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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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In the field of forensic science, there is currently not a field test that can be used to differentiate between venous blood and menstrual blood at a crime scene. However, it is well known that there are differences between the two kinds of blood. Menstrual and venous blood have different component proteins, which helps to explain their different functions in the human body. One of these proteins is Human Chorionic Gonadotropin (hCG), which is found during a women's menstrual cycle [1,2]. Using the beta form of this protein, this study focuses on finding the hCG-β protein in menstrual blood, seeing if it is also in venous blood, and comparing the level in which the protein occurs to see if it is possible to develop a differentiating assay for identification purposes. After obtaining Institutional Review Board (IRB) approval, each volunteer was asked to sign a consent form and donate both venous and menstrual blood. The venous blood was collected by a certified phlebotomist in EDTA treated vials, and the menstrual blood was collected using sterile cotton swabs by the volunteers themselves. Both kinds of blood samples were then stored in a refrigerator at 2^{o}C until used. The venous and menstrual blood DNA were then extracted using the QIAamp® DNA Investigator Kit following kit protocols – *Isolation of Total DNA from Small Volumes of Blood or Saliva,* and *Isolation of Total DNA from Surface and Buccal Swabs* – for the venous blood and menstrual blood, respectively[3]. The extracted DNA was then quantified using the NanoDrop ^{TM} One UV/Vis Spectrophotomer and the Qubit® 3.0 fluorometer. The extracted DNA was then treated to an EpiTect® Bisulfite Treatment according to the kit protocols. After, the converted DNA was prepped for qPCR using a Custom Taqman^{TM} Gene Expression Assay and run through qPCR. The assay probe was created using a known hCG-β sequence found in the National Center for Biotechnology Information Database, with primer3 software[4].

Relative qPCR was run on the Applied Biosystems ® 7500 Real-Time PCR System to determine each sample's cycle threshold (C_T) values. The results showed that not only did the protein present itself in menstrual blood, it also presented itself in venous blood. Each blood sample was run with five replicates of venous and menstrual blood. The average C_T values of each type of blood was different – with menstrual blood having a C_T value range from 34-36 cycles and venous blood having a range from 32-34 cycles. When the standard deviation of each type of blood was calculated, it concurred with the idea that the C_T values were not overlapping. This proved to be statistically significant ($p<0.01$) and will further be examined with a larger sample size and more testing. This research showed that there is a possible difference in menstrual and venous blood regarding hCG-β protein levels, as a result of bisulfite conversion. Future research will be to determine if these results are also shown when collecting samples from dried blood stains on different surfaces. This research and an abstract were submitted to the Northeastern Association of Forensic Scientists Annual Meeting and is pending approval for a poster presentation at the conference.

Sources

1. Bauer, Patzelt, *Evaluation of mRNA markers for the identification of Menstrual Blood.* Journal of Forensic Science. 2002.
2. Zimmerman et. Al *Epithelial Human Chorionic Gonadotropin is Expressed and Produced in Human Secretory Endometrium During Normal Menstrual Cycle*. Biology of Reproduction. 2009.
3. QIAamp® DNA Investigator Handbook, Qiagen, June 2012
4. **https://www.ncbi.nlm.nih.gov/nuccore/NC_000019.10?report=genbank&from=49022869&to=49024375&strand=true**