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Determining the Sex of the Fetus through the Serum of Pregnant Mothers at 8-12 Weeks Was Determined by the 4 STR Markers (DYS393, DYS460, DYS388, DYS391) Specific Chromosomes Y

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ABSTRACT

Non-invasive sex-determination methods, such as the use of ultrasound waves, are performed in the second trimester of pregnancy, which is relatively late in women who are at risk for X-related illness (sex), But today by creating new non-invasive methods in the second month, one can identify the sex of the fetus. Therefore, in the present study, the aim of this study was to find a non-invasive method for determining the sex of the fetus through cff DNA based on the STR marker at the Y chromosome level. This cross-sectional study was performed in the second six months of 1396. For this purpose, 23 blood serum samples were collected from the mother and DNA extraction was performed using DNA extraction kit. Primers were extracted from the paper(Butler, etal. 2002). Then real time reaction was performed on the DYS393 DYS396 DYS396, DYS388, DYS39 genes and finally the data were analyzed by SPSS software using chi square and ANOVA. The results in this study indicated that marker 388 was more specific than other markers with 73.9% then the marker 460 with 69.6% and the marker 391 with 69.6% and the marker 393 with 52.6%. Therefore, the specificity of marker 388 is better than other markers for gender determination it is also suggested that this study should be done with a higher statistical population in order to obtain more accurate results and use STR markers for further examination so that information can be obtained in the future.

Key words: Repetitive Short Sequences, Molecular Markers, Fetal Sex Determination

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INTRODUCTION

The determination of fetal sex in addition to its importance in early detection of some genetic genetic disorders is seen by over 99% of couples in their curiosity about their child's gender. The accuracy of the diagnosis in cases where there is a risk of a sexually transmitted disease [1[1]]. Non-invasive sex-determination methods, such as the use of ultrasound waves, are performed in the second trimester of pregnancy, which is relatively late in women who are at risk for X-related illness (sex), but today by creating new non-invasive methods in the second month, one can identify the sex of the fetus [1]. Also after embryo fertilization and implantation in the mother's womb blood-related intercourse between the mother and the mother begins by pairing. Gradually this relationship has spread so that many of the material in the blood of the fetus reaches the mother's blood. One of the most important maternal cells and embryo DNA that can open the knot of many prenatal diagnostic problems [1].

[1] Non-invasive prenatal test

The development of noninvasive methods of prenatal diagnosis (NIPT) by identifying fetal cells in the mother's blood by Bianchi et al. [2[2]]. They showed that the amount of fetal cell transfer from fetus to mother increases in embryonic aneuploidy and there are also many studies to isolate all types of nucleated embryonic cells including nucleus erythrocytes, granulocytes, lymphocytes and possibly cells [3,4]. In 1990 Bianchi and colleagues used the anti-transferrin antibody using FACS to isolate the nucleus of erythrocyte [5]. Two years later, Ganshirt and colleagues used an overwrite to isolate these cells [6]. Since the results from these methods are not acceptable many researchers are still undergoing changes to the protocol and the change in antibodies is aimed at optimizing the conditions for the isolation of embryonic cells using these methods but among other methods such as separation based on centrifugation of the density of ficoll and percolum and lectin-based separation have also been used [7,8]. In 1997 scientists discovered the presence of fetal DNA in the mother's blood. This important discovery carried out by Dnnis Lo, was the basis for non-invasive methods, especially for the detection of aneuploids [9]. This opened up a new horizon in prenatal non-invasive diagnoses. The free analysis of embryonic fetal DNA is technically much easier to enrich and isolate fetal cells and the amount of free fetal DNA is much larger than that of fetal cells. Another advantage of this method is that the half-lives of these DNAs are very short and less than 2 hours, so DNA samples from the previous pregnancies will not be retained.

The mechanism of DNA entry to the mother's bloodstream has not been completely detected, but according to studies and the difference between maternal and embryonic DNA in size, it is believed that the source of this DNA is the apoptosis of the embryonic cells and the entry of the corresponding DNA through the mating into the mother's blood stream [10]. Therefore, in the present study the aim of this study was to find a non-invasive method for determining the sex of the fetus that is both highly cost-effective and economically viable using maternal fetal DNA (cff DNA)[3] . To this end, STR markers at the level of Y chromosomes will be used at the level of these DNAs. If the embryo is male reproduction of these markers will be done using PCR and if the embryo is female and the Y chromosome does not exist, reproduction will not be done. DNA separation from the mother's blood will also be done using THP[4] which has not been used in studies in this area while similar studies have been shown to show that the separation of free DNA in plasma by this method is 2-fold as compared with the common QIAmp kits [11].

MATERIALS AND METHODS

Sample collection

In this descriptive cross-sectional study, 23 blood serum samples were collected from pregnant women (obstetrics and gynecology clinic) and then transferred to the Genetic Laboratory of Tonekabon Azad University.

Molecular examination by Real Time PCR

Table 1: Primer sequence used in this research

Size	Primer sequence	Primer type
175	F:5-GAATTCATGTGAGTTAGCCGTTTAGC-3 R: 5- GAGGCGGAGCTTTTAGTGAG -3	DYS388(15)
133	F: 5- GTGGTCTTCTACTTGTGTCAATAC -3 R: 5- GAACTCAAGTCCAAAAAATGAGG -3	DYS393(15)
121	F: 5- TTCAATCATACACCCATATCTGTC -3 R: 5- GATAGAGGGATAGGTAGGCAGGC -3	DYS391(15)
120	F: 5- GAGGAATCTGACACCTCTGACA -3 R: 5- GTCCATATCATCTATCCTCTGCCTA -3	DYS460(15)
Size	Primer sequence	Primer type
175	F: 5-GAATTCATGTGAGTTAGCCGTTTAGC-3 R: 5-GAGGCGGAGCTTTTAGTGAG-3	DYS388(15)
133	F: 5- GTGGTCTTCTACTTGTGTCAATAC -3 R: 5- GAACTCAAGTCCAAAAAATGAGG -3	DYS393(15)
121	F: 5- TTCAATCATACACCCATATCTGTC -3 R: 5- GATAGAGGGATAGGTAGGCAGGC -3	DYS391(15)
120	F:5- GAGGAATCTGACACCTCTGACA -3 R:5- GTCCATATCATCTATCCTCTGCCTA -3	DYS460(15)

Extraction of serum DNA was performed individually using pzp kit and extracted for confirmation. Quantitative analysis (with spectrophotometer) and qualitative (agarose gel 1%) were used and also the primers of the paper [15] were extracted and then examined on the NCBI site in the Blastn program and the most suitable primer sequence was selected Table [1]. Then the reaction volume for Real time included 4 µl DNA (250 ng) and 20 µl volume, 10 µL Mixer Master (X1), 1 µL Mixer Primer (10 pmol). The temperature cycle consists of initial oscillation at 95 ° C for 15 seconds, followed by 33 cycles at 95 ° C for 15 seconds, 55 ° C for 20 seconds, 72 ° C for 20 seconds and a final cycle was 72 ° C for 5 minutes for all genes and was tested by Bio Rad

[2] Fluorescence activated cell sorting

[3] Cell free fetal-DNA

[4] Triton heat phenol-chloroform

Real time PCR then the data were analyzed using SPSS software using chi-squared test and ANOVA test.

RESULTS

The results of this study showed that 65.2% of girls and 34.8% of them were boys (Chart 2). Also in the review of 388 markers 73.9% of girls and 26.1% of them were boys (Chart 3). Also the marker was 391, 65.2% female and 34.8% male (Chart 4) and 393 markers, 69.6% female and 30.4% boys (Chart 5). Also the marker of 460, 69.6% of girls and 30.4% of them were boys (Chart 6) and the results of the review using the Real Time technique are shown in Figure 1.

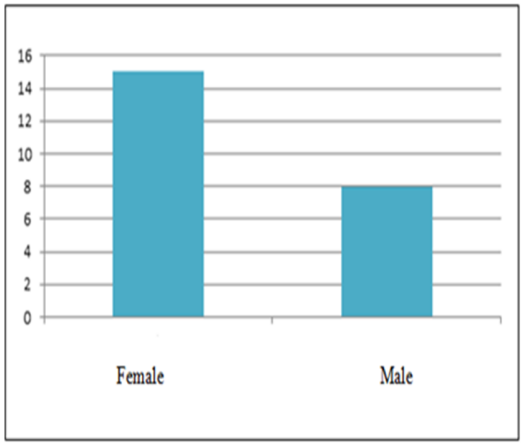

Chart 2: Gender bar graph

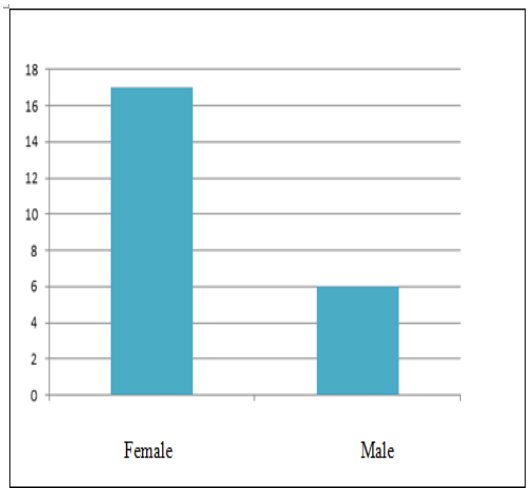

Chart 3: Marker Review Results 388

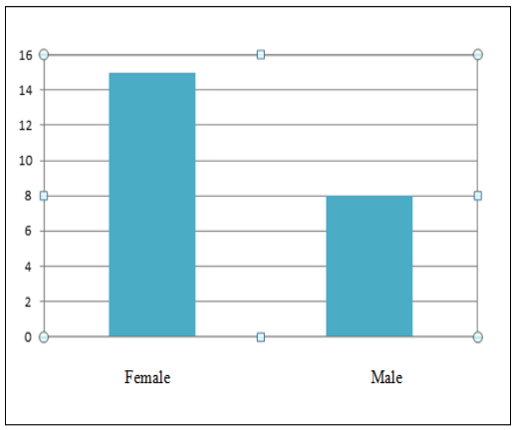

Chart 4: Marker Review Results 391

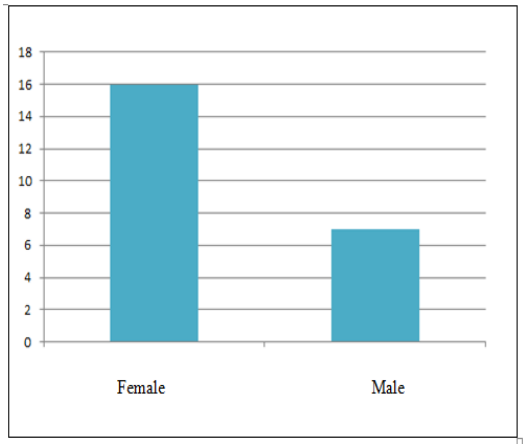

Chart 5: Marker Review Results 393

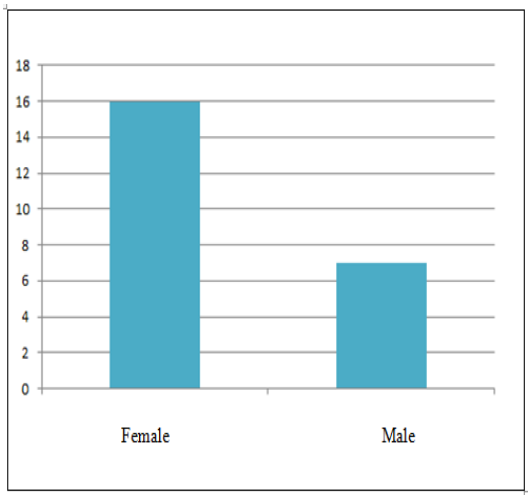

Chart 6: Marker 460 results

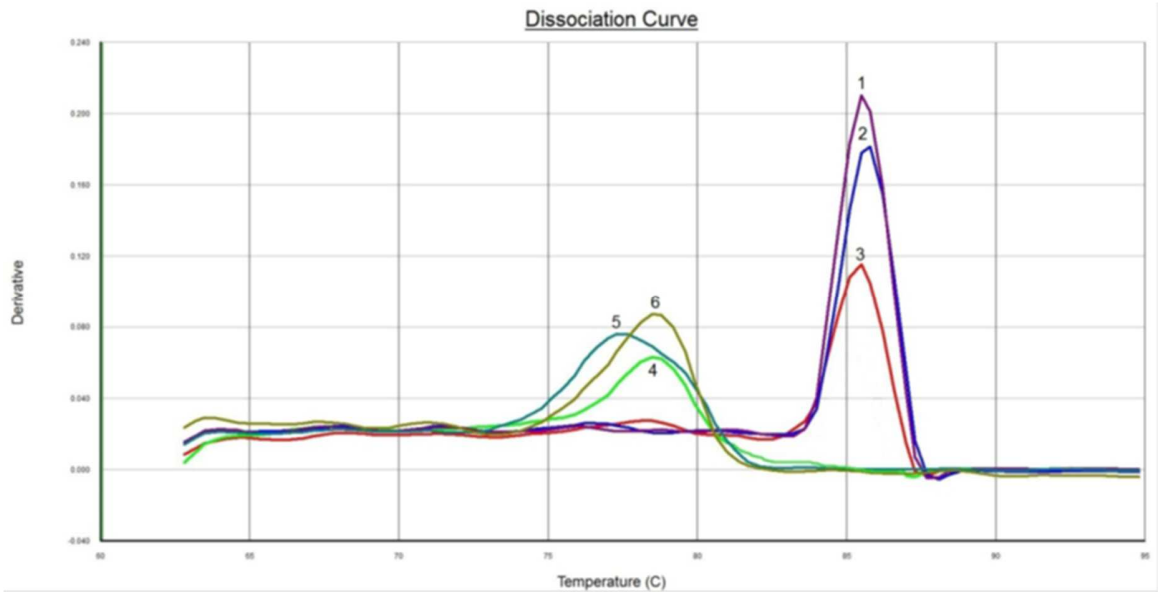

Figure 1: Analysis of some DNA extracted from the mother's serum using the Real Time technique

DISCUSSION

Since this study is both cost-effective and cost-effective to find a non-invasive method for determining the sex of a fetus which is both highly accurate and cost-effective. Because non-invasive sex determination methods such as the use of ultrasound waves occur in the second trimester of pregnancy this detection time is relatively late in women whose embryos are at risk for X-related diseases (sex). In order to find a non-invasive method for determining the sex of the embryo the STR markers used at the level of the Y chromosome Y were used and at the level of the DNA because STR is a short sequence of repetitive sequences in the Y chromosome that has inheritance from the father to all boys. If in the present study, if the fetus is male, reproduction of the markers will be performed using PCR and if the embryo is female and there is no Y chromosome, reproduction will not be done. Therefore according to the findings of this study, the four markers in the sex determination of the ratio of other non-invasive methods are taken earlier and also the specificity of marker 388 is better than other markers for sex determination. These results are of great importance in non-invasive methods for gender determination since, the determination of the sex of the fetus in addition to its importance in the early detection of some genetic diseases,is viewed by 99% of couples in terms of curiosity of their child's gender and the accuracy of diagnosis in cases where the risk of a sexually transmitted disease is important [1]. Studies such as Hanna et al. Conducted in 2015.

As in the present study, the determination of embryo's sex by noninvasive method was performed using 16 STR in Y chromosome and sex determination with specificity and sensitivity was 100% [12]. Also in 2012, Aghanori and his colleagues looked at the possibility of sexually exploring the embryo by noninvasive methods and comparing the PCR and Real-time PCR methods. In this regard; In simple PCR, 19 strains of STR were used but in real time PCR only DYS14 (Y marker) was performed and the sensitivity and specificity of the PCR method were 95% and 98% respectively and the Real TimePCR method was equal to 91% and 100 and acknowledged that since the sensitivity of the conventional method is greater than the quantitative method, the simple method can be used in the clinic for gender

determination [13] and in 2012 ong and colleagues used 17 strains of STR on the Y chromosome to determine the sex of the fetus. The group used two sets of AmpFlSTR Identifier amplification kit and Mini STR Amplification kit for amelogenin gene and respectively obtained results with accuracy of 92% and 96%. Also the use of the AmpFlSTR Yfiler kit yielded a precision of 100% [14].

Therefore according to the results of the present study and researchers in this field, it can be said that the use of the method of determining the sex of the fetus through the serum of pregnant mothers at 8-12 weeks through 4 STR to determine the appropriate and high-precision gender. Because the free analysis of fetal free DNA is technically much easier than enriching and separating fetal cells as well as the amount of fetal free DNA that is much more than fetal cells. And the use of this method is that the half-lives of these DNAs are very short and less than 2 hours so DNA samples from the previous pregnancies will not remain.

CONCLUSION

Given the findings of this study and the specificity of marker 388 relative to other markers, We assume that the non-invasive early detection of embryonic genus using maternal plasma cffDNAs can provide women with the risk of having a baby and X-related genetic disorders in the shortest time. In order to prevent unnecessary invasion I hope that in the near future this method will be used to identify specific genetic diseases. It is also suggested to use STR markers for further investigation so that information about these markers can be used in the future.

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