Molecular Biomarkers: The Development of mRNA Multiplex RT-PCR Assay For The Definitive Identification of Semen

Thesis

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By

Lamis Mohamed Nader

M.B., B.Ch.M.Sc.

ASSISTANT LECTURER OF FORENSIC MEDICINE AND CLINICAL TOXICOLOGY
Faculty of Medicine, Cairo University

Supervised by

Prof. Dr. Manal Mohy El Din Ismail

PROFESSOR OF FORENSIC MEDICINE AND CLINICAL TOXICOLOGY
Faculty of Medicine – Cairo University

Prof. Dr. Amany Salah Mohamed

ASSISTANT PROFESSOR OF FORENSIC MEDICINE AND CLINICAL TOXICOLOGY
Faculty of Medicine – Cairo University

Prof. Dr. Dina Sabry Abd El Fatah

ASSISTANT PROFESSOR OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
Faculty of Medicine – Cairo University

Prof. Dr. Taymor Mostafa Ibrahim

PROFESSOR OF ANDROLOGY & STDs
Faculty of Medicine – Cairo University

Faculty of Medicine
Cairo University
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List of Abbreviations

A	Adenine
ACPP	Acid phosphatase, prostate
ALS	Alternative light source
AMI	Acute myocardial infarction
AMV-RT	Avian myeloblastosis virus- reverse transcriptase
ANOVA	Analysis of variance
AP-1	Activeator protein-1
AQPs	Aquaporins
B2M	Beta-2 microglobulin
C	Cytosine
CB	Cannabinoid receptor
CCL9	ligand 9
CT	cycle Threshold
C-to-U	Cytidine-to-uridine
DNA	Deoxynucleic acid
DNase	Deoxynuclease
dsRNA	Double stranded RNA
EB	Ethidium Bromide
EPO	Erythropoietin
FDD-PCR	Fluorescent mRNA differential display PCR
fsTnI	Fast sTnI
FWD	Freshwater drowning
G	Guanine
GAPDH	Glyceraldehyde 3 phosphate dehydrogenase
Gas5	Growth arrest specific 5
GLUT1	Glucose transporter 1
GP	Genome profiling
GTC	Guanidine thiocyanate
HB A	Haemoglobin A
HBB	Hemoglobin beta
HBD-1	Human beta-defensin1
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIF-1	Hypoxia-inducible factor 1
HIV	Human immunodeficiency virus
hnRNA	Heterogeneous nuclear RNA
hPL	Human placental lactogen
HPRI	Human Placental Ribonuclease Inhibitor
HTN3	Histatin
ICH	Idiopathic cerebral hemorrhage

IEGs	Immediate early genes
IL	Interleukin
KLK	kallikrein 3
MAT	Membrane aspiration test
MCE	Microcapillary electrophoresis
MCP-5	Mast cell protease-5
miRNAs	Micro RNAs
MKP-1	Mitogen-activated protein kinase phosphatase -1
MMLV	Moloney murine leukemia virus
MMP	Metalloproteinases
mRNA	Messenger RNA
MtDNA	Mitochondrial DNA
MUC 4	Mucin 4
MUP-1	Major urinary protein-1
NASBA	Nucleic acid sequence-based amplification
NB	Northern blot
ncRNAs	Non-protein-coding RNAs
NPE	Neurogenic pulmonary edema
P	Phosphate
PBGD	porphobilinogen deaminase
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PI	Postmortem immersion
PIC	Preinitiation complex
PlGF	Placenta growth factor
PMI	Post-mortem interval
PP	Pyrophosphate
PRM1	Protamine 1
PRM_2	Protamine 2
PSA	prostate-specific antigen
qRT-PCR	quantitative reverse transcription-PCR
RNA	Ribonucleic acid
RNAP	RNA polymerase
RNase	Ribonuclease
ROC	Recessive operating characteristics
RPA	Ribonuclease protection assay
RQ	Relative Quantification
rRNA	Ribosomal RNA
RT	Reverse transcriptase
RT-PCR	Reverse transcriptase- polymerase chain reaction
SAP	Seminal acid phosphatase
SD	Standard deviation
SEMG1	Semenogelin 1

SDS	Sequence detection software
SIDS	Sudden infant death syndrome
siRNAs	Small interfering RNAs
SnRNAs	Small nuclear RNAs
SOCO	Scene of Crime Officer
SP	Surfactant protein
SPTB	Spectrin
ssTnI	Slow sTnI
STATH	Statherin
SWD	Salt water drowning
TAE	Tris-Acetate EDTA
TFBSs	Transcription factor binding sites
TGM4	Transglutaminase 4
THC	Tetrahydrocannabinol
TIA	Thin-layer immunoassay
TnI	Troponin I
tRNA	Transfer RNA
U	Uracil
UV	Ultraviolet
VEGF	Vascular endothelial growth factor
WL	Wood's Lamp
Y-STR	Y chromosome short tandem repeats
βHCG	Beta human chorionic gonadotrophins

ABSTRACT

Background and rationale:

Semen is one of the most common body fluids found at the crime scene. Protamine-1 (PRM1), Protamine-2 (PRM2) and semenogelin-1 (SEMG1) are specific mRNA markers found only in semen so if it is possible to detect these markers in a forensic specimen by RT-PCR, this could be helpful to verify the presence of semen.

Aim of the Work:

This work aims at identifying semen from other body fluids in forensic samples by molecular assessment of specific RNA biomarkers

Subjects and methods:

Semen samples were collected from 50 inpatients at the andrology section and outpatients at the andrology clinic that were divided into 3 main groups including: normozospermic, oligozospermic and azoospermic groups and only 10 blood samples were taken from the same patients to assess the presence of the markers in blood. The next steps of work were RNA extraction from semen and blood, PCR amplification of genes, detection of the amplified gene using agarose gel electrophoresis and performing gene expression analysis of the 3 genes using quantitative RT-PCR.

Results:

(PRM1), (PRM2) and (SEMG1) were only detected in semen samples and were totally absent in blood samples. PRM1 was the most specific and reliable marker for semen identification followed by PRM2 and SEMG1 was the least specific. These markers are not only used for identification but also for detection of the semen profile of the suspect. There was a direct positive correlation between sperm count and RNA expression. Light and humidity are detrimental factors for RNA stability.

Conclusion and Recommendations:

Each tissue or cell type makes a unique constellation of mRNAs, some specific for only that tissue or cell type. Therefore, analysis of the "RNA profile" in a sample can uniquely identify the fluid or tissue of origin.

RT- PCR is valuable in semen identification by gene expression analysis of PRM1, PRM2 and SEMG1. PRM1 is the most specific and reliable marker used for semen identification. Light and humidity are important factors affecting RNA expression.

Key words:

Semen, Identification, RT-PCR, Protamine-1, Protamine -2, semenogelin-1, RNA stability

INTRODUCTION

Forensic identification consists of two major steps. First, a determination must be made as to whether the questioned and the known marks are indistinguishably alike. The second step is to evaluate the meaning of their appearing indistinguishably alike. The first step might seem straightforward, but it is fraught with error risks. In forensic odontology, skin might be temporarily stretched or compressed at the time a bite is inflicted, and examiners must try to account for whether and how much that altered the appearance of a bite. Examiners must avoid mistaking class characteristics for so-called individualizing characteristics (**Thompson and Cole, 2007)**.

They must distinguish differences that are real ‘‘unexplained’’ from those that are artifactual (differences are always present; examiners must decide what to make of them). These and other problems were explained in greater detail by Bowers**(Bowers,2010)**. For most areas of forensic identification, all of this is entirely subjective, which means that there are no objective standards to guide these judgments. Solutions to these problems can be found. For example, research is being conducted in an effort to make the evaluation of DNA profiles in electropherograms more data-based and objective **(Krane et al., 2008).**

Once it is properly determined that a questioned and a known are indistinguishably alike, the second step is to evaluate the meaning of that finding. Many forensic identification examiners skip the second step entirely, simply assuming that finding a match at the first step in and of itself establishes their common source. Rather than calculating the frequency of such matches in the relevant population or sub-population, based on data, examiners simply assume identity. This is where the assumption of uniqueness substitutes for science. The flaw in this assumption is shown by morphometric studies of simulated bite marks which demonstrate that as many as 15% of participants could generate bite patterns that were indistinguishable from each other**(Blackwellet al., 2006)**.

At least three research strategies could be adopted to move forensic identification science forward onto more secure ground, which can be termed the DNA model, the black box model, and the basic research model**(Saks, 2010)**.

1- The DNA model

DNA typing has provided the most defensible approach to forensic identification yet developed. Physical attributes of the objects of interest are measured, data are collected on the variation of these attributes in a reference population, and the probability of a coincidental match is determined and reported. No assumption of uniqueness is necessary and none is employed. Objective data are collected and used to guide judgments about the relative rarity of the questioned and known samples.

DNA typing thus provides a model for how the rest of forensic identification science could and should carry out its work. Difficulties concerning measurement of complex patterns will have to be surmounted, requiring the help of fields which deal with such problems. While none of this obviates problems at the first stage (comparison), those also can be and are beginning to be studied and standards developed to reduce or remove problematic subjectivity from the comparison process**(Saks and Koehler, 1991)**.

2- The black box model

Another model is to leave the process of judging similarity and drawing inferences of its improbability to the examiner using conventional subjective methods, but to harness those human ‘‘black boxes’’ to a program of serious research. By systematically presenting examiners with different problem tasks within their various domains (e.g., for handwriting examiners: signatures, cursive writing, hand printing, with different degrees of writing complexity, intra-writer variation, and so on), a ‘‘map’’ can be drawn that describes which examination tasks can be performed at what level of accuracy. By systematically varying the adequacy of the questioned evidence (e.g., for fingerprint examiners: the completeness of the latent print, the quality of the latent), the level of accuracy associated with that amount and quality of evidence can be estimated, and limits can be defined beyond which an opinion have not be ventured**(Dror et al., 2006)**.

3- The basic research model

Each subfield of forensic identification is rich with beliefs about the nature of the evidence and the process of examining and drawing inferences. These beliefs can be regarded as hypotheses that need to be tested. Specific studies can be designed to test them. If forensic identification is like other fields – as varied as medicine or arson investigation – many of the hypotheses will turnout to be correct and many will turn out to be incorrect. It is important to learn which are which**(Saks, 2010)**.

Some traditional fields have taken steps along the lines of the above models. For example, a movement within the field of firearms examination has begun to develop what in effect are empirically based statistical floors of accuracy. By comparing the frequency of consecutive matching striae in pairs of bullets that are known matches versus pairs that are known non-matches ,minimum consecutive matching striae can be stated. This is somewhat of a hybrid approach, crossing the DNA model with the basic research model**(Biasotti et al., 2010).**

Body fluid traces recovered at crime scenes are among the most important types of evidence to forensic investigators. They contain valuable DNA evidence which can identify a suspect or victim as well as exonerate an innocent individual**.** Besides, determination of the type and origin of biological samples found at crime scenes can provide important clues for crime scene reconstructions by supporting a link between sample donors and actual criminal acts. The presence of certain body fluids can be used as excellent indicators of the sequence of events which occurred. For example, blood stains can indicate some form of physical struggle, assault or murder, and detection of semen or vaginal fluid can indicate the involvement of some form of sexual encounter or assault. The common body fluids found at crime scenes are blood, semen, saliva, vaginal fluid, urine, and sweat (**Ja Hyunet al., 2012*)*.**

Semen is one of the other most commonly encountered body fluids at crime scenes. Sexual assault is usually a hidden crime where the only witnesses are the victim and the assailant. For a variety of reasons, even the victim may be unable to provide a detailed account of the assault or the identity of the perpetrator. Often the case history deteriorates into one person's word against another. With such limited initial information, the physical and biological evidence collected from the victim, from the crime scene, and from the suspect will play a pivotal role in the objective and scientific reconstruction of the events in question .The total volume of semen for each ejaculation of a human male ranges between 2 and 5 ml (0.12 to 0.31 cubic inch); in stallions the average ejaculate is about 125 ml (7.63 cubic inches). In human beings each ejaculation contains normally 200 to 300 million sperm, making semen ideal for DNA profiling **(Giuliano and Clementa, 2005).**

Molecular forensic science has been predominated by DNA research in the past decade. Furthermore, RNA was and is notorious for its allegedly rapid postmortem and in vitro decay which has kept forensic scientists from investing time and money into this field**(Bauer, 2007).**

However, times are changing and the enormous potential of RNA technologies together with reports of unexpectedly high stability in certain conditions has stimulated forensic researchers all over the world to start exploring the RNA world. Messenger ribonucleic acid (m RNA) is a single strand nucleic acid involved in transcription and translation processes. It provides a matrix for protein synthesis. A typical mammalian cell contains 10–30 pg of total RNA, of which only 1–5% is mRNA. A single cell contains approximately 360,000 mRNA molecules, which constitute 12,000 different transcripts that differ in their percentage representation in the general mRNA pool. While DNA is identical in each cell of an organism, the mRNA profile demonstrates what genes are expressed and what proteins are synthesized **(Alberts et al., 1994).**

The first step of identifying a particular body fluid is highly important since the nature of the fluid itself is very informative to the investigation, and the destructive nature of a screening test must be considered when only a small amount of material is available. The ability to characterize an unknown stain at the scene of the crime without having to wait for results from a laboratory is another very critical step in the development of forensic body fluid analysis. Driven by the importance for forensic applications, body fluid identification methods have been extensively developed in recent years**(Kelly and Igor, 2009).**

Semen can be detected using an alternative light source(ALS) such as ultraviolet light. It is a routine procedure to search for semen and other fluids using this simple and non-destructive method (**Sensabaugh, 1982).**