Examining the Possible Difference Between Venous and Menstrual Blood Through the Methylation Status of the hCG-β Protein as a Means of Identification

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Introduction

At the present moment, there is not a distinctive field test used at crime scenes to differentiate between menstrual blood and venous blood in Forensic Science. In order to create a test to differentiate this, differences in the kinds of blood must first be investigated. One of these differences are the proteins that are found in the two kinds of blood. Beta Human Chorionic Gonadotropin (hCG-β) is found in menstrual blood[1], and will therefore be the protein examined. If there is a significant difference found in the methylation of venous blood and menstrual blood , this can help aid in the blood's analysis using *q*PCR.

Materials and Methods

After being Institutional Review Board (IRB) approved, participants signed a consent form. Then venous blood was collected by a trained phlebotomist at the University of New Haven Health Services. The blood was collected into EDTA tubes. Menstrual blood was collected using sterile cotton swabs. Samples were stored at 2°C until use.

DNA was then purified and extracted from both the venous and menstrual blood. Venous blood DNA was extracted using the protocol *Isolation of Total DNA from Small Volumes of Blood or Saliva* and menstrual blood was extracted using the protocol *Isolation of Total DNA from Surface and Buccal Swabs*. Both of these protocols were included in QIAamp ® DNA Investigator Kit[2]. The DNA was then quantified using both the NanoDrop™ One UV/Vis Spectrophotometer, and the Qubit® 3.0 Fluorometer.

The extracted DNA was then treated to a bisulfite conversion using the EpiTect® Bisulfite Kit following kit protocols. The converted DNA was then prepared for *q*PCR using a Custom Taqman™ Gene Expression Assay, and once prepared, each sample was run five times through the *q*PCR. The assay used was created using Primer3 website, and a known hCG-β sequence from the National Center for Biotechnology Information Database[3] . Relative *q*PCR – which was run on Applied Biosystems 7500 Software – was used to find each sample's cycle threshold (C_T) value. After each sample was run multiple times, the average C_T value was determined for each sample. *p*-values were calculated for comparison of the two sample types.

Results and Discussion

The overall average C_T value of venous blood was 33.285 with the standard deviation being 0.740. The overall average C_T value of menstrual blood was 35.110 with the standard deviation being 0.824. There was a consistent trend in C_T values, with menstrual blood having a larger C_T value than venous blood. A two tailed T-Test was conducted to compare the C_T values of the menstrual blood and venous blood. From the T-Test, the *p*-value was calculated, with the value being 0.00044.

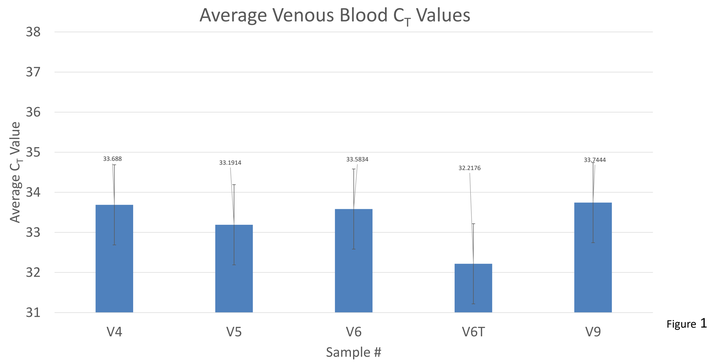

Figure 1

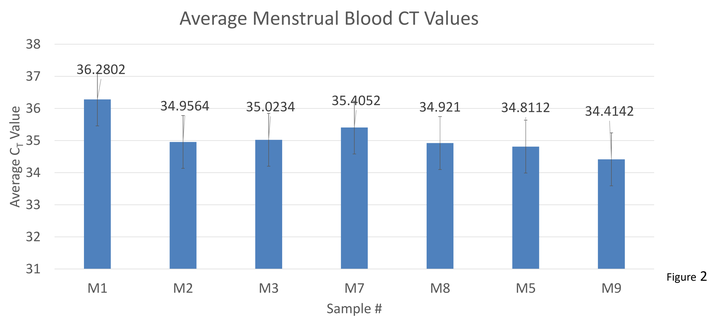

Figure 2

Ultimately, this study showed that there is a statistically significant difference between venous blood and menstrual blood that can be demonstrated through *q*PCR analysis. This difference is seen from their corresponding C_T values and low *p*-value. The *p*-value being below 0.01 provides support that the difference in methylation status in the hCG-β protein is statistically significant.

Non-bisulfite treated portions of theses samples were initially run via relative *q*PCR and, importantly, it was determined that the resulting C_T values of both sample types, venous and menstrual, overlapped with no statistically significant differences (p=0.123).

Conclusions

This research demonstrated that there is a statistically significant difference in the levels of hCG-β between venous blood and menstrual blood via methylation status. Venous blood had an average hCG-β C_T value of 33.285, while menstrual blood had an average hCG-β C_T value of 35.110. This difference in the C_T values is significant and can, with more research, be used in the future to help more effectively differentiate between venous and menstrual blood.

With future research, it is important to get both venous and menstrual blood samples from the same person to see if the C_T values alter largely, or if they are closer together. In this study, only two pairs of blood samples came from the same participant (V5 and M5, and V9 and M9), so a conclusion can not be made about their comparative C_T values alone. Future research would also include a bigger sample size, to see if the results are consistent. In addition, RNA will be extracted from the blood samples, reverse transcribed, and run through *q*PCR to see if the results are consistent.

Future Work

- Run more samples to see if these results are consistent with a larger sample size.
- Run venous and menstrual blood samples from the same donor to see if C_T values are comparable.
- See if these results are consistent when blood is collected from dried stains.

References

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