

## Research Article

# Ethyl Glucuronide Determination in Nails for Alcohol Consumption Screening in Adolescents

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Submitted: 14 April 2015

Accepted: 17 July 2015

Published: 20 July 2015

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**OPEN ACCESS****Keywords**

- Ethyl-glucuronide
- Biomarkers
- Nails
- Alcohol consumption
- Adolescents
- Emergency Room

**Abstract**

Alcohol is the most consumed substance of abuse in our environment and at a premature age. Alcohol consumption and abuse in adolescents can lead to a number of health and social issues. Determination of ethanol consumption in adolescents by questionnaire is not a reliable method of screening. Biomarkers of ethanol in alternative biological matrices are useful tools in prevalence studies.

The aim of our study was to establish the real prevalence of alcohol consumption in preadolescents and adolescents through the evaluation of ethyl glucuronide, an ethanol biomarker, (EtG) in nails. We conducted a prospective study in 127 children between 11 and 16 years at Hospital del Mar (Barcelona) and 107 adolescents between 13 and 18 years at Hospital Virgen de la Arrixaca (Murcia) who consulted for any reason at the Emergency Department. They answered a questionnaire about alcohol and other drugs of abuse consumption and then samples of fingernails were obtained. Twenty-nine samples in Barcelona (22.8%) and 21 samples in Murcia (20%) were positive for EtG. A total of 93.1% of patients that were positive for EtG in nails in Barcelona and 33.3% in Murcia didn't report alcohol consumption in the questionnaire. In conclusion, we found that alcohol consumption in preadolescents and adolescents in our area appeared to be a topic of concern and questionnaires fail to detect chronic consumption of alcohol in the majority of cases. For this reason we suggest EtG determination in nails could be a screening tool for chronic and sustained alcohol use in adolescents.

**ABBREVIATIONS**

EtG: Ethyl Glucuronide

**INTRODUCTION**

The most declared consumed substance of abuse by adolescents in Spain between 14 and 18 years old is alcohol (81.9%) [1]. The average ages at first time consumption are between 13 and 16 years, usually starting with alcohol or tobacco. Prevalence of drunkenness during the last month varies from 26% in 14 years old to 63% in 17 years old adolescents. Self-reported binge drinking, known as drinking 5 or more glasses

in 2 hours, during the last month also increases with age (20% at 16 years old, 51% at 17 years old) [1]. The consumption of alcohol from an early age can lead to a wide range of health and social deleterious effects. Heavy drinking is associated to liver disease, cancer and also worsens illnesses such as diabetes and cardiovascular disease. In addition, alcohol abuse correlates with higher incidence of family problems and traffic accidents among others [2].

Self-reported questionnaires which aim to quantify alcohol intake, are still 'gold standard' for assessing alcohol use pattern [3,4]. The applicability of these tools must be assessed in relation

to adolescents and be complemented with other relevant items such as quantity, frequency and pattern of consumption (binge drinking, age of onset, polydrug use, etc.) from questionnaires currently used in our country to analyse consumption in children [1].

In health care scenarios, adolescents usually do not tell the truth when being asked about their alcohol intake by a professional, because they fear their parents or tutors may know their answers. Furthermore, the use of biomarkers in biological alternative matrices, such as nails or hair, which are able to assess acute and chronic use of alcohol, is crucial.

EtG is a minor metabolite of ethanol produced by conjugation pathways that has been studied as a biomarker for alcohol consumption in different matrices such as serum, urine, meconium, placenta, hair and nails [5-8]. It can be found in urine or blood as a biomarker of acute consumption but it can also be found in hair or nails indicating chronic consumption [8]. Fingernail, a layered keratinised structure, originates at its matrix and grows along the nail bed at approximately 0.1 mm/day. Analytes are incorporated into the fingernail by blood flow in the nail matrix and from the nail bed underneath as the fingernail grows [5]. Another way of incorporation into nails, not yet well studied could be through sweat. Jones and colleagues found that fingernails could be an alternative to hair and even preferred because of higher concentrations present [5]. Moreover, Morini and colleagues also found that EtG is accumulated in nails at a higher rate than in hair and it could also be a useful marker for binge drinking [6].

The aim of our study was to establish the prevalence of alcohol consumption in preadolescents and adolescents through EtG in nails in comparison with standard questionnaire.

## MATERIALS AND METHODS

This study was conducted in the Paediatric Emergency Department of the Hospital del Mar in Barcelona and Hospital Virgen de la Arrixaca in Murcia. Both hospitals are located in urban areas with low socioeconomic status (SES) and a high percentage of immigrants. Fingernail samples were collected from children between 11 and 16 years at Hospital del Mar and adolescents between 13 and 18 years at Hospital Virgen de la Arrixaca, admitted to the Emergency Department for a variety of general medical complaints and after hand washing with a non alcoholic cleaning product. The study was conducted in two different hospitals to have a more varied population and also a broader age range. They were asked about past year ethanol consumption using CRAFFT questionnaire. Its confidentiality was explained to the children and it was conducted without their parents' presence.

The study was approved by the Local Ethics Committee (CEIC-PSM) and a signed informed consent was obtained from the accompanying parent and the adolescent.

Nails were tested for EtG by liquid chromatography tandem mass spectrometry. All the available methods for the analysis of ethyl glucuronide (EtG) in nails employed LC-MS-MS with electrospray ionization, but used different sample preparation procedures [5,7,9,10]. Using a simple extraction in aqueous

solution of fingernails, followed by a direct injection into a liquid chromatographic tandem mass spectrometric system (LC-MS/MS), an analytical procedure already validated and previously published by Morini and colleagues [4,6] achieved low LODs (2pg/mg) and LOQs (8 pg/mg).

## RESULTS AND DISCUSSION

Out of 234 samples tested in Barcelona and Murcia, 21.4% were positive for EtG in nails (22.8% in Barcelona and 20% in Murcia separately) (Table 1). From all females analysed, 23.9% were positive for EtG in nails whereas only 19% of males were positive, a non statistically significant difference. When we analysed the positive group we saw a similar percentage under (20.4%) and over (21.6%) 12 years old. As we analysed the proportion of positives for EtG in nails by age, we found a peak of consumption in adolescents around 15 years old (47.8%) in both Murcia and Barcelona.

Finally, we found that from all the children that were found positive for EtG in nails, 93.1% in Barcelona and 33.3% in Murcia had reported that they did not drink alcohol in the previous months. These differences between both cities can be explained by the average age in both groups, 12.7 years old in Barcelona versus 15.7 years old in Murcia, due to the fact that younger children may deny more often their alcohol consumption.

As a metabolite of ethanol, EtG is a useful biomarker of alcohol consumption, and can be found not only in blood and urine but also in hair and nails making it an indicator of both acute and chronic consumption. To our knowledge, this is the first study using EtG in nails as a biomarker to assess chronic alcohol exposure in adolescents. Other studies have used hair as a screening tool of exposure to other drugs of abuse in children [11,12]. In these studies, unsuspected high prevalence of paediatric exposure to drugs of abuse has been found repeatedly through the years.

Various studies have examined EtG in nails as a long-term alcohol biomarker. Firstly, Morini and colleagues validated EtG in nail assay and then compared it with hair and meconium in pregnant women and their babies, focusing in prenatal exposure [6,13]. Secondly, Berger and colleagues reported that EtG had not only qualitative but also quantitative value, especially in nails [7]. Consequently, it has also been suggested that EtG testing in nails used along with self-reported questionnaires could be a perfect tool for monitoring treatment programs, organ transplant

**Table 1:** Results by EtG in nails compared to declaration by questionnaire.

	Barcelona (n=130)	Murcia (n=114)	Total (n=234)
Age (yo)	12.7	15.7	14.2
EtG positives in nails (%)	22.8	20	21.4
EtG positives and negative by questionnaire (%)	93	33.3	63.8
Gender of EtG positives (%)	21.9 female 15 male	25.9 female 22.3 male	23.9 female 19 male

care, research trials and screening in pregnant women [7,8,13]. Because of higher EtG levels in nails compared to hair, nails may potentially be the preferred matrix to differentiate teetotallers from moderate alcohol consumers [13,14].

On the contrary to our findings, Morini and colleagues<sup>6</sup> reported that EtG concentrations in fingernails correlated with self-reported alcohol consumption, though it is not yet possible to find proper cut off. This difference could be explained because their study was conducted among teetotallers, social drinkers and heavy drinkers without specifying gender or age. Another study carried out by Keten and colleagues [10] in 16 males, mean age of 45.5 years old, also correlated EtG levels in fingernails and alcohol intake behaviour previously assessed.

Finally, differences between ages have been found in our study. We have found that 20.4% of the preadolescents tested were positive for EtG in nails, 30.8% of girls and 8.7% of boys, none had admitted consuming alcohol. In our country, preadolescents start high school at the age of 12. Preventive campaigns of alcohol consumption and other drugs are usually focused on adolescents at high school, forgetting that it might be late since consumption may start much earlier.

In our study we aimed to provide a useful screening tool for alcohol abuse in preadolescents and adolescents. The use of biomarkers in matrices which assess chronic use of alcohol may help in detecting situations of premature use of alcohol that could help to prevent its social and health consequences.

## CONCLUSION

Our study shows a high prevalence of chronic alcohol consumption in preadolescents and adolescents. We found that questionnaire is a non useful tool to screen consumption of alcohol in preadolescents and adolescents in a health care scenario. EtG in nails is a useful biomarker of chronic alcohol consumption. These results support the implementation of different strategies. On the one side, preventive strategies should be implemented by recommending interventions of public health and social services to raise awareness of risks regarding to alcohol and other drug consumption in children and adolescents and also providing guidelines for paediatricians, nurses and other professionals dealing with adolescents. On the other side, screening protocols for alcohol consumption should be implemented in emergency departments, where children and adolescents are usually visited. Although acute alcohol consumption can be accessed through measurement of blood and breath levels of ethanol, this does not indicate chronic alcohol use, or binge drinking habits. For this reason EtG determination in nails could be recommended as a screening tool for alcohol abuse that should be used by clinicians dealing with adolescents. Furthermore, we would like to remark the importance of implementing these tools and strategies in preadolescents, especially in low socioeconomic environments, where alcohol consumption may start before 12 years of age. Paediatricians and teachers in primary schools should be aware of this situation so that prevention strategies could be implemented successfully.

## ACKNOWLEDGEMENTS

This study was supported by grants from Plan Nacional Sobre Drogas (2011/13) (and Red de Salud Materno-Infantil y del Desarrollo (SAMID) (RD12/0026/0003), FEDER, Instituto Carlos III (Spain), and from Generalitat de Catalunya (Spain) (2014SGR584).

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### Cite this article

Mercadal-Hally M, Falcon M, Luna A, Sánchez-Roig L, Morini L, et al. (2015) Ethyl Glucuronide Determination in Nails for Alcohol Consumption Screening in Adolescents. *J Subst Abuse Alcohol* 3(3): 1035.