A Forensic Study of Mycosis and its Relation with Amphetamine Addiction Tests

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Abstract

The abuse of amphetamines is of national concern from a public health perspective. This issuing a nowadays important subject, because the problem of amphetamine drugs has recently been raised to a very dangerous level. Amphetamines are generally prescribed as a potent central nervous system (CNS) stimulant that is used in the treatment of attention deficit hyperactivity disorder (ADHD), narcolepsy, and obesity. The present study aimed to test the ability of Fungi to bio-convert methamphetamine that may change the metabolite pattern and/or concentration of the drug. Total of 53 blood samples were collected (10 samples from drug addict prisoners at Al-Samawa prison, 30 samples from healthy (non-symptomatic), 13 sample phlegm of respiratory system patients). Samples were cultured on Blood agar then purified on Sabouraud dextrose agar. Thereafter, each isolate was cultured in 5ml Sabouraud dextrose broth containing 6mg/ml methamphetamine drug and incubated at 37°C. Samples were tested periodically, by thin layer chromatography (TLC) using methanol and ammonia (100:1.5) as a mobile phase. The separated spots were visualized by spraying with iodoplatinate or Marquis Reagent the results revealed that 11 sample was able to convert the drug after 14 days of incubation. The TLC method found more advantageous in detecting drug bio-conversion. Add to that, it is inexpensive, fast and easy to manipulate in forensic labs.

Keywords: Methamphetamine, Fungi, TLC.

Introduction

There are different kinds of microorganisms known as "normal microflora" found in the human body. In addition to bacteria, different kind of fungi mainly of the genus Candida is very common [1]. In immunocompromised patients, fungi of the genera Aspergillus, Fusarium, Mucor, Curvularia can further be found [2, 4]. No positive results were obtained using Blood culture method. However, PCR technique has showed 70% of immunosuppressed patients and 30% of non-symptomatic persons were infected with fungi using ITS1-ITS4 primer pair [5].

Through overview of the latest developments in the world’s illicit drug markets by focusing on the production of, trafficking in and consumption of the main illicit drug types and their related health consequences, It is estimated that a total of 246 million people, or 1 out of 20 people between the ages of 15 and 64 years, [6].

Testing for drugs of abuse in biological fluids and tissues is an international phenomenon, practiced in many different settings for a variety of reasons, tests of Blood, Urine, Hair, Oral Fluid and Other Specimens [7]. In a published paper [8], species of fungal colonies of the genera Chaetomium, Bjerkandera Coroliopsis, Enterocarpus, Penicillium and Trichoderma found in decomposed body samples after death. In another work by [9] some fungi are able to produce phase I metabolites by hydroxylation, N-and O-dealkylation and N-oxidation. Most of these metabolites were similar to those produced in human, but some fungi produce new fungal metabolites (NFM) that could be used as markers of fungal colonization.

It is necessary to verify the presence of fungal infection in forensic medicine that include analysis of drugs and toxins for post-
mortem or pre-death cases, because the fungal metabolism can alter the chemical properties of the drug or reduce its concentration and thus can’t be detected or at least give false results [10]. Very few studies manipulated fungal infection and drug interaction, Therefore, the aim of the present study was to investigate Methamphetamine drug and mycosis interactions.

**Materials and Methods**

**Solutions and Media**

Iodoplatinate [11] The spraying agent (Iodoplatinate) was prepared by Dissolving 0.15g potassium chloroplatinate with 3g potassium iodide in 100ml hydrochloric acid.

Marquis agent [11] It was prepared by mixing one part of 38% formaldehyde with five parts of 98% sulphuric acid (v/v).

**Media Preparation**

All culture media were prepared according to the manufacturer directions (HIMEDIA). Chloramphenicol was added in 250mg/l to prevent bacterial growth Next, Autoclaved at 121ºC for 15 min.

**Methodology**

**Sample Collection**

Total of 53 blood samples were collected in the EDTA tubes that include (10 samples collected from drug addict prisoners at Al-Samawa prison. A questionnaire format was filled including: age, gender, drug type and arresting period, 30 samples collected from asymptomatic people, 13-phlegm samples of respiratory system patients.

**Blood Culture**

Blood culture was done in triplicates and as following. About 0.5ml of blood were inoculated in 10ml Brain heart infusion broth tubes then incubated at 37ºC for two weeks or until growth appear. Thereafter, 0.5ml of culture broth was spread on Blood agar media and incubated at 37ºC for a week. Successive cultures were sub-cultured on Sabouraud dextrose agar for purification.

**Sample Cultivation and Drug Preparation**

A part of the fungal colony was transferred into the Sabouraud dextrose broth (SDB) tubes (5ml) in triplicates by aseptically punching out 7mm disc of the culture then incubated at 37ºC for 3 days. Thereafter, the tubes were transferred to the poisons and drugs division, Medicolegal institute, ministry of health, Baghdad where the research continued by adding methamphetamine (dissolved in Methanol) to each tube in 6mg/ml (TLC, LOD) final concentration in addition to control tubes (media + methamphetamine and media only). The tubes were incubated at 37ºC with periodical test by TLC every 7 days for any drug change.

**Thin Layer Chromatography (TLC) [11]**

TLC was performed on silica gel plates (20cm x 20cm, 0.2mm thin layer) were used. About 20μl of the sample to be examined, were spotted onto the TLC plate approximately 2cm from the bottom and 1.5cm apart. The plate was developed in a solvent saturated glass TLC tank using the standardized solvent system (Methanol:ammonia, 100:1.5 v/v). When the mobile phase reached approximately 2cm from the top of the plate, the plate was removed from the TLC tank and allowed to dry. Then a suitable dyeing agent was applied (Iodoplatinate or Marquis Reagent). The $R_f$ was calculated by dividing the distance spot had travelled from the origin by the distance travelled by the mobile phase.

**Results and Discussion**

**Blood Culture**

Blood culture from drug (amphetamine) addict samples has showed no results even when Blood agar plates were incubated more than two weeks, this might be due to that the addicts are not infected or infected with uncultivable fungal strains. Positive blood culture results were obtained with 15 (50%) of asymptomatic samples and 13 (100%) positive results were obtained with samples of respiratory system patients as shown in Table (1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
<th>Positive results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug addict prisoners</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0%</td>
</tr>
<tr>
<td>asymptomatic</td>
<td>30</td>
<td>15</td>
<td>15</td>
<td>50%</td>
</tr>
<tr>
<td>phlegm of respiratory system patients</td>
<td>13</td>
<td>13</td>
<td>0</td>
<td>100%</td>
</tr>
</tbody>
</table>
Thin Layer Chromatography (TLC)

The methamphetamine drug appeared with $R_f=0.43$ using Iodoplatinate spray (Figure 2). By using Marquis Reagent, two spots appeared, one of methamphetamine (Brown) with $R_f=0.45$ and the other (Yellow) with $R_f=0.54$ that is most probably belongs to Dextrose sugar since it appeared with the medium only control lane (Figure 1, 2, 3).

After 7 days of incubation, Fungi showed no effect on the methamphetamine concentration or there is no bio conversion sign (Figure 2). After 14 and 21 days of incubation, 11 fungal isolates showed a dramatic change in methamphetamine concentration that might be bio converted to other forms that are not detected (Figure 3 and 4 respectively). One of the rare studies had found that Amphetamine resulting from the N-dealkylation of N-n-propylamphetamine was metabolized by a strain of filamentous fungus Cunninghamella echinulata to 4-hydroxyamphetamine, Nacetylamphetamine and the N-oxidation products N-hydroxyamphetamine and 1-phenyl-2-propanone oxime [12, 13].
Figure 4: TLC result with Marquis Reagent after 21 days of incubation am: Methamphetamine stander. Almost all lanes show dramatic reduction in drug spot

Conclusion

Fungemia detection is very important in drug addiction detection in forensic cases since fungi found play a vital role in the bio-conversion of amphetamine and might change the metabolic pathway of the drug. Addicts with fungal infection that alters and/or consume drug lead to reduce the concentration of drugs (methamphetamine) in their blood and this increases the desire for an additional dose of drugs. However, it must be pointed out that continuing to reduce the concentration of drug substance in the blood (when the addict is under a treatment program), it might help the adduct to quit addiction gradually.

References