Analysis of benzodiazepine metabolite in the urine of Wisma Anggrek Resident, Mutiara Sukma Psychiatric Hospital, West Nusa Tenggara

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Abstract. Benzodiazepines, including group IV psychotropic substances that are often abused. To ensure that benzodiazepine abuse has been carried out, a screening test for the presence of benzodiazepines metabolites is carried out in the urine sample. The accuracy of the analysis is largely determined by the time of sampling. False negatives can occur when the urine sampling is done before benzodiazepine metabolites are present in the urine. This study aims to determine the appropriate sampling time so that the test results can provide appropriate test results. On residents of Mutiara Sukma who were given clobazam therapy, the clobazam metabolite test was carried out in the urine. Urine sampling was done after the therapy was given, that is after 6, 9, 12, 15 and 18 hours. Clobazam metabolite were positive in the urine sampled after 9 hours of consumption and were still positive until 18 hours later.

Keywords: Benzodiazepine metabolite; Time of sampling; Urine

1. Introduction
Benzodiazepines are a large class of drugs with some 35 and many more are available throughout the world. They are commonly use by the community as minor tranquillizers, hypnotics, muscle relaxants and as anticonvulsants. They are often abused by the young illicit drug user often in large doses causing profound behavioral effects. Their continued abuse leads to dependence. The older population are not immune to using benzodiazepines and are also often dependent on their effects. Benzodiazepines may also cause or contribute to sudden death if misused [1].

Drugs of abuse testing is conducted both for medical purposes and legal causes. Urine is the specimen of choice because it contains ample concentrations of the drug and its metabolite(s) and can be easily collected. As urine drug screening is used for many different purposes and patient populations, selection of appropriate cutoffs is of great importance[2].
The common approach to urine drugs of abuse testing is an immunoassay screen. Immunoassays, as opposed to chromatographic or mass spectrometry methods, are clinically desirable because they provide a rapid turnaround time and are more easily integrated into the laboratory workflow [3]. Immunoassays are designed to detect a drug at a pre-determined concentration (cut-off values). However, confirmation of the drug may also have the same cut-off as screening cut-off or a different cut-off. Moreover, each drug has a specific detection window in urine. Benzodiazepine window of detection in urine are until 2 days for short acting and until 10 days for long acting [2]. Some data only describe the test deadline detection window but do not specify when the inspection should be carried out after taking benzodiazepines.

Mutiara Sukma Psychiatric hospital serves detoxification of drugs abusers as Wisma Anggrek resident. Clobazam as an intermediate benzodiazepines is given if Wisma Anggrek resident has difficulty sleeping. This research was conducted to determine the appropriate sampling time so that the qualitative test results of the benzodiazepines could give actual results. If the sampling is carried out before the benzodiazepine metabolites have reached in the urine, it is possible to give a false negative results.

2. Methods

This research is a pre-experimental study to determine the appropriate sampling time for the benzodiazepam group of intermediate acting drugs. The samples of this study were residents of the orchid homestead in the pearl sukma mental hospital in NTB who were given benzodiazepine drug therapy during May 2020. The type of drug given was Clobazam which is an intermediate acting benzodiazepine group. A total of 5 residents were given clobazam therapy because they had difficulty sleeping as the sample

2.1. Sample

The sample of this study were residents of Wisma Anggrek resident in Mutiara Sukma Psychiatric Hospital who were given benzodiazepine therapy during May 2020. The type of drug given was clobazam which is an intermediate acting benzodiazepine group. The dose given is once a day at night. During May 2020, a total 5 residents were given clobazam therapy. Urine sampling was carried out starting 6 hours after the therapy

2.2. Procedure

2.2.1. Tools and material. Benzodiazepine test strip and pot urine

2.2.2. Testing. Prepare the tools and materials used. The test strips were left at room temperature for 5 minutes. The test strip is dipped in the urine sample vertically for 10 to 15 seconds. The test strip is lifted and placed on a flat surface and the results are read after 5 to 10 minutes.

2.2.3. Interpretasi Hasil. A Positive result is indicated by the formation of 1 pink band on the control line (C). Negative results are indicated by the formation of 2 pink bands on the control line (C) and the test line (T). The results of the examination are invalid and must be repeated if no pink bands are formed on the control line (C) and the test line (T), or only a pink band is formed on the test line (T).
3. Result and Discussion

For all samples, the results of the benzodiazepine test began to show positive results in the urine sampled at the same time, 9 hours after the therapy was given.

Benzodiazepine are divided into 3 groups based on their metabolic rate, namely long acting, intermediate acting and short acting. In this study, clobazam is used, which is a benzodiazepine class of drugs that has an intermediate metabolic rate. If the metabolic rate affects the metabolites produced, the duration of detection of each metabolite will be different [4].

The IUPAC name of clobazam is 8-chloro-5-methyl-1-phenyl-1,5-benzodiazepine-2,4-dione with trade names castilium, clarmyl, clopax, or frisium. Clobazam is easily absorbed after oral administration, peak plasma concentrations are reached after 1 to 4 hours. Clobazam is highly lipophilic and rapidly crosses the blood-brain barrier. Metabolites found in serum include N-desmethylclobazam which is considered active. About 90% of the dose is excreted in the urine in 17 days and about 2% is eliminated in the stool. The active metabolite clobazam is present in a higher concentration than the parent drug [5]. The half-life is the time it takes so that half of the drug is removed from the body. Factors affecting the half-life are absorption, metabolism and excretion of drugs [6]. The pharmacokinetic half-lives of benzodiazepines are mostly used to determine medical use. The half-life of clobazam in the plasma averages 25 hours with a span of 10 to 58 hours [7].

Urine usually contains a wide range of benzodiazepine metabolites, often accompanied by a small amount of the parent drug. It is very important to assess individual benzodiazepine target metabolites in people exposed to these drugs. The clearance of benzodiazepines from the body is reduced by hepatic disease, although the greatest effect occurs with drugs metabolized by the P450 system whereas lorazepam and oxazepam which are metabolized by glucuronidation have very little impact on liver disease. Clobazam is also metabolized in the liver by demethylation and hydroxylation. Kidney disease in particular affects benzodiazepines which are metabolized to active drugs and which exhibit high levels of protein binding. Old age has an effect similar to that of liver and kidney disease due to reduced output of major organs and changes in volume distribution. Conjugated benzodiazepines such as oxazepam, lorazepam and temazepam were least affected by age [7].

Differences in peak clobazam concentrations in people with liver disease. A total of 15 patients with liver disease and 6 healthy volunteers were given a single oral dose of 20 mg of clobazam. Peak plasma concentrations in healthy volunteers, patients with viral hepatitis and patients with cirrhosis were achieved within 1.7 hours, 3 hours and 2.5 hours, respectively [8]. Norclobazam is the active metabolite of clobazam. Clobazam and its metabolites are excreted in the urine and feces, with the rate of elimination of clobazam increasing with age. There is some evidence that hepatic impairment can affect the pharmacokinetics of clobazam metabolism, most notably the elimination of norclobazam. This is reflected in the prescribing information, which recommends that patients with hepatic impairment are given reduced doses of clobazam [9].

The results of the study did not show any differences in the results for 5 respondents. Respondents in this study were 5 people with adult age ranges of 28, 18, 28, 19 and 22 years, respectively, not recorded as having liver or kidney disease so that the excretion of metabolites or drug residue clearance occurred in the same time frame.

Selection of the correct specimen is an important step in toxicological investigations. The circumstances surrounding the case, the availability of specimens, the nature of the investigation and even legal or legal issues can determine which specimens to choose, and for what purposes. Time is an important factor in specimen collection, especially in ante mortem cases where some drugs have a short detection time [10].
The results of this study are very useful for a medical laboratory technology expert to determine the appropriate sampling time in cases of psychotropic abuse in the intermediate acting benzodiazepine group, especially clobazam.

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References