Determination of Toluene in Antemortem and Postmortem Biological Specimens of Volatile Abusers and Evaluation of User Profile in İstanbul

Uçucu Bağımlılarının Antemortem ve Postmortem Biyolojik Örneklerinde Toluenin Belirlenmesi ve İstanbul'daki Kullanıcı Profilinin Değerlendirilmesi

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ABSTRACT Objective: It is known that the amount of toluene in postmortem biological specimens of volatile abuser cases is an important evidence for proving primarily useful data when providing evidence about cause of death. The aim of this study was to analyze antemortem and postmortem blood and urine specimens of volatile abusers in terms of forensic toxicology and obtaining the abusers' profile in Istanbul region. **Material and Methods:** Headspace Gas Chromatography-Mass Spectrometer (HS-GC/MS) technique was used for analysis of toluene in blood and urine samples of twenty five antemortem and six postmortem cases. Chromatographic separations were acquired by using a capillary column of HP-INNOwax, and n-butanol was used as internal standard (IS). **Results:** The limit of detection (LOD) and the limit of quantification (LOQ) values were predicted as 0.01 mg/L and 0.1 mg/L, respectively. In antemortem cases, toluene amounts in urine and blood samples were found between 0.32 and 4.39 mg/L; 0.48 and 15.49 mg/L, respectively. According to the survey results, the average beginning age for glue and thinner abusers was 14.4 years. Among the survey participants, 80% had abused drugs and alcohol together with glue and/or thinner. In postmortem cases, toluene amounts in blood were found between 3.2 and 51.3 mg/mL. **Conclusion:** In this study, amount of toluene in both antemortem and postmortem biological specimen was determined with a rapid and accurate method. It is thought that the determination of blood toluene concentration play an active role in the treatment of abusers. On the other hand, establishment of volatile substance abusers' profile showed that the users abused other illicit drugs together with volatile substances. This situation confirmed that abuse of thinner and glue was known as transient phenomenon tousing narcotics.

Key Words: Toluene; inhalant abuse; forensic toxicology

ÖZET Amaç: Uçucu madde istismar eden postmortem vakaların biyolojik örneklerinde toluen miktarının ölüm nedeninin belirlenmesinde önemli bir kanıt olduğu bilinmektedir. Bu çalışmanın amacı uçucu istismar eden vakalarda antemortem ve postmortem kan ve idrar örneklerinin adli toksikolojik açıdan analiz edilmesi ve buna bağlı olarak İstanbul'da uçucu madde kullanıcılarının profillerinin belirlenmesidir. **Gereç ve Yöntemler:** Bu çalışmada Headspace Gaz Kromatografisi-Kütle Spektrometresi (HS-GC/MS) yöntemi ile yirmi beş antemortem ve altı postmortem olguda toluen analizi gerçekleştirilmiştir. Kromatografik yolla toluen, HP-INNOwax kapiler kolonu kullanılarak ayrılmış ve n-butanol Internal Standart (IS) olarak kullanılmıştır. **Bulgular:** Yöntemin tespit sınırı (LOD) ve miktar tayin sınırı (LOQ) değerleri sırasıyla 0,01 mg/L ve 0,1 mg/L olarak belirlenmiştir. Antemortem örneklerde kan ve idrarda toluen miktarları sırasıyla 0,32 ve 4,39 mg/L; 0,48 ve 15,49 mg/L olarak bulunmuştur. Yapılan anketlere göre, uçucu madde kullanımına ortalama başlama yaşı 14,4 olarak belirlenmiştir. Ankete katılanların %80'inin uçucu madde istismarının yanı sıra, uyuşturucu ve alkol de kullandığı belirlenmiştir. Postmortem vakalarda, kanda belirlenen toluen miktarı 3,2 ve 51,3 mg/mL gibi geniş bir aralıkta belirlenmiştir. **Sonuç:** Bu çalışmada, hem antemortem hem postmortem biyolojik örneklerdeki toluen miktarı hızlı ve kolay bir yöntem ile belirlenmiştir. Kan toluen miktarının belirlenmesinin bağımlı hastaların tedavisinde etkin rol oynayacağı düşünülmektedir. Diğer taraftan kullanıcı profillerinin belirlenmesi ile bu kullanıcıların uçucu organik bileşik ile birlikte pek çok madde kullandığını ortaya çıkarmıştır. Bu da uçucu organik maddelerin, uyuşturucu kullanımına bir geçiş maddesi olduğunu doğrulamaktadır.

Anahtar Kelimeler: Toluen; inhale kötüye kullanım; adli toksikoloji

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Since there is no blood-brain barrier for hydrophobic substances like toluene and xylene due to their lipid solubility and volatility, they may affect and directly intoxicate all mammalians including humans. Volatile substances (VS) are also known as a "gateway" to other drugs of abuse. Therefore, the vapor of VS is usually inhaled intentionally to become intoxicated. In recent years, drug and volatile substance abuse (VSA) has become a problem as an increasing threat in Turkey, as well as all over the world.[1-3] This is because VS have low cost, are legal, easily accessible and usable in household products in Turkey. Abusing of minimum dosage of VS may cause euphoria and some kind of behavioral disorders, while higher dosage causes the high-risk comas and convulsions and the highest dosage may cause sudden death.[4] The wounds as eczema due to chronic abusing of volatiles, occur on the face, especially around the mouth and nose.[5]

During the autopsy, the characteristic sharp smelling of these volatile substances is usually observed. But, also only scent characteristic is not enough for an adequate report without an analysis. The necessity of toxicological analysis is unavoidable because of the insufficiency in anatomic autopsy findings.[3] Therefore; forensic specialists have to confirm the presence of toluene in the biological samples as well as other concomitant illicit drugs. During the systematic toxicological analysis, qualitative and quantitative data of toluene in body fluids assist to clarify the principle cause of death, and sometimes may help "forensic toxicologist" to search the suspicious traffic accident[6] or date rape cases which are supposedly caused under the effect of toluene.[4,7]

Each society of population is affected by VSA problem directly or indirectly. An increasing number of VSAs together with alcohol, medicinal and illicit drugs were found during studied years.[8]

In present study, we aimed to detect toluene in blood and urine samples of twenty five antemortem and six postmortem cases which investigated in the frame of this work with the cooperation of Alcohol and Drug Research, Treatment and Training Center (AMATEM) and Institute of Forensic Medical Council, Ministry of Justice, Turkey during six months interval by HS-GC/MS. We also aimed to determine the abusing profile of other concomitant illicit drugs by a survey.

MATERIAL AND METHODS

MATERIALS AND REAGENTS

Benzene, Toluene, n-butanol and Methanol as organic solvents were purchased from Merck Chemical Company, Germany and used without previous purification. Deionized water was prepared with Millipore Water Purifying System (Millipore Corp, USA).

CASE SELECTION

Twenty five antemortem biological samples were collected from volunteers who admitted to the AMATEM the day after they abused glue and/or thinner. Six postmortem blood samples were collected from Morgue Department of the Council of Forensic Medicine in these six months period. This research has been approved by the Committee of the Council of Forensic Medicine, the Ministry of Justice in 2000. Written consents were obtained from antemortem participants and a survey was applied to the patients which included questions such as "amount of volatile substance used, if exist other substances used, usage period and duration of the usage of volatile substance".

ANALYTICAL PROCEDURE

A Hewlett Packard 6890 GC equipped with 5973B mass selective detector was used in the study. HP-INNOwax capillary column (30 m x 530 μm i.d. x 1 μm film thickness of polyethylene glycol stationary phase) was utilized for chromatographic separation. n-butanol was used as internal standard (IS).

The samples were placed in 20 ml headspace vials by adding 1 ml of sample and 0.5 ml of IS (50 mg/L). Parameters of the instrument were as follows: Analysis time (min): 11; Vial equilibration time (min): 10; Vial pressurize 0.20; Sample loop fill time (min): 0.20; Loop EQTime: 0.05; Inject time (min): 0.30; Vial mixing: High.

Temperature program was set as follows: 40°C initial for 6 min, ramp of 10°C/min up to be 140°C, hold for 3 min. The carrier gas was high quality helium with a pressure value of 2.3 psi and a flow rate of 3.4 mL/min. The injector and transfer line temperatures were 180°C and 110°C, respectively.

Standard stock solution of toluene was prepared by dissolving the compound in methanol. The linearity range was determined as 0.1-80 mg/L. The calibration curve was obtained with diluted standard solutions as 0.1, 3, 10, 20, 40, and 80 mg/L in incremental amounts with 6 replicates of each points. Accuracy and precision were also calculated by these data.

RESULTS AND DISCUSSION

During the six months of this study period, 25 VSAs were found in Istanbul and interviews were applied. Among 25 abusers, 14 (56%) were glue abusers, 8 (32%) were thinner abusers, and 3 (12%) were both thinner and glue abusers (Figure 1). The abused products show variability in each country. For example, Wu et al. reported abused agents were as glue, shoe polish, toluene, lighter fluid, and gasoline, in United States.[9] There are only two types of VS in Turkey as shown in Figure 1 according to our study.

In recent years, drug abuse and VSA are an important and increased problem in Istanbul, Turkey as all over the world. The user profile of VSAs were evaluated with this survey and with limited participants that twenty two (88%) antemortem cases were below thirty years old and only three of twenty five cases (12%) were female abusers. Here we reported the average age was as 23.7 among the VSAs (in the range of 18-32 years), also average age in some Japanese studies reported as 19.6.[10] This difference may be due to a number of effects as socioeconomic and familial problems, etc.[11] In the present study the beginning age was found 14.4 (in the range of 6-24) years (Figure 2) while another study in Turkey reported as 13, furthermore the study achieved in United States determined it as five or six years old.[3,12]

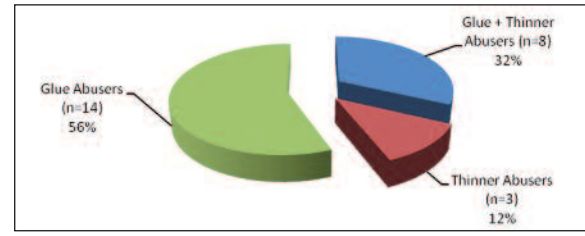

FIGURE 1: Glue and thinner abusers profile.

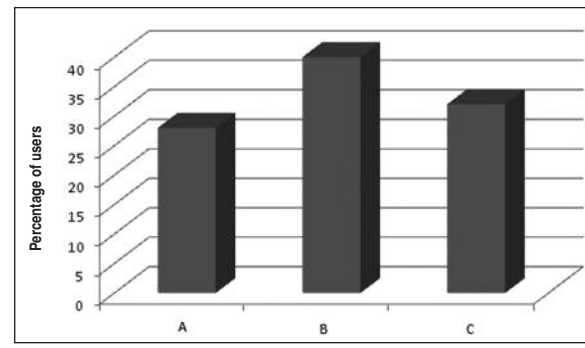

FIGURE 2: Distribution of abusing periods for users: (A) 1-5 year-abuser (28%), (B) 6-10 year-abuser (40%), (C) 11-20 year-abuser (32%).

The highest abusing duration was determined as 40% in the range of 6-10 years (Figure 2). Additionally, in the same period, 6 postmortem cases were found in Istanbul. The average age for postmortem users was 20.8 (in the range of 10-34 years). From 1971 to 1981, 140 deaths associated with VSA were identified in the United Kingdom.[4] However, collected six postmortem samples were collected during this study. Postmortem cases' rate related with VSA in our country is similar with other countries.[13]

The association between VSA and alcohol-drug usage also deserves mention. According to survey, about 80% of participants had abused other medicinal, illicit drugs and alcohol together with VS as shown in Figure 3. The survey helped to obtain information about their alcohol and medicinal drugs usage such as benzodiazepines. Moreover, both glue and thinner abusing cases' results informed us that they have also used illicit drugs such as tetrahydrocannabinol (THC), cocaine etc (Figure 4).

According to the method of HS-GC/MS, the limit of detection (LOD) and limit of quantification (LOQ) of toluene were 0.01 mg/L and 0.1 mg/L, re-

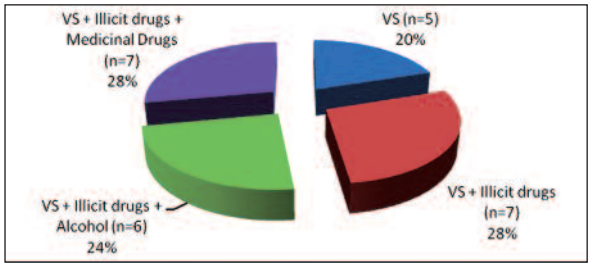

FIGURE 3: Profile of alcohol, medicinal and illicit drugs used together with VS.

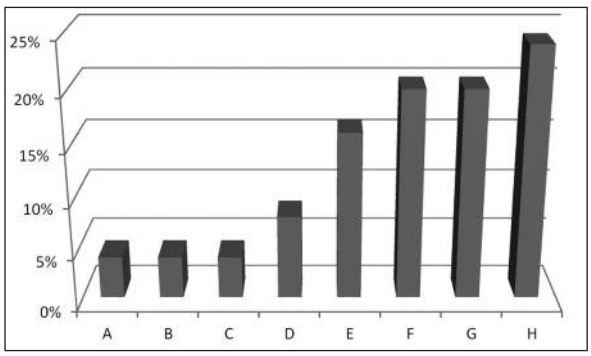

FIGURE 4: Alcohol and the drugs used together with VS: (A) amphetamine, benzodiazepine and cocaine, (B) tetrahydrocannabinol (THC) and amphetamine, (C) amphetamine and alcohol, (D)THC, amphetamine and benzodiazepine, (E) amphetamine and benzodiazepine, (F) THC and alcohol, (G) none, (H) THC.

spectively. Ramsey and Flanagan reported that LOD for toluene in blood given by HS-GC/MS were 0.1 mg/L.[14] In our study, LOD for toluene was found 0.01 mg/L as determined by HS-GC/MS methods.[15,16] Accuracy value was found 12.5% bias and precision value was found 9.8% RSD (relative standart deviation) in 20 mg/L concentration. The linearity range was 0.1-80 mg/L. Calibration curves were linear over these range and r^2 value for the regression analysis calculated as 0.998. The chromatographic parameters used for toluene and n-butanol provided retention times of 10.2 min. and 12.5 min., respectively (Figure 5).

Headspace (HS) unit on Gas Chromatography (GC) offers the greatest specificity, sensitivity and accuracy for quantitative analysis of toluene and other volatiles in different matrices. The sample in HS unit can be collected directly into the vial without extraction, preventing analyte loss.[17]

We determined the toluene amount by fully automated HS-GC/MS method in whole blood and urine samples. This HS-GC/MS method can provide benefit in clinical diagnosis of acute poisoning to confirm suspected chronic VSA or in investigation of rape or other assault, also is useful for the investigation of suspicious deaths. This sensitive and simple assay is not labor intensives.[17]

The toluene amounts in antemortem urine samples were found between 0.32 and 4.39 mg/L (Table 1), in antemortem blood samples ranged between 0.48 and 15.49 mg/L (Table 2). However, toluene in antemortem urine samples was only determined in five cases.

Park et al. reported toluene blood concentration as 46% of the all cases ranged between 1.0 and 5.0 mg/L and 39% of those ranged between 0.1 and 1.0 mg/L.[15] In our study, toluene blood concentration in antemortem cases was found as 60% ranged between 0.5-7 mg/L and 32% of those not detected toluene.

In two of six postmortem cases, toluene was not detected from the blood samples (Table 3), although they were known as VSAs according to information given by their relatives. Baselt and Cravey reported postmortem blood toluene concentrations were 10-20 mg/L.[18] In our study, postmortem blood results were found that they have broad differences (from ND to 51.3 g/L) since some of them were brought to the autopsy room long after they died. Also these differences of results

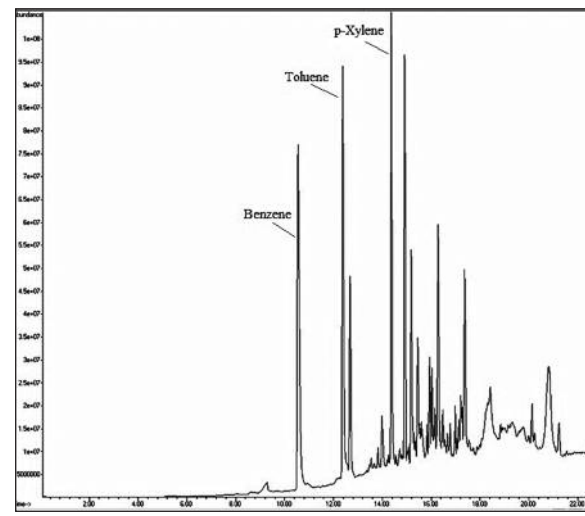

FIGURE 5: HS-GC/MS chromatogram of a commercial thinner product.

TABLE 1: The toluene concentration in antemortem urine analyzed by HS-GC/MS.

Case #	Concentration (mg/L)
1	4.39
2	0.89
3	0.70
4	0.32
5	0.35

TABLE 2: The toluene concentration in antemortem blood analyzed by HS-GC/MS.

Case #	Concentration (mg/L)
1	ND
2	0.96
3	1.48
4	1.33
5	ND
6	ND
7	0.99
8	ND
9	ND
10	2.8
11	1.34
12	2.38
13	1.1
14	6.6
15	3.88
16	ND
17	ND
18	ND
19	15.49
20	0.66
21	0.81
22	0.48
23	2.17
24	0.64
25	1.23

may be due to factors such as disease or metabolism discriminates, development of tolerance to VS and etc. Toluene-blood concentrations higher than 10 mg/L may be lethal although higher concentrations have been found in habitual abusers.[19] Exposure to a concentration of 100y subjects at rest and about 1.16 mg/L in six subjects during light exercise, after 20 to 30 min.[19] The following postmortem tissue concentrations of toluene were reported in a 16 years old boy found dead with a plastic bag over his head: blood 20.6 mg/L, brain 297 μg/g, liver 89 μg/g; acetone was also detected in blood at a concentration of 3 mg/L.[20]

GC/MS analysis of commercial form of thinner used in Turkey showed that some paint thinners were mixtures of solvents consisting of toluene but especially the major compounds were benzene and o-xylene (Table 4). However, none of the samples of the cases was found to contain measurable amounts of related components. Since the most toxic one is benzene among these solvents, the authorities should consider this situation for further study on related subject.

Especially, dependence among adolescents is prevalence.[12,21] In our study, the adolescent period was determined as the beginning of abusing to VS. Habitual user's abuse potential is directly related to

TABLE 3: The toluene concentration in postmortem blood analyzed by HS-GC/MS.

Case #	Concentration (mg/L)
1	28.9
2	ND
3	ND
4	3.2
5	4.3
6	51.3

TABLE 4: Thinner components and their percentages.

Compound name	% Amount (percentage)
Dimethylhydrazine	0.4
Benzene	8.6
Toluene	7.68
Hexamethylcyclotrisiloxane	3.7
p-Xylene	1.5
o-Xylene	7.1
Benzimidazol	6.7
Phenol	3.4
Decane	2.0
Trimethylbenzene	1.9
Dichlorobenzene	4.7
Cyclopentasiloxanedecamethyl	4.1

their ability to produce intoxication and repeated abuse may result in psychological dependence or other harmful health effects. Monitoring VS usage in patients under treatment depends on the presence of toluene in blood specimens as a marker for recent VS usage. This may be useful to evaluate the efficacy of patient's treatment because the absence of the VS in blood probably indicates the giving up. Blood toluene determination is useful as part of the treatment-compliance plan. In order to follow this situation it is important for a forensic toxicologist to perform a complete screening for both medicinal and illicit drugs and not only focus on VS.

CONCLUSION

In this study, toluene amount in both antemortem and postmortem biological specimens were determined with a rapid and accurate method. It is thought that the determination of blood toluene concentration play an active role in the treatment of abusers. On the other hand, establishment of volatile substance abuser profile showed that the users abuse other illicit drugs together with VS. This situation confirmed that thinner and glue abuse is known as transient phenomenon. We hope that both drug and solvent abuse problem may be solved with national and international drug control and prevention programs and developmentally sensitive educational strategies.

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