Maternal capillary blood: A new source of circulating cell-free fetal DNA for noninvasive prenatal testing

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Abstract

Noninvasive prenatal testing (NIPT) is currently limited to maternal venous blood collection by trained phlebotomists, which can restrict accessibility to prenatal screening. We aimed to identify a new source of circulating cell-free fetal DNA that would be convenient for broad population screening and reliable enough for noninvasive prenatal diagnosis. Maternal capillary blood samples were obtained from pregnant women (6-24 weeks gestation) by finger stick. Plasma was separated from whole blood by centrifugation, and circulating cell-free DNA was isolated using a commercial DNA extraction kit. Real-time quantitative PCR was performed to detect fetal DNA using a multi-copy sequence on the Y chromosome. An endogenous control gene was used to measure total cell-free DNA (maternal and fetal). We detected cell-free DNA in all maternal capillary blood samples. Y-chromosome specific sequences were detected in all pregnancies confirmed to have a male fetus. All gender results were in concordance with known fetal sex, without false-positive or false-negative results. The overall diagnostic accuracy was 100%, and provided 100% sensitivity and specificity. This new method for prenatal diagnosis of fetal gender from maternal capillary blood is simple, accurate, and reliable. The results of this study demonstrate that fetal DNA detection using maternal capillary blood is highly feasible and easily adaptable for population screening. This method simplifies collection of maternal blood and should increase the accessibility of NIPT.

Introduction

The potential of utilizing maternal plasma for noninvasive prenatal diagnosis was first described in 1997 by Dennis Lo.[(1)] Lo was the first investigator to demonstrate the presence of cell-free fetal DNA in maternal plasma.[(1)] The maternal plasma samples in Lo's study were obtained from pregnant women via venous blood draw.

Almost twenty years later, current noninvasive prenatal testing still require venous blood sampling for the detection and analysis of cell-free fetal DNA. Venous blood sampling requires a licensed phlebotomist and a centralized laboratory which can limit access to NIPT. A simpler and more convenient method for collecting maternal blood for noninvasive prenatal testing was investigated in this study.

This study identifies a new source of cell-free fetal DNA, capillary blood via lancet finger stick, that can be used for noninvasive prenatal diagnosis. This new source of cell-free fetal DNA has the potential to increase the accessibility of noninvasive prenatal testing.

In addition, we conducted a validation study to show that at-home collection of capillary blood via lancet finger stick (SneakPeek® Early Gender Test) could be used to accurately determine gender of the fetus with our assay.

Materials and Methods

After obtaining informed consent, venous and capillary blood samples were obtained from pregnant women (6-24 weeks gestation). For the validation study, capillary blood samples were collected from 153 pregnant women using SneakPeek® sample collection kits. Venous blood was collected via standard venipuncture and capillary blood was collected via finger stick. Plasma was separated from whole blood via centrifugation. Cell-free DNA was extracted and purified using a commercial DNA extraction kit.

Following isolation of cell-free DNA, real-time quantitative PCR was performed to detect the presence of male cell-free fetal DNA using a multi-copy target sequence on the Y chromosome. In addition to the Y-target, an endogenous control gene was used to measure the amount of total cell-free DNA (maternal and fetal) present in the sample.

Gender calls were made using the SneakPeek® algorithm. The algorithm incorporates Ct values of the Y-target sequence and endogenous control gene.

A survey was used to confirm gender determination. Participants confirmed gender calls via ultrasound, CVS, or NIPT. Participants were contacted by telephone or email survey.

Self-reported gender results were used to determine the test performance of the algorithm for samples collected at-home.

Results

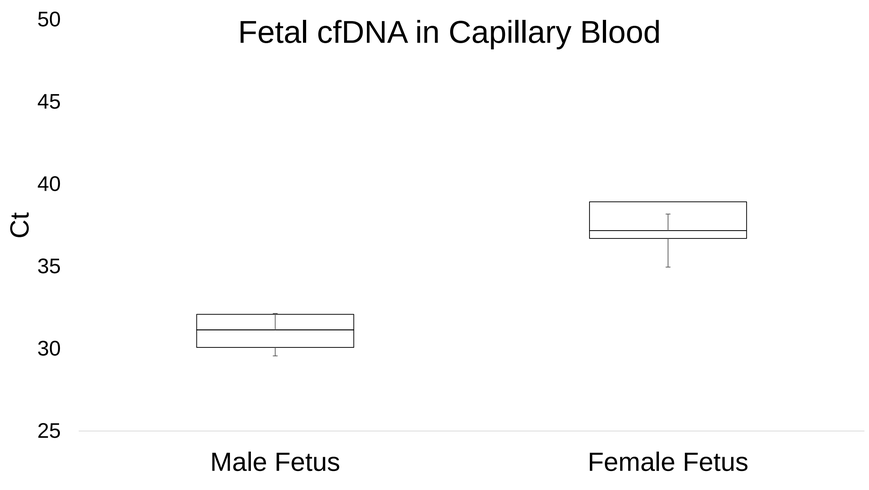

Figure 1.Cycle threshold results for Y-target PCR

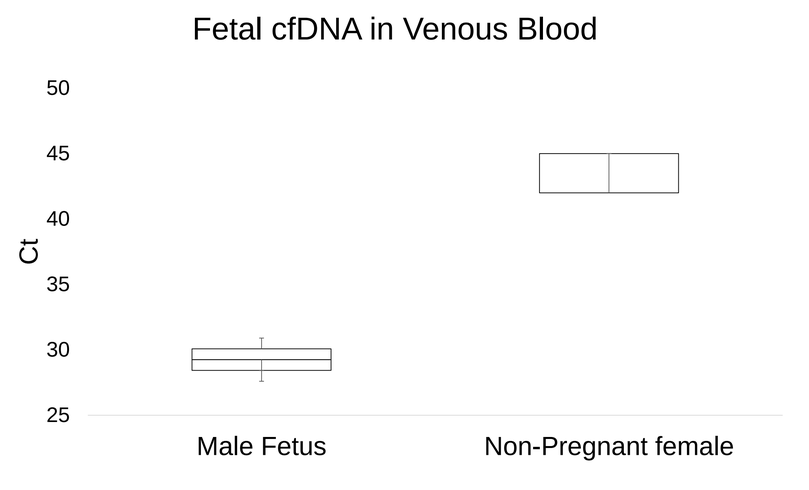

Figure 2. Cycle Threshold results for Y-target PCR

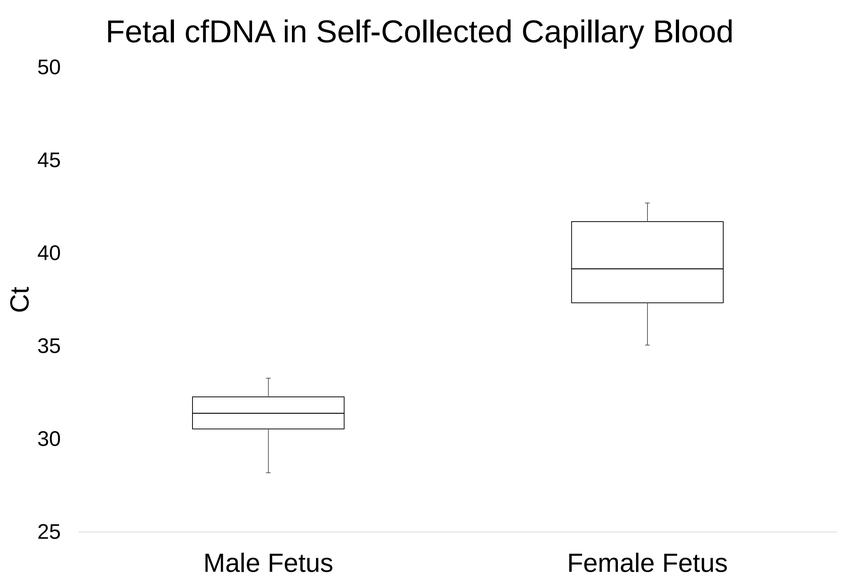

Figure 3. Cycle threshold results for Y-target PCR

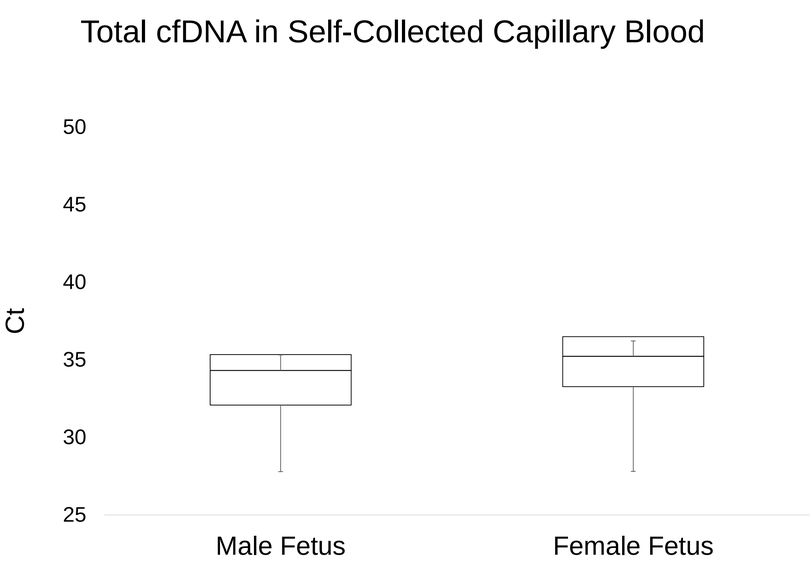

Figure 4. Cycle threshold results for endogenous control PCR

Table 1. Statistical Parameters of the Validation Study	
Samples Analyzed	153
Female Fetuses	48
Male Fetuses	74
False Positives	2
False Negatives	0
Sensitivity	100%
Specificity	96%
Accuracy	98%
Inconclusive	29

Conclusion

Cell-free DNA (cfDNA) was detected in plasma samples from both venous and capillary blood sources. Total cfDNA levels were similar between venous and capillary plasma samples. Y-chromosome cell-free fetal DNA was successfully detected in 100% of samples from confirmed male bearing pregnancies (both venous and capillary blood). Fetal cfDNA levels were similar between venous and capillary plasma samples demonstrating that capillary plasma is a viable source of cell-free fetal DNA.

Amplification of Y-chromosome DNA from capillary blood plasma enabled highly accurate gender determination. This new method of using maternal capillary blood for prenatal diagnosis of fetal gender is simple, accurate, and reliable.

The results of this study demonstrate that cell-free fetal DNA detection using maternal capillary blood is feasible and has the potential for being adapted to broad population screenings.

A validation study showed that the SneakPeek® early gender test algorithm was 98% accurate. The two false positive results could be attributed to a number of causes: sample contamination with male DNA; inaccurate survey responses; or poor sample quality. The inconclusive calls fell in the range of Ct values that did not meet a threshold for definitive detection of the Y-chromosome.

The ability to detect Y-chromosome cell-free fetal DNA in maternal plasma from both capillary and venous blood demonstrates that both sources of maternal plasma can be used for prenatal diagnosis.

This new source of cell-free fetal DNA greatly simplifies the collection of maternal blood and has the potential to increase the accessibility of noninvasive prenatal testing. To our knowledge, this is the first study to show the isolation of cell-free fetal DNA and accurate gender determination from maternal capillary plasma.

Reference

[1] Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. *Lancet*. 1997;350(9076):485–487.