value. The results showed a poor to slight agreement between the techniques, with a Kappa value of 0.032 for the comparison between DYS14 and Doppler results and 0.028 between DAZ and Doppler results; both values indicate slight agreement. In contrast, the agreement between SRY and Doppler results was poor because all SRY values were positive (Table 4).

Statistical analysis not computed due to the constant value of *SRY*.

Independent sample T-test was done between the expected fetal gender in the first trimester by sex determining genes, as well as doppler results and levels of both testosterone and estrogen, as shown in Table 5. The most (nearly significant) important results were between estrogen levels and DYS14 expected gender, while all other correlations were non-significant.

Table 3: Timing of samples collection.

Week of first trimester	Number of samples
1st	6
2nd	11
3rd	7
4th	11
5th	7
6th	11
7th	14
8th	11
9th	13
10th	12
11th	4
12th	3
Total	110

Table 4: Degree of agreement between the results of qPCR for SRY, DYS14, and DAZ genes and Doppler results in first-trimester pregnancy.

Gene	Expected gender		P-value	Kappa value	Degree of Agree-ment
	Male	Female			
SRY	110	0	*	0.000 *	Poor
DYS14	106	4	0.134	0.032	Slight
DAZ	108	2	0.075	0.028	Slight
Doppler	67	43			

Table 5: Correlation between the levels of Testosterone and Estrogen hormones and gender expected results by qPCR and Doppler results in first trimester pregnancy.

Hormones	*SRY*	*DYS14*	*DAZ*	Doppler	P-value
Testosterone	*	0.778	0.453	0.163	
Estrogen	*	0.068	0.879	0.338	

* Statistical analysis not computed due to constant value of *SRY*.

According to [2], real time results of sex determining genes should be negative for Y-chromosome genes (SRY, DYS14, and DAZ) to consider the female gender, and vice versa. This is one of the most applications of use of cffDNA analysis, however, several other applications were present, such as disorders in single genes [17] . were the first study relay the using of SRY and DYS14

due to presence of DYS14 in multicopy, and the study of [17] indicates its efficacy of presence of cffDNA in pregnancy blood.

Present studies show poor relationship and agreement between the results of real time PCR and doppler results, and this were contrary to most of the results of previous studies, as the results of [18] show perfect agreement between the results of DYS14 and ultrasound results during 12 week of pregnancy and this were also confirmed by several other previous studies [19], [20]. The results of this contrary in the present study may be due to sampling time of first trimester, as the present study collect the sample randomly from 1st week to 12th week of pregnancy while the other previous studies indicated that the accurate results were obtained at 12th week of pregnancy, and the present study as shown in Table 1 show that 35 sample were in 4th week and below and this may be the significant results of the poor agreement between the results of expected fetal gender by genes and doppler, and this approved by several previous studies, as the results of [6] which aimed to show optimal gestational age for sex determining and show that the accuracy of sex determination at 5th week of pregnancy were 50% compared to 80% at 7th week and 100 at 9th week of pregnancy, and this indicates that the accuracy of molecular determination of fetal gender affected greatly by gestational age, and this was also approved by several other studies [21], [22].

Although there is a strong relationship between the sex of the fetus with the levels of other hormones such as testosterone, this relationship with progesterone is not completely clear and the results of previous studies in this regard were few and varied significantly, while the results of a study [12]. were negative in the existence of a relationship between fetal sex and progesterone levels.

Some previous studies reported no significant differences in progesterone levels in pregnant women, whether the sex of the fetus was male or female [11], [23] but the results of a study showed that progesterone levels decrease in pregnant women carrying a female fetus, and this was later confirmed by the study [14], [24]. indicated a significant decrease in the levels of progesterone hormone in the blood serum of pregnant women in the female sex compared to its levels in pregnant women in the male sex.

The present study shows no correlation between the levels of both testosterone and estrogen and expected fetal gender by both doppler and genes, and the main reason for this may be due to gestational age of collected samples.

4 CONCLUSIONS

Determination of fetal gender in first trimester of pregnancy gives highly false positive results and contrary results with expected gender in doppler ultrasound, as well as estimation of concentration of testosterone and estrogen in this time will not give good indication for fetal gender. Using the SRY gene to detect fetal sex in the first trimester of pregnancy provides accurate and reliable results in determining fetal sex. The SRY gene cannot be used alone to detect fetal sex in the first trimester of pregnancy. It must be used in conjunction with the DYS14 gene, which has excellent support for male sex detection, especially when used with the SRY gene. The DAZ gene, used to detect fetal sex in the first trimester of pregnancy, produces unreliable results and has a relatively weak potential, making it insufficient for reliable detection. Results have shown a correlation between elevated estrogen levels and pregnancy with a female fetus. This can be used as a biological, non-genetic indicator for detecting fetal sex Testosterone levels were low in the first trimester of pregnancy and there were no significant differences between males and females.

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