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Original communication

Demonstration of ethyl glucuronide in dental tissue samples by liquid chromatography/electro-spray tandem mass spectrometry

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ABSTRACT

Introduction: Ethyl glucuronide (EtG) has been studied in various tissues and body fluid for determination of alcohol intake. However, no study, dealing with EtG analysis in dental tissue, was performed so far. In this study, we aimed to demonstrate EtG levels in dental tissue.
Materials and methods: Michigan Alcohol Screening Test (MAST) was performed to 29 participants. Following the test, cases were divided into three groups as non-hazardous alcohol users, alcohol abusers and 6 controls who verbally declared that they were abstainers. A total of 29 tooth specimens, obtained from participants, was included in the study. These specimens were analyzed using LC/MS/MS.
Results: All of the participants included in the study were male. According to the MAST outcomes 14 of the participants were non-hazardous alcohol users, and 9 were alcohol abusers, while 6 patients verbally declared that they were abstainers. Dental tissue analyses revealed EtG levels ranging between EtG < LOD and 23.39 pg/mg. EtG levels were observed to be <LOD in dental specimens of 6 abstainer cases. A significant correlation was found between EtG levels measured in the dental tissues and MAST outcomes on the statistical analyses ($r = 0.914$).
Conclusion: The findings of the present study demonstrated that dental tissue can be used for detection of alcohol intake, using LC/MS/MS.

1. Introduction

Alcohol use continues to rise throughout the world.[1] Thereby, alcohol related issues are still of high importance in daily forensic practice. Alcohol is metabolized following intake in living ones, while microbial activity and fermentation of glucose after death may lead to postmortem production of alcohol under some circumstances in the corpses, which might cause serious problems during evaluation of forensic cases.[2–5] Determination of levels of consumed alcohol is unreliable in some cases because of alcohol levels possibly produced by microbial activity. And detecting an accurate level of ethanol became impossible in such cases. Therefore, ethyl glucuronide (EtG), a minor metabolite of ethyl alcohol, is used for this purpose.[6–8] By a number of researchers, EtG has been previously demonstrated in various tissues and body fluid for revealing alcohol intake.[6,9–12] However, to date, no study dealing with EtG analysis in dental tissue was performed.

Dental tissue is one of the specimens used in forensic medical applications and analyses.[13] Because it is a live tissue that give response to physiological and pathological alterations with its micro-circulation.[14–16] Dental tissue is of high importance due to its durable structure in case of the deterioration of the body integrity caused by diseases, postmortem changes and severe traumatic events.[13,17]

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In this study, to the best of our knowledge as the first study in this field we aimed to demonstrate EtG levels in dental tissue using LC/MS/MS method and to reveal its correlation with alcohol intake behavior.

2. Materials and methods

2.1. Ethics statement and subject

The tooth specimens used in this study were obtained from the patients presented to Dental Clinic of Mustafa Kemal University, Medical Faculty for examination. Extracted teeth of the patients in whom the decision was made for tooth extraction were taken after informing them about the study. Informed consent was given by all participants. Michigan Alcohol Screening Test (MAST) was performed to 29 participants. Following the test, cases were divided into three groups as non-hazardous alcohol users, alcohol abusers and 6 controls who verbally declared that they were abstainers. Twenty-nine tooth specimens obtained from these patients were included in the scope of the study. The specimens were preserved in clean Eppendorf tubes and consequently analyzed with LC/MS/MS method. The protocol of this study was approved by the Mustafa Kemal University Ethics Committee.

2.2. Chemicals, reagents and materials

EtG and deuterium-labeled EtG-d5 standards (internal standard) were obtained from Medichem (Stuttgart, Germany). All solvents were hypergraded for LC–MS LiChrosolv and purchased from Merck KGaA (Darmstadt, Germany). Deionized water was obtained from the Milli-Q (Millipore, Bedford, USA) water purification system.

Stock solutions of EtG (10 μg/ml) and d_5-EtG (2.5 μg/ml) were prepared in methanol and they were stored at −20 °C. Working standard solutions used for calibration and quality control samples were prepared by 2, 5, 10, 20, 50, 100, 200, 1000 and 2000 ng/ml. All working solutions were stored in a refrigerator when not in use.

2.3. Specimen preparation

Extracted teeth were cut from the root part containing dentin. After the addition of 5–6 steel ball bearings (2 mm diameter) to each vial, the capped vials were placed in a Mini-Bead-Beater-8, a high energy cell disrupter (BioSpec Products, Bartlesville, OK, USA) for approximately 3 min or until powdered.

The powderized specimen of 50 mg was weighed with a sensitive scale and placed in a tube. A mixture of 50% acetonitrile/50% water was added on it, and this mixture was kept in an ultrasonic bath at 25 °C for 2 h. Then, internal standard of 50 μl was added on it and mixed with vortex. Then it centrifuged at 4000 rpm for 10 min. 2 ml was extracted from the upper part and placed in the autosampler vials. The specimens were separately subjected to extraction with mixtures of water/acetonitrile/methanol, acetonitrile/water of 80% and acetonitrile/water of 50% and the best result was obtained with the mixture of 50% acetonitrile/50% water.

2.4. LC–MS/MS conditions

The specimens were analyzed using an Agilent Technologies 1200 system that consisted of a G1367C autosampler, a G1379B degasser, G1312B binary pump. Separation was achieved using two Zorbax Hilic Plus (4.6 × 100 mm, 3.5 micron particle size) serial connected column. Reverse–reverse chromatographic technique was used. The column was held at 25 °C in a G1316B Thermostatted Column Compartment (Wilmington, DE, USA). The solvent system was a gradient that consisted of A (1 mM NH4Ac) and B (acetonitrile), using a flow rate of 0.8 mL/min. The solvent program held B at 65% from 0.0 min to 2.2 min. Solvent B was decreased to 20% between 2.3 and 9.5 min. Solvent B was increased to 20% at 5.1 min and held at 65% until 10.0 min. The detector was Agilent Technologies 6460 Triple Quad LC/MS System using electro-spray ionization (ESI) in the negative mode (Wilmington, DE, USA). The capillary voltage was set at 4000 V, the nozzle voltage set at 0 V and the desolvation gas (nitrogen) was heated to 350 °C with a flow of 11 l/min. Nebulazator pressure: 50 psi. The sheath gas (nitrogen) was heated to 350 °C and delivered at 11 l/min.

The internal standard (ETG-d5) was monitored using the m/z 226.0 > 75.0 (quantification ion) transition and the m/z 226.0 > 85.0 (qualifying ion) transition. The m/z 221.0 > 75.0 (quantification ion) and m/z 221.0 > 85.0 (qualifying ion) transitions were used to monitor ETG. All three transitions used a fragmentor voltage of 100 V and collision energy of 12 V. All data were processed using MassHunter B.04.01 (Wilmington, DE, USA).

Start	RT	End	Height	Area	Signal To Noise
1.986	2.045	2.13	14	62	12.4

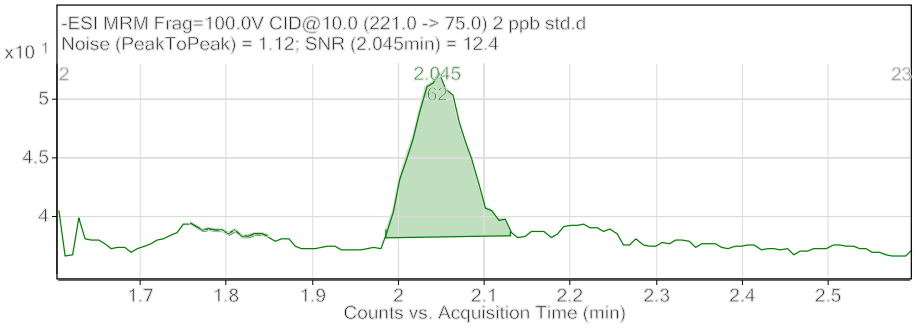

Fig. 1. Integration peak list.