FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 45, No. 2, 2007
pp. 135-136

Vitreous humour as a potential DNA source for postmortem human identification

Ireneusz Sołtyszewski[1], Anna Niemcunowicz-Janica[2], Witold Pepiński[2], Magdalena Spólnicka[3], Renata Zbiec[3], Jerzy Janica[2]

[1]Department of Criminalistics and Forensic Medicine, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland
[2]Department of Forensic Medicine, Medical University of Bialystok, Bialystok, Poland
[3]Department of Biology, Central Forensic Laboratory of Police, Warsaw, Poland

Abstract: Purpose: The aim of this study was the assessment of vitreous humor as a potential DNA for forensic human postmortem identification. Material and methods: Vitreous humor samples were collected using two alternative approaches from 25 corpses of either sex during autopsies. DNA was extracted by standard organic method. Recovered DNA was quantitiated fluorometrically. AmpFlSTR SGM Plus kit and ABI 310 Genetic Analyzer (Applera) were used to obtain genetic profiles. Results: Different DNA yields were quantitated in vitreous body depending on cause of death and sampling approach. Conclusion: Vitreous humor is a potential DNA for forensic human postmortem identification depending on a sampling method used.

Key words: Vitreous humour - DNA quantitation - Forensic genotyping

Introduction

Vitreous humour is a clear gelatinous substance that fills the space between the lens and the retina of the vertebrate eyeball. The solution is 99% water including salt ions, sugars, phagocytes and a network of collagen fibres [4]. According to extensive literature vitreous humour (VH) can be used as an alternative and complementary biological fluid in the determination of ethanol levels or psychoactive substances in decomposed and nondecomposed bodies [1,3,7]. Decomposition of cells, tissues and organs result from processes of autolysis and putrefaction. Autolysis involves enzymatic degradation of cells or tissues, putrefaction is caused by saprophytic microbial organisms. Postmortem changes are correlated with extrinsic and intrinsic conditions including age and weight, antemortem diseases and injuries [5]. DNA typing as well as ethanol level determination in severely decomposed bodies is often difficult due to water loss from the circulatory system, putrefaction and autolytic processes [6]. The authors attempted to assess vitreous humour as an alternative DNA source in genetic identification of human corpses and remains and in verification of toxicologic samples origin.

Correspondence: W. Pepiński, Dept. of Forensic Medicine, Medical University of Bialystok, ul. Waszyngtona 13, 15-230 Bialystok, Poland, e-mail: pepinski@amb.edu.pl

Material and methods:

The vitreous humor samples were collected from 25 corpses of either sex during autopsies. Causes of death and postmortem intervals are summarized in Table 1. Periphereal blood samples collected from respective cadavers were used as genotyping references. VH specimens were collected by puncture through the both eyaballs through internal angle at approximately 12mm distance from corneal nimbus with sterile syringe needles of 1mm diameter. The specimens were aspirated to sterile syringes of 1.5ml volume. Two different approaches were used for the specimen transfer to DNA extraction tubes: I. the VH aspirates from right eyeballs were discharged through syringe needles, II. the VH aspirates from left eyeballs were discharged directly from syringes after needle detachment.

DNA was extracted by organic method using 2% SDS 1 M DTT and proteinase K. Recovered DNA was quantitiated fluorometrically using PicoGreen dsDNA Quantitation Reagent (Molecular Probes) and Fluoroskan Ascent FL (Thermo Electron Corporation, Inc.). For quantification human DNA this method could replace currently used Quanti-Blot (Applera). The commercially available kit AmpFlSTR SGM Plus (Applera, USA) was used according to the manufacturer's instructions. The kit contains the reagents necessary for amplification of: the following loci: D3S1358, VWA, D16S539, D2S11338, D8S1179, D21S11, D18S51, D19S433, TH01, FGA and gender marker - amelogenin.

Table 1. Causes of death and PostMortem Intervals for the experimental corpses

Cause of death	No of corpses	PMI (hours)
road traffic accident	8	24-96
drowning	4	48-133
suicide by hanging	5	24-26
morbid condition	5	24-50
fatal poisoning	3	24-34

Positive (calibrated DNA fenotype) and negative (no DNA) controls were using during all methods steps. DNA samples were amplified using GeneAmp PCR System 9700 (Applera, USA). Genotyping was performed in a 310 ABI Genetic Analyzer (Applera, USA).

Results and discussion

DNA quantitation results in VH aspirates discharged through syringe needles (Approach I) are presented in Table 1. DNA concentrations measured by Fluoroskan were significantly higher than those measured by QuantiBlot. The fact may be attributable to considerable contents of exogenous (microbial) DNA due to circumstances of death and a time interval before autopsy as well as a higher sensitivity of the fluorimetric method. Consequently, the highest total DNA yields were obtained from VH specimens collected from drowning victims. No significant differences were found among human (primate) DNA concentrations regardless of cause of death. Our results correspond with the those reported by Josephi et al. who collected samples from cadavers in different stages of decomposition [2]. Full SGM Plus profiles typed in all VH aspirates discharged through syringe needles (Approach I). The profiles matched those typed in reference blood samples. No DNA was found in VH specimens discharged directly from syringes (Approach II) regardless of the quantitation method used. The obtained results indicate that VH itself is not human DNA source. The authors suggest that DNA quantitated in VH specimens collected using Approach I is an artifact due to the sampling method.

Table 2. DNA quantitation results in VH aspirates discharged through syringe needles

Cause of death	DNA quantitation method	
	Fluoroskan (total DNA) ng/µl	QuantiBlot (primate DNA) ng/µl
road traffic accident	0.82 – 11.20	0.63 – 2.50
drownig	67.1 – 93.0	0.63 – 1.25
suicide	0.84 – 5.80	0.63 – 2.50
morbid condition	2.35 – 7.40	0.63 – 1.25
fatal intoxication	1.46 – 6.40	0.31 – 2.50

Conclusions

1. Reliability of DNA quantitation in VH during forensic autopsies depends on a sampling method used.
2. DNA content in the isolates may result from endogenous and/or exogenous (microbial) contamination.

References

[1] Felby S, Olsen J. Comparative Studies of Postmorten Ethyl Alcohol in Vitreous Humour, Blood and Muscle. J Forensic Sci, 1969; 14: 93-101
[2] Josephi E, Braito U, Tutsch-Bauer E. DNA extraction from body fluids of cadavers. Beitr Gerichtl Med, 1990; 48: 1-5
[3] Raszeja S, Kreuger A, Olszewska I. Stężenie alkoholu etylowego w ciałku szklistym oka i mazi stawowej. Arch Med Sad i Krym, 1979; 21: 61-66
[4] Stevens A, Lowe JS. Histologia człowieka. Warszawa: PZWL; 2000
[5] Takayama T, Nakamura I, Watanabe Y, Yamada S, Hirata K, Nagai A, Bunai Y, Ohya I. Quality and quantity of DNA in cadaver's serum. Legal Med, 2003; 5: 180-182
[6] Trela FM. Badania porównawcze stężeń alkoholu w żółci, w ciałku szklistym i we krwi pobranych ze zwłok. Arch Med Sad i Krym, 2000; 50: 227-233
[7] Winek CL, Esposito FM. Comparative study of ethanol levels in blood versus bone marrow, vitreous humour, bile and urine. Forensic Sci, 1981; 17: 27-361

Received: December 18, 2006
Accepted after revision: January 31, 2007