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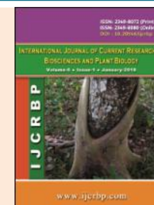

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Amplification of the Y1.7, Y1.8 and GAPDH genes for sex identification in human by using multiplex PCR

Maytham Abdul Kadhim Dragh[1]*, Talib Ahmed Jaayid[2] and Zainab Sabeeh Hasan[3]

[1] Biological Department, College of Science, University of Misan, Misan, Iraq
[2] Animal Production Department, Agriculture College, University of Basrah (UoB), Basrah, Iraq
[3] Animal Production Department, College of agriculture, University of Maisan, Maisan, Iraq

***Corresponding author; e-mail:** meatham71@yahoo.com

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ABSTRACT

Pre-natal embryo sexing was done using circulatory blood of pregnant women at early stages of pregnancy, before the 4th month of pregnancy, in which 20 samples were obtained the objective of the study was to establish and evaluate multiplex PCR for detection of Y-chromosome – specific sequence of fetal DNA in maternal blood circulation during pregnancy in which identification of fetal gender become possible before the 4th month of pregnancy, this pre-test can be used to determine whether invasive prenatal diagnosis, such as amniocentesis and chorionic villi sampling should be performed on a fetus having a risk of X- linked recessive inheritance. The results of the study showed the appearance of a band with 57bp (represent the primer GAPDH) In all samples which represented the control. The second band with the 100bp (represent the primer Y1.7 and Y1.8) as well as the first band (57bp) appeared in male fetuses since it represent a gene found only on the Y chromosome. The application of a non-invasive method like multiplex PCR by using maternal blood for embryo sex identification reveals a reliable and accurate method for prenatal diagnosis.

Introduction

Fetal cells isolated from maternal blood represent a good source of fetal chromosome or DNA obtained non-invasively by maternal vein puncture that would avoid the complication and disadvantage of amniocentesis and chorionic villus sampling (CVS) which currently are the gold standard samples for prenatal diagnosis. The possible fetal cell types that can be isolated from maternal blood included trophoblasts, lymphocytes and nucleated red blood cells (Pongsritasana et al., 2006).

Fetal Nucleated Red Blood Cells (FNRBCs)

Fetal cells such as trophoblasts, lymphocytes and nucleated red cells (erythroblasts) have been shown to exist in maternal blood during pregnancy (Bianchi et al., 1990; Geifman-Holtzman et al., 1994). Nucleated red blood cells seem to be the promising cell type for prenatal diagnosis because

they are relatively short-lived, and therefore the likelihood that these cells originated in prior pregnancies is reduced (Adinolfi, 1991; Geifman-Holtzman et al., 1994).

It has previously been shown that fetal-nucleated red blood cells may be present in the maternal blood as early as 8 weeks of gestation, thus permitting first trimester diagnosis of fetal aneuploidy (Simpson and Elias, 1993). Retrieval of fetal cells from maternal blood can be enhanced by enrichment techniques involving fluorescence-activated cell sorting and magnetic-activated cell sorting (Price et al., 1991; Zheng et al., 1993). However, most enrichment and purification techniques are time-consuming and require expensive equipment.

Fetal DNA and PCR method

Recently, there has been much interest in the use of DNA derived from blood for the molecular diagnosis of genetic abnormalities and tumors (Mulcahy et al., 1996; Lo et al., 1997). Furthermore, it has been shown that fetus-specific DNA exists in maternal circulation (Lo et al., 1997; Zhong et al., 2000) and can be detected as early as 7 weeks of gestation (Lo et al., 1998; Al-Yatama et al., 2001a) These studies demonstrate that DNA from cells dying in the developing embryo passes through both placental and kidney barriers to appear in the maternal circulation; the size of DNA fragments in serum and plasma is sufficient to provide polymerase chain reaction (PCR) analysis, and the amount of fetal DNA in the mother's plasma is high enough to serve as a target for the detection of multiple as well as single copy genes (Al-Yatama et al., 2001b).

Materials and methods

This study was conducted in the genetic engineering laboratory/ biology department/ college of science/Misan University.

Samples

Peripheral blood cells from male donors were used for standardization experiments. To obtain whole blood, peripheral blood (5–10 ml) was collected from 20 women donors carrying male fetuses during pregnancy. The samples were kept in well labeled EDTA tubes in refrigerator until DNA extraction step was began.

Isolation of DNA from whole blood

DNA from whole blood was isolated using standard procedures. In brief, 500 µl of whole blood was treated with red cell lysis buffer and centrifuged. The pellet was resuspended in 16 µl of x5 proteinase-K buffer, 4 µl proteinase-K enzyme (10 mg/ml), 4 µl 20% sodium dodecyl sulfate and 30 µl distilled water. The tube was incubated with slow rotation at 55°C for 2–4 h. The DNA was then precipitated with 70% ethanol, the pellet air-dried, dissolved in 50 µl TE buffer and 10 µl was used for PCR.

Primers for the detection of Y-chromosome specific sequence

To amplify Y-chromosome-specific DNA sequences, three pairs of primers were used, below a table showing the designation and the nucleotide sequence of the primers and the size of target DNA to be amplified.

The names and sequences of the primers used in the study as well as the size of DNA to be amplified and their location on the chromosomes. Y1.7 / Y1.8 are present in a single copy in the Y chromosome (Lo et al., 1990; Melkonyan et al., 1997).

PCR and detection of amplified DNA

By using the oligonucleotide primers described above, Y-chromosome- specific DNA fragments were amplified according to the standard procedures described previously by (Mustafa et al., 1995). Each reaction mixture (100µl) contained 200 nM of each relevant primer and 2.5 U of Ampli Taq DNA polymerase, PCR buffer, dNTPS and target DNA. Amplification was carried out in a thermal cycler (Perkin Elmer System 2400). Each cycle of the PCR amplification consisted of denaturation step at 95˚C for 15 seconds and Annealing step at 60˚C for 30 seconds and extension step at 72˚C for 45 seconds The PCR program was 42 cycles**.** The amplified DNA was analyzed by agarose gel electrophoresis according to standard procedures (Mustafa et al., 1995). The bands for specific DNA were observed under UV light and photographed.

Results

The results showed that DNA fragments of expected size were amplified with all the three primer pairs in standard PCR (Table 1). The results of the study showed the appearance of a band at the base pair 57bp represent the GAPDH gene in all the samples which refer to the internal control. The second band appear on the 100bp represent the Y1.7 and Y1.8 gene only in male fetuses, since it represent a gene found only on the Y-chromosome.

PCR amplification of fetal DNA from maternal blood: 1) standard marker, 2,4,5,6,8,9,11,12,15) women pregnant with female fetuses, only the GAPDH gene was amplified at the 57 bp.(one band); 1,3,7,10,14) women pregnant with male fetuses, both the GAPDH and Y1.7, Y1.8 gene was amplified at the 57 and 100 bp respectively (two bands) (Fig. 1).

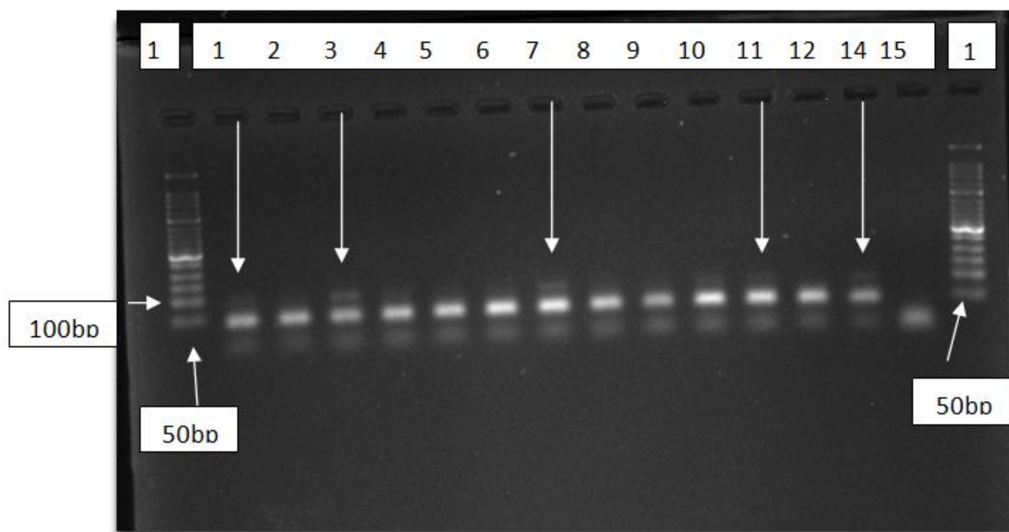

Fig. 1: PCR amplification of fetal DNA from maternal blood: 1)standard marker, 2, 4, 5, 6, 8, 9, 11, 12, 15) women pregnant with female fetuses, only the GAPDH gene was amplified at the 57 bp (one band); 1, 3, 7, 10, 14) women pregnant with male fetuses, both the GAPDH and Y1.7, Y1.8 gene was amplified at the 57 and 100 bp respectively (two bands).

Table 1. Amplification of DNA fragments of expected size with three primer pairs in standard PCR.

Name	Sequence of primer	Base pair	GenBank
Y1.7	5´ - CATCCAGAGCGTCCCTGGCTT -3´	100 bp	AF505528.1
Y1.8	5´ - CTTTCCACAGCCACATTTGTC -3´		
GAPDH	5´ - CCTAGTCCCAGGGCTTTGATT - 3´	57 bp	NM_002046
GAPDHa	5´ - CCCCACACACATGCACTTACC - 3´		

Discussion

In this study, we have demonstrated the presence of Y-chromosome-specific DNA sequences in the maternal specimens of women bearing male fetuses by using highly sensitive multiplex PCR. In previous reports, in which standard PCR was used, it was shown that Y-chromosome specific DNA sequences were detected in 80–87% of maternal plasma samples during pregnancy (Mulcahy et al., 1996; Honda et al., 2001). Our results demonstrate improved sensitivity by showing the detection of Y-chromosome-specific DNA in 96% of plasma specimens from pregnant women bearing male fetuses. This could have been due to the improved sensitivity of multiplex PCR as compared to standard PCR. This is because in multiplex PCR the initial starting template for the second round of PCR is actually the PCR product, and therefore the target region is in high copy number compared to the genomic template used in the first round of PCR (Al-Yatama et al., 2001b). These results could be explained on the basis that the sensitivity of amplification of a target in PCR, in addition to target copy number, also depends on the primers and size of the amplified DNA etc. (Mustafa et al.,

1999). The multiplex PCR results using specimens from pregnant women suggest that whole blood, serum and plasma samples are useful in determination of fetal sex and could be applicable to noninvasive prenatal diagnosis of genetic Y-linked abnormalities, single gene disorders or determination of fetal sex, etc. (Al-Yatama et al., 2001b).

Conclusion

The results of the present study suggest that the highly sensitive multiplex PCR was reliably used in fetal sex determination and prenatal diagnosis.

Conflict of interest statement

Authors declare that they have no conflict of interest.

Acknowledgement

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