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Chapter

Obstetric Markers as a Diagnostic Forensic Tool

Adithi Shetty and B. Suresh Kumar Shetty

Abstract

The field of Forensic diagnostics is evolving very rapidly keeping in pace with the emerging technology in the various fields. Several biomarkers up to the molecular level have been discovered which aid in solving cases. Pregnancy diagnosis from traces of blood could aid in solving cases of finding a missing pregnant lady or illegal abortions. But the challenge posed could possibly be the minimal amount of blood obtained for diagnosis. Here comes in the role of RT PCR diagnosing mRNA which is pregnancy specific, i.e., for hPL and beta hCG. The additional advantage would be that a small quantity suffices. Even if the blood stain is dried and degraded, the detection rate is good. This could add weightage to the investigation as a vital clue or change the course of investigation. The other areas of application of obstetric biomarkers are sexual assault, maternal substance abuse and paternity testing.

Keywords: Crime scene, mRNA, bloodstains, RT-PCR, noninvasive paternity testing, STR, SNP

1. Introduction

Forensic diagnostics is one area which is developing in an extremely fast rate and has changed the way in which investigations are handled. The principles of the various disciplines -immunology, biotechnology, biochemistry, molecular biology etc. is integrated into the various diagnostic modalities developed to solve a case scientifically in order to achieve the final result [1]. But pregnancy diagnostics is one area in forensics which has a lot of gray areas.

The possible cases could be sexual assault of a pregnant woman, criminal miscarriages, feticides, drug and alcohol abuse, paternity detection. Crime scenes often yield samples – biological human body fluids – vaginal fluid, saliva, skin and liquid tissues- semen and blood. These provide the necessary genetic material which could help in firstly, establishing the identity of the concerned individual and also aids in establishing the cellular origin of the concerned material. Hence, the investigators need appropriate and effective tools to aid in detection of cellular origin of the sample [2].

Bloodstains from pregnant women can be diagnosed using obstetric markers and aid in solving cases related to criminal miscarriages, feticides, infanticides and identification of missing pregnant women. The earlier markers worked on the principle of immunodetection of protein specific for pregnancy needing large amount of bloodstains for detection with lower sensitivity rates. The possibility of utilization of placental derived mRNA in plasma of the mother using RT-PCR in detection of blood stains proved to be promising [3].

Alcohol intake during early pregnancy causes a teratogenic effect on the fetus is a known fact. But unfortunately there is no well documented screening method in pregnancy to detect alcohol use. One marker that is sensitive and specific which is gaining importance is Phosphatidylethanol 16:0/18:1 [4]. The other markers being evaluated are non-oxidative direct ethanol metabolites such as ethyl glucuronide (EtG), ethyl sulphate (EtS) and fatty acid ethyl esters (FAEEs) [5].

Likewise, use of obstetric markers in assistance to solve the various forensic cases have been studied for some time now. Earlier the use of these markers were downplayed, but now they are having an emerging role in aiding to solve crimes.

2. Obstetric markers for bloodstains

2.1 History of the various biomarkers

Bloodstains at the site of crime are often obtained. But is there a way to detect if the bloodstains belong to a pregnant woman? The answer is yes. But, of course a lot of research has been done in order to find the "ideal" detection method. The base for the research is the use of methods to detect hormones or associated proteins specific for pregnancy and puerperium.

2.1.1 Pregnancy Hormones

The earliest attempts for detection of **pregnancy hormones** in bloodstains started in the 1900s. The first hormone researched was choriogonadotropins hormones as it was one of the ways to establish that the bloodstain belonged to a pregnant lady. In 1932, Goroncy invented a test based on the Aschheim-Zondek test, which detected pregnancy. He made modifications to it and thought that it could prove valuable as an investigation. Although the drawback of this test was frequent false negative results. So it was not a very effective method to detect if bloodstains belonged to a pregnant woman.

After that many techniques were employed for the same- Hemagglutination Inhibition test, crossed electroimmunodiffusion procedure. In the Hemagglutination test, the test cells are hCG sensitized RBCs. Suspected stain extract with the control was incubated with antihCG titres and then addition of test cells was done. But this method was cumbersome and was discarded.

2.1.2 Pregnancy Specific Proteins

The detection of **pregnancy specific proteins** in bloodstains to determine the pregnancy status was also attempted. The 4 proteins detected were – PAPP A, HPL, SP1 and Pregnancy associated alpha 2 glycoprotein. In 1971, Bohn reported that by Immunodiffusion, four proteins could be detected in pregnant serum. The first component was identical to hPL, one another was an a2-glycoprotein, while the remaining two were glycoproteins. In the pregnancy serum, four antigens were detected by Gall and Halbert in 1972. They named them as pregnancy associated plasma proteins A, B, C and D, or PAPP-A, -B,-C and –D. In 1973, Bohn coined the term SP1 for the pregnancy specific 8 – glycoprotein [6]. A lot of research was done to establish which of the hormones or proteins were the best indicator of pregnancy with high specificity [7].

Strejc et al. published a paper in 1989 wherein he detected SP 1 by the process of Immunoprecipitation using self-produced antiserum and it was considered a reliable marker, though, the drawback was that the detection was after 4th month of pregnancy and the reliability was 91–95%.

Then came the detection of hCG by the Enzyme technique, which proved to be faster and sensitive and specific [8]. Initially, the Polyacrylamide disc gel Electrophoresis was employed by Oya et al., to examine cysteine aminopeptidase(CAP) and leucine aminopeptidase (LAP), which were detected only after fourth month of gestation. In 1973, Oya et al. demonstrated that alkaline phosphatase which is heat stable was detected in pregnancy after fourth month. Only disadvantage was the large quantity of blood required. But this method was reliable and utilized [9].

2.1.3 Placental Messenger RNA (mRNA)

The big breakthrough came with the promising technique of detection of **Placental mRNA** for beta -hCG & hPL by RT-PCR in the bloodstains from pregnant women [10]. A lot of research was done in the 21st century with respect to this.

What is mRNA? mRNA is the step in between protein-encoding DNA translation and the proteins production by ribosomes [11]. Placenta expressed genes based mRNA transcripts easily detected in maternal plasma prove that the source of fetal nucleic acid release is placenta [12]. Only placental trophoblasts express hPL and beta hCG mRNA which is detected in maternal plasma.

Method of Detection of mRNA: In order to detect presence of these mRNA, RT-PCR is the method of choice. Advantages of the RT-PCR technique is manifold. First it is highly specific and sensitive. The second advantage is that it can be designed to be human specific, proving to be advantageous over the immunological tests of pregnancy which have high chances of false positivity [13].

The controversy was that mRNA was thought to be highly unstable and was considered to be degraded quickly making its use as a possible detection tool in old stains questionable. But few studies have refuted this dogma and there are instances wherein the mRNA has been detected in bloodstains as old as 16 years [14].

An ideal mRNA-based test should be in detectable quantities early in pregnancy as well as throughout pregnancy with rapid clearance after delivery. Out of the known 11 genes which were reported to have pregnancy-specific expression patterns, only 2 genes have been thoroughly studied - β-subunit of the hCG and human placental lactogen [13]. Let us look at both the molecules closely to determine which is ideal.

Human Placental Lactogen: The various parts of the hPL mRNA is depicted in **Figure 1**. As per the demonstration, RT-PCR detection of hPL was noted throughout pregnancy increasing until delivery and it was undetectable within 24 hours following delivery, which makes it an ideal test [13]. The added advantage is that, when there is a mixed sample, it can be designed to be human specific [10].

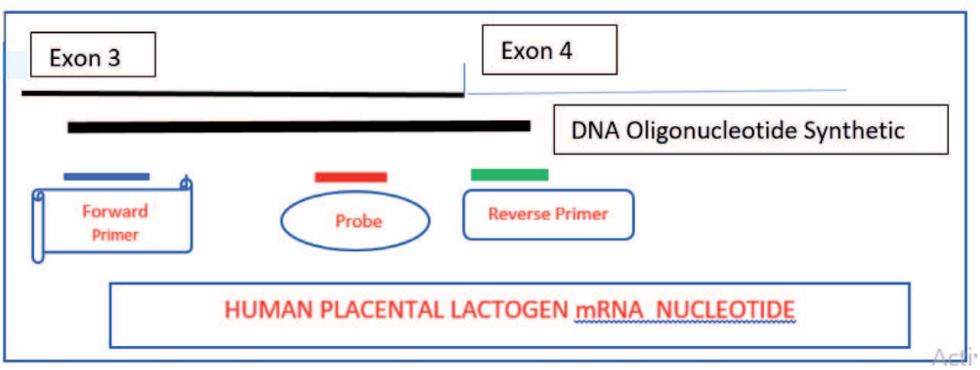

Figure 1.
Human placental lactogen mRNA nucleotide (source: [15]).

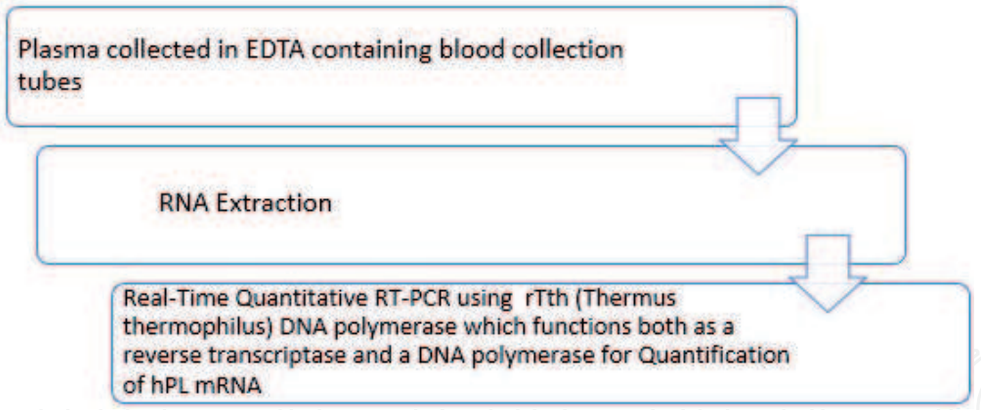

Figure 2.
Method describing the quantification of mRNA using RT PCR technique.

Method of RT PCR for quantification of mRNA [15]:

As in **Figure 2**, the exact methodology of quantification of mRNA using RT PCR technique is described.

Advantages of hPL mRNA: A study done by Gauvin et al. demonstrated the detection of transcripts of hPL in as little as 0.25 cm2 of dried bloodstain, showing that this test has a high sensitivity. The other advantage noted was that the hPL transcript was demonstrated in bloodstains as old as 56 days at room temperature. All these factors- stability and high sensitivity imply that RT-PCR hPL mRNA assay is an ideal marker for pregnancy related forensic diagnostics [10].

β-subunit of the hCG: With respect to the other marker – βhCG, it was noticed that the mRNA concentration reduced as pregnancy advances [16]. The RT-PCR of βhCG mRNA levels are detected in the first trimester where their levels are the highest. After which, the levels decreases as pregnancy advances making its detection difficult. So to conclude, by itself βhCG mRNA may not be reliable as a biomarker when you have to consider the entire duration of pregnancy [13].

Also research has been done in the area to estimate the gestational age from the bloodstains. A study based on the rationale that the use of time-wise reverse expression intensity pattern of the hPL and βhCG transcripts could predict the period of gestation from the pregnant woman's bloodstains. Gauvin et al. tested this hypothesis and found that there was a significant positive relation in women with gestational ages between 8 and 20 weeks. But the biggest disadvantage faced was that the RT-PCR assay for βhCG is less sensitive when compared to hPL.

3. Obstetric markers in substance abuse

3.1 Obstetric markers in alcohol abuse

Alcohol abuse in the mother is a problem which affects the fetus drastically. The teratogenic effects of alcohol on the fetus are well known causing Fetal Alcohol Syndrome, a severe form of affection of the Fetal Alcohol Spectrum Disorder. Also the pregnant woman can commit crimes under the influence of alcohol.

The biomarkers specific to alcohol abuse in pregnancy are ethyl glucuronide (EtG), fatty acid ethyl esters (FAEEs) and ethyl sulphate (EtS) which are non-oxidative direct ethanol metabolites. These remain positive in maternal serum and urine for FAEEs for up to 24 h in serum and EtG in urine up to 5 days. These are promising. Also carbohydrate-deficient transferring (CDT) and phosphatidylethanol in blood of the mother are being evaluated.

3.2 Obstetric markers In drug abuse

Drug abuse in pregnancy is not uncommon. But unfortunately, the teratogenic effect of drugs can prove disastrous on the fetus. Use of cocaine prenatally causes preterm labour, abruption, congenital anomalies and low birth weight babies. Cannabis metabolites use causes difficulty in memory and learning. Opiate exposure prenatally results in withdrawal symptoms in neonates. Evaluation of these drugs in hair of the mother and meconium of the neonate can be done by standard chromatography methods. Information based on maternal hair depends on its length, while the exposure during pregnancy results in these drugs getting accumulated in the meconium, which needs to be analyzed as soon as its passed once fetus is born [17]. The forensic implications are that these pregnant women on drug use exhibit depression, anxiety, psychological struggle, can commit crimes under the drug influence and are liable for arrest and can be tried in the court according to the prevailing laws of the countries where they are prosecuted [18].

4. Obstetric markers in paternity suits

Paternity testing in the prenatal period could be required in cases of pregnancy resulting from rape. Earlier, the paternity testing depended on invasive procedures such as chorionic villus sampling and amniocentesis. These procedures could pose a risk to the wellbeing of the mother and fetus. In order to find a relatively safer testing method, non-invasive methods using the cell free fetal DNA (cffDNA) were investigated [19].

Initially the genetic markers investigated were short tandem repeat (STR) loci. But, the increased stutter amount hindering allelic assignments and decreased size of DNA fragments- maternal & fetal proved to be a hindrance [20]. Also only Y-chromosome STRs (Y-STRs) could be used which restricted the application to male fetuses only and not in female fetuses. The chances of false paternity exclusions were increased [21].

Then the Single nucleotide polymorphisms (SNPs) were investigated. A recent study done by Tam et al. developed a systematic SNPs selection procedure which reduced the number of target-SNPs for sequencing analysis to an average of 148 effective SNPs to calculate the probability of paternity. But the possible drawback is that in order to perform the test, a large number of loci is required [22]. But this is the mainstay for noninvasive paternity testing as of now.

With the number of SNPs to be tested on an average being 148, it can be cumbersome. So research is on to find a better genetic marker. Hence, the use of microhaplotypes is being researched. Microhaplotypes are the regions of ~200 bp containing two or more SNPs and at least three different haplotypes. Microhaplotypes with only 15 regions and with admixtures of DNA are being researched to determine paternity in a non-invasive manner [23].

5. Medicolegal implications

Obstetric markers is emerging as an important aspect in the forensic diagnostics. The pregnant women may face crimes like rape, physical harm against them. While, they may undergo criminal abortions, illegal feticide or have a substance abuse issue for which they can be held liable. The various obstetric markers- use of mRNA in bloodstains, use of biomarkers in substance abuse or the noninvasive genetic

markers for paternity testing, play an important role in solving the investigations and cases. Amongst the tests performed to determine if the bloodstain is that of a pregnant woman, the detection of mRNA of hPL is superior to other DNA analysis methods. Amongst the non-invasive paternity testing methods which is gaining prominence, the SNP detection is far superior to the STR detection. The emergence of use of Microhaplotypes which would further simplify the paternity detection is still under research.

6. Future research avenues

Obstetric markers in forensic diagnostics is an area of potential research. The possible areas would be to identify newer biomarkers which can be detected in bloodstains for a longer duration. Another potential area is to discover a technique which could estimate the gestational age from the blood stains obtained.

7. Conclusions

The field of forensic diagnostics has a widespread implication on solving cases related to pregnancy using specific obstetric markers. These markers are based on molecular technology. Bloodstains of pregnant women are best detected by RT PCR quantification of mRNA hPL. The ideal biomarkers for maternal alcohol abuse is still under investigation. Noninvasive Prenatal diagnostics is a helpful diagnostic aid as it does not harm the mother or fetus. Presently, SNP targets are being used for paternity detection and microhaplotypes as biomarkers are being investigated.

Acknowledgements

We are grateful to Kasturba Medical College, Mangalore and Manipal Academy of Higher Education, Manipal for instilling in us a scientific temperament and encouraging us to do more research.

Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

mRNA	Messenger ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
PS-beta-G, TSG, PAPP-C (SP 1)	Schwangerschaftspezifisches beta-1-Glykoprotein
hCG	Human chorionic gonadotropin
EDTA	Ethylenediamine tetraacetic acid
cffDNA	Cell-free foetal DNA
STR	Short tandem repeat
SNPs	Single nucleotide polymorphisms

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References

[1] Anjum Gahlaut et al. International Journal of Chemical and Analytical Science 2014,5(1),6-10

[2] Forensic DNA Applications: An Interdisciplinary Perspective chap15-Forensic tissue identification with Nucleic acids

[3] Sakurada, Koichi et al. "Current Methods for Body Fluid Identification Related to Sexual Crime: Focusing on Saliva, Semen, and Vaginal Fluid." Diagnostics (Basel, Switzerland) vol. 10,9 693. 14 Sep. 2020, doi:10.3390/diagnostics10090693

[4] Finanger T, Spigset O, Gråwe RW, Andreassen TN, Løkken TN, Aamo TO, Bratt GE, Tømmervik K, Langaas VS, Finserås K, Salvesen KÅB, Skråstad RB. Phosphatidylethanol as Blood Biomarker of Alcohol Consumption in Early Pregnancy: An Observational Study in 4067 Pregnant Women. Alcohol Clin Exp Res. 2021 Feb 15. doi: 10.1111/acer.14577. Epub ahead of print. PMID: 33586791.

[5] Chiandetti, A., Hernandez, G., Mercadal-Hally, M. et al. Prevalence of prenatal exposure to substances of abuse: questionnaire versus biomarkers. Reprod Health 14, 137 (2017). https://doi.org/10.1186/s12978-017-0385-3

[6] Sourcebook in Forensic Serology, Immunology, and Biochemistry 126-129 8.2 Identification of Retroplacental Blood, Blood Shed at Parturition and the Forenslc Dlagnosls of Pregnancy In Bloodstains

[7] Sourcebook in Forensic Serology, Immunology, and Biochemistry – Unit II

[8] Allejo G. Human chorionic gonadotropin detection by means of enzyme immunoassay: a useful method in forensic pregnancy diagnosis in bloodstains. J Forensic Sci. 1990 Mar;35(2):293-300. PMID: 2184196.

[9] Sourcebook in Forensic Serology, Immunology, and Biochemistry 126-129 8.2 Identification of Retroplacental Blood, Blood Shed at Parturition and the Forenslc Dlagnosls of Pregnancy In Bloodstains

[10] Gauvin J, Zubakov D, van Rhee-Binkhorst J, Kloosterman A, Steegers E, Kayser M. Forensic pregnancy diagnostics with placental mRNA markers. Int J Legal Med. 2010 Jan;124(1):13-7. doi: 10.1007/s00414-008-0315-6. Epub 2009 Jan 16. PMID: 19148664; PMCID: PMC2795858

[11] Pardi, N., Hogan, M., Porter, F. et al. mRNA vaccines — a new era in vaccinology. Nat Rev Drug Discov 17, 261-279 (2018). https://doi.org/10.1038/nrd.2017.243

[12] Enders K. O. Ng, Nancy B. Y. Tsui, Tze K. Lau, Tse N. Leung, Rossa W. K. Chiu, Nirmal S. Panesar, Lydia C. W. Lit, Kam-Wing Chan, Y. M. Dennis Lo. mRNA of placental origin is readily detectable in maternal plasma. Proceedings of the National Academy of Sciences Apr 2003, 100 (8) 4748-4753; DOI: 10.1073/pnas.0637450100

[13] Kayser M. Forensic pregnancy testing: a special case in molecular diagnostics. Expert Rev Mol Diagn. 2009 Mar;9(2):105-107. doi: 10.1586/14737159.9.2.105. PMID: 19298133.

[14] Zubakov, D., Kokshoorn, M., Kloosterman, A. et al. New markers for old stains: stable mRNA markers for blood and saliva identification from up to 16-year-old stains. Int J Legal Med 123, 71-74 (2009). https://doi.org/10.1007/s00414-008-0249-z https://doi.org/10.1007/s00414-008-0315-6

[15] Tsui NB, Ng EK, Lo YM. Molecular analysis of circulating RNA in plasma. Methods Mol Biol. 2006;336:123-134. doi: 10.1385/1-59745-074-X:123. PMID: 16916258.

[16] Okazaki S, Sekizawa A, Purwosunu Y et al. Measurement of mRNA of trophoblast-specific genes in cellular and plasma components of maternal blood. J. Med. Genet.43(9), e47 (2006).

[17] Shankaran S, Lester BM, Das A, Bauer CR, Bada HS, Lagasse L, et al. Impact of maternal substance use during pregnancy on childhood outcome. Semin Fetal Neonatal Med. 2007;12:143-150.

[18] Serino Ma D, Peterson Md BS, Rosen Md TS. Psychological Functioning of Women Taking Illicit Drugs during Pregnancy and the Growth and Development of Their Offspring in Early Childhood. J Dual Diagn. 2018 Jul-Sep;14(3):158-170. doi: 10.1080/15504263.2018.1468946. Epub 2018 Sep 5. PMID: 29694295; PMCID: PMC6202263.

[19] Christiansen, S.L., Jakobsen, B., Børsting, C. et al. Non-invasive prenatal paternity testing using a standard forensic genetic massively parallel sequencing assay for amplification of human identification SNPs. Int J Legal Med 133, 1361-1368 (2019). https://doi.org/10.1007/s00414-019-02106-01

[20] Fordyce SL, Mogensen HS, Borsting C, et al. Second-generation sequencing of forensic STRs using the Ion Torrent HID STR 10-plex and the Ion PGM. Forensic Sci Int Genet. 2015;14:132-140

[21] Zhang S, Han S, Zhang M, Wang Y. Non-invasive prenatal paternity testing using cell-free fetal DNA from maternal plasma: DNA isolation and genetic marker studies. Leg Med (Tokyo). 2018; 32: 98-103.

[22] Tam JCW, Chan YM, Tsang SY, et al. Non-invasive prenatal paternity testing by means of SNP-based targeted sequencing. Prenat Diagn. 2020;40(4):497-506.

[23] Wang, J.Y.T., Whittle, M.R., Puga, R.D. et al. Noninvasive prenatal paternity determination using microhaplotypes: a pilot study. BMC Med Genomics 13, 157 (2020). https://doi.org/10.1186/s12920-020-00806-w