

# Morphine/Codeine Ratio, a Key in Investigating a Case of Doping

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## Abstract

**Introduction:** Consumption of codeine can lead to positive urine test for morphine in athletes. Morphine is classified as a prohibited doping drug while Codeine is not. Morphine/codeine ratio is used in forensic medicine to distinguish the consumption of codeine from abuse of morphine and other narcotics.

**Case Presentation:** We present an athlete with positive urine test for morphine with a history of consumption of codeine. The disciplinary committee came to conclusion that the athlete had not consumed morphine and did not violate doping code based on morphine/codeine ratio.

**Conclusions:** Analysis of codeine to morphine metabolism rate is needed when we are using morphine/codeine ratio to rule out abuse of narcotics. WADA should consider analysis for the CYP2D6 alleles (main metabolizer of codeine) in case of including morphine/codeine ratio in future prohibited list. The possibility of ultra-rapid CYP2D6 cannot be ruled out in certain results of morphine/codeine near the cut point.

**Keywords:** Doping in Sports, CYP2D6, Morphine, Codeine

## 1. Introduction

Codeine or 3-methylmorphine (a natural methylated form of morphine) is widely used to manage mild to moderate pain and to relieve mild cough. Codeine is the most commonly used opiate in the world with a wide safety margin. Codeine is found in combination preparations with acetaminophen, aspirin or ibuprofen in many over-the-counter drugs used for cold symptoms and analgesia. These over-the-counter drugs are widely used by athletes for a wide range of conditions such as sport injuries to common cold. Codeine is not classified as prohibited drug by world anti-doping agency (WADA) but morphine (along some other narcotics) is one of the possible metabolites of codeine that is prohibited in the time of competition. Codeine is distinguished from morphine by the methylation of hydroxyl group on C3 of the aromatic ring. Codeine mainly metabolizes to codeine-6-glucuronide (C6G) by uridine diphosphate glucuronosyl transferase (UGT2B7); but codeine can also undergo metabolism to morphine in the liver via cytochrome P450 2D6 (CYP2D6). Patients who took codeine have shown both codeine and morphine in their plasma and urine (1). Determining the origin of morphine found in a doping control urine sample of athletes can be challenging. The disciplinary committee of doping rules based on

whether the finding is due to ingesting codeine or morphine by the athlete. Intake of morphine can violate doping control code, but consumption of codeine is allowed due to the WADA current code.

Morphine/codeine (Mor/Cod) ratio is widely used in forensic medicine to differentiate between consuming codeine and morphine (2, 3). Mor/Cod ratio below 1 is considered as a sign of codeine only intake, whereas the ratio above 1 is considered as a sign of using morphine or heroin (4, 5). but the number of one is not absolute to determine the source of morphine, some suggested that in individuals with ultra-rapid CYP2D6 metabolism, Mor/Cod ratio can be higher than 1 in even sole consumption of codeine.

Importance of this case report lies in the presence of Mor/Cod ratio in WADA 2015 monitoring program. This report can help WADA decide about its policy for doping control regarding narcotics. We have searched the PubMed database until February 2015 using key words: Morphine, Codeine, and Opioid Metabolism

## 2. Case Presentation

The doping case regarded the result of urine sample analysis of a 25-year-old male Futsal Premier League

player in season 2014 - 2015, Iran. He had no history of anti-doping rules violation. He was selected randomly for sampling during a match, mid-2014. His sample was positive for morphine, and was alleged to violation of article 6 of FIFA anti-doping regulations. FIFA anti-doping regulations refer to the WADA prohibited list to define prohibited drugs. The player denied using narcotics including morphine in testimony but admitted that he had taken a number of acetaminophen-codeine tablets (300 mg and 10 mg, respectively), the day before match for controlling tooth pain. He did not remember the exact number or the exact time of ingesting the tablets. The reported concentrations of morphine and codeine in his urine sample were 54.5 µg/ml and 52.7 µg/ml respectively. Mor/Cod ratio was 1.03 in this case, this Mor/Cod ratio was in agreement of Mor/Cod ratio reported by He et al. (6) 24 hours after codeine consumption. Considering the fact that codeine is not among the WADA 2015 prohibited list, the positive result for morphine in urine sample was attributed to codeine consumption.

He presented no significant past medical history, specifically hepatic disease. The football player denied taking any drugs or substances but the acetaminophen-codeine tablets in the 2 months before testing.

The disciplinary committee came to the conclusion that the player had not violated anti-doping rules. The player could establish how the prohibited substance entered his system and had no intention for enhancing performance. Therefore, according to article 21 of FIFA anti-doping regulations the doping case was dismissed.

### 3. Discussion

The disciplinary committee's conclusion was based on assumption that the athlete can be an individual with ultra-rapid CYP2D6 metabolism. A study by He et al. (6) has shown that in the individuals with ultra-rapid CYP2D6 metabolism, Mor/Cod ratios were below 1, median (range): 0.108 (0.045 - 0.236) in plasma, 12 hours after codeine consumption. But it could be above 1, median (range): 0.635 (0.184 - 1.060) after 24 hours. UGT2B7 isozyme conjugates codeine and morphine to their corresponding 3- and 6-glucuronides. He et al. (6) also measured morphine 3-glucuronide (M3G), morphine 6-glucuronide (M6G), codeine-6-glucuronide (C6G) as forms other than free morphine and free codeine.  $(\text{Mor} + \text{M3G} + \text{M6G}) / (\text{Cod} + \text{C6G})$  ratio also have shown comparable results to (Mor/Cod) ratio. Another study by Kirchheiner et al. (7), using inverse ratio  $(\text{Cod} + \text{C6G} / \text{Mor} + \text{M3G} + \text{M6G})$  in plasma and urine samples has reported the inverse ratio to be 9 (6 - 16) in ultra-fast metabolizers 12 hours after codeine consumption.

CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) is a member of the cytochrome P450 oxidase system, CYP2D6 is one of the enzymes which is involved in the metabolism of foreign chemical substance or substances presented with higher than normal concentrations in the body as well as morphine. CYP2D6 accounts for

small fraction of hepatic CYPs (< 2%) but metabolizes and eliminates about 25% of drugs which are used in clinic (8).

Some drugs like rifampicin and dexamethasone can increase the CYP2D6 metabolism through induction of CYP450 isozymes. Some other drugs like selective serotonin reuptake inhibitors (SSRIs), paroxetine and fluoxetine and the antidepressant, bupropion, and the class I antiarrhythmic agent, quinidine are CYP2D6 inhibitors and can reduce or even completely block the CYP2D6 metabolism. People with multiple copies of the 2D6 gene also produce more CYP2D6 and will metabolize drugs faster than others. There is considerable evidence on association between CYP2D6 genotype and variability in codeine metabolism to morphine. CYP2D6 metabolism in a subject can be described as poor metabolizer (with little or no metabolism), intermediate metabolizers (with a metabolism rate between poor and extensive), extensive metabolizer (with normal metabolism) or ultra-rapid metabolizer (with greater than normal metabolism due to multiple copies of CYP2D6 gene). The possible genomic or environmental mechanisms leading to the substantial difference among subjects with the same metabolizer group are unknown. Fatal intoxication, such as respiratory depression demanding airway intubation, can develop in days after consumption of codeine in subjects with multiple functional alleles of CYP2D6 (ultra-rapid metabolism). Poor CYP2D6 metabolizers present lower analgesia after consumption of codeine. Clinical Pharmacogenomics Implementation Consortium (CPIC) guidelines recommend against administration of codeine to ultra-rapid CYP2D6 metabolizers because of higher risk of adverse drug reactions due to morphine intoxication. And it is also recommended to use an alternative analgesic other codeine in poor CYP2D6 metabolizers because it is widely assumed that morphine is an active metabolite of codeine which is responsible for its analgesic effects (9).

In a clinical setting, a subject with CYP2D6 phenotype is usually determined by the administration of a selective CYP2D6 substrate (e.g. debrisoquine) and then measurement of plasma concentration of that substrate metabolite (4-hydroxydebrisoquine in case of using debrisoquine) (10). Debrisoquine hydroxylation phenotype has been the most used test in humans to evaluate CYP2D6 activity. Two CYP2D6 activity phenotypes have been described using debrisoquine hydroxylation: poor and extensive metabolizers. Ultra-rapid metabolizers have very low debrisoquine metabolic ratio among the extensive metabolizer subjects.

CYP2D6 variability can be otherwise determined by genotyping, because the CYP2D6 allele is the genetic basis of variability of CYP2D6 metabolism. Cytochrome P450 Nomenclature Committee have defined more than 100 CYP2D6 alleles. Subjects with certain alleles show different levels of CYP2D6 enzyme activity. Pharmacogenomic testing can be used to determine the CYP2D6 allele of a subject (11). CYP2D6 pharmacogenetics can grow into a valuable instrument to predict drug side effects, interactions or

metabolisms. Nevertheless, for some reason, research in this field failed to bloom. A lot of CYP2D6 allele phenotypes have only been predicted based on their genetic variations or based on in vitro studies instead of clinical trials. Genotype test may also fail to cover rare CYP2D6 variants. Subjects with rare CYP2D6 variants may be labelled as having “wild type” as default which can have different properties than that certain rare CYP2D6 variant.

Ethnicity is a factor in CYP2D6 variable function. There is a difference in the prevalence of different CYP2D6 alleles among different populations. Northern African and middle eastern populations show higher prevalence of ultra-rapid CYP2D6 metabolizers (12), the same ethnicity that the aforementioned player possessed.

The disciplinary committee was unable to conduct clinical or genetic testing to determine CYP450 metabolism due to some limitations. The committee ruled that no violation of doping code occurred with presumption of innocence as a law principle. The committee was unable to determine the metabolism rate CYP2D6 of the given athlete; and considering the possibility of ultra-rapid CYP2D6 metabolism in the athlete, the committee had no satisfactory evidence of violation of doping code.

Morphine/codeine ratio is used along with a number of narcotics (Hydrocodone, mitragynine, tapentadol and tramadol) in WADA 2015 monitoring program. WADA monitoring program aims to detect misuse patterns among sportspersons. As we mentioned, interpretation of morphine/codeine depends on determination of CYP2D6 metabolism. We could not exclude the possibility of ultra-rapid CYP2D6 metabolism in case of our specific athlete because of near the cut point result of morphine/codeine ratio. In athletes with results close to the cut point of morphine/codeine ratio we cannot rely on the results because of possibility of ultra-rapid CYP2D6 metabolism. If WADA is going to include morphine/codeine ratios above 1 (the cut point) in prohibited list, they should consider that this number can vary in certain athletes with ultra-rapid CYP2D6 metabolism. We suggest that WADA consider that clinical and/or pharmacogenomic testing for CYP2D6 metabolism is needed in case that WADA is planning to use Morphine/Codeine ratio in investigation for violation of doping code. Including Morphine/Codeine ratio without considering varied CYP2D6 metabolism can lead to legal difficulties in future cases of doping. Polymerase chain reaction tests (PCR) analyze the alleles of CYP2D6 (7) to determine the genotype; the genotype corresponds to a score in a semi quantitative gene-dose system that gives a number to each consisting allele of genotype (13). The result of that scoring system shows the metabolism rate of CYP2D6 system. Aforementioned Problems in pharmacogenomic testing (lack of clinical data on some alleles and problems in detecting rare alleles) and side effect of drugs on CYP2D6

metabolism should also be considered in future decisions regarding using morphine/codeine ratio in doping control by WADA.

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