medicinska revija
medical review

UDK: 615.2/.3.099
ID BROJ: 209792780

Atanasov V. et al.. MD-Medical Data 2014;6(3): 283-286

*Prikazi bolesnika/*
*Case Reports*

# FORENSIC TOXICOLOGY REPORT ON FATAL METFORMIN AND CLONIDINE OVERDOSE

# LETALNO PREDOZIRANJE METFORMINOM I KLONIDINOM – PRIKAZ SLUČAJA

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*Key words*
metformin, clonidine, metformin-associated lactate acidosis (MALA)

*Ključne reči*
metformin, klonidin, metformin-udružena laktatna acidoza (MALA)

*Abstract*

A 19-years-old girl was admitted to Emergency Department (ED) after ingestion of different drugs by anamnestic data, (empty blister packs): 15 g metformin, 625 mg hydroxyzine, 750 mg dipyridamole, 3 mg clonidine and 400 mg chlorthalidone (combined antihypertensive drug), 8 g acetylsalicylic acid, 63 mg drospirenone /0.63 mg ethinyl estradiol. Initially the patient was in altered mental status and normal vital signs, but very fast deterioration of the general status was observed. Severe acidosis was occurred (pH 6.51, BE -29.4mmol/L). It is the first case of metformin-poisoning where extreme hypoglycemia (0.21 mmol/L) was followed by hyperglycemia (31.05mmol/L). The heart rate decreased up to 30 b/min and 22 hours after admittance the patient died. Ante-mortem and post-mortem samples (blood, urine, gastric content/blood, tissue samples) were analyzed. Here we report the results from forensic toxicological analysis indicating fatal metformin/clonidine overdose and the interpretation of the results is discussed.

## *INTRODUCTION*

The toxicological analysis is a combination of screening and confirmatory tests which are used mainly in clinical and forensic practice to provide information about the presence and quantity of drugs (xenobiotics) in biological samples. In clinical toxicology, it aims to answer as fast as possible the presence of drugs (or poisons) and their concentration range (therapeutic/normal, toxic or comatose-lethal) in suspected cases of intoxication or drugs misuse. In forensic toxicology (especially in post-mortem toxicology), the toxicology test is the main part of forensic toxicology report and should provide information concerning i) the presence of any drugs or poisons in the samples; ii) the amount of the poison; iii) the time of ingestion; iv) description of the effects of poison(s) and their influence (relation) to the case; v) assistance to medical examiner in explanation of the toxic injures or poisoning-related death.

In the present case the widely used oral antidiabetic drug metformin is a key-factor responsible for the fatal poisoning of young woman. It should be noted thatin last years the metformin usage (and overdosage, respectively) increases the number of cases accompanied by life-threatening lactate acidosis.

Metformin is a small (MW 165 g/mol) and water-soluble (as hydrochloride) molecule and therefore it can theoretically be extracted by dialysis. The drug is characterized by 50% bioavailability, volume of distribution higher than 3 L/kg (single oral dose of 850 mg results in 654 ± 358 L). Metformin is not bound to proteins and its main route of elimination is renal tubular secretion without hepatic metabolism.

Metformin-associated lactate acidosis (MALA) is the most frequent reported side effect of this drug with an estimated incidence of 2-15 cases per 100,000 patients each year with an associated mortality rate up to 50 % [1-4]. The exact mechanism of MALA is still unclear. It has been accepted that the lactate formation is a result of anaerobic intracellular metabolism [5,6]. Lactic acidosis is defined when the serum lactate concentration is higher than 5 mmol/L and pH of arterial blood is less than 7.35 [7]. The typical symptoms of lactic acidosis include drowsiness,

lethargy, and hypoglycemia[8]. The symptoms of severe MALA are nonspecific – tachycardia, hypotension, tachypnea, mental status changes [4]. In acute MALA pH is reported to be 6.87 ± 0.11 [9]. Hypothermia [2] and hyperkalemia [10] are also reported.

The correction of metabolic acidosis is crucial for the treatment. It should be started with hemodialysis (CVVH, CVVHD) [2,4,10,11]. Sodium bicarbonate ($NaHCO_3$) infusion alone is not able to compensate the acidosis sufficiently [12]. Bicarbonate therapy for severe lactic acidosis remains controversial therapy according to some authors [3].

The other key-drug in the present case is the antihypertensive drug clonidine, which was taken under trade name Chlophadon® (Sopharma, Bulgaria). It is a combined drug containing clonidine (0.150 mg as hydrochloride) and chlorthalidone (20 mg). The drug is widely used in Bulgaria for treatment of arterial hypertonia. The clonidine toxicity results mainly in bradycardia and hypotension. Cardiac bradyarhythmias can occur as well as developing AV block [13].

## *CASE REPORT*

19-years-old woman was found after ingestion of different drugs byher grandfather. In ED she was not able to specify the amount of the drugs - Chlohadon® (clonidine/chlorthalidone), Atarax® (hydroxyzine), Metfogamma® (metformin), Antistenocardin® (dipyridamole). She vomited twice. On the next day her family informed about additionally found blister-packs of Aspirin-protect® (acetylsalicylic acid) and Yasmin®(drospirenone/ethinylestradiol). From the physical examine: the patient was somnolent and drowsy; pale and dry mucosa; blood pressure 80/60 mm Hg, heart rate 60 b/min; a weak abdominal pain on palpation was noticed; normal peristaltic was found. Hypothermia was observed.

Laboratory data: Urine – positive for glucose, acetone and albumin; pH 6. Blood count– WBC 10.4/14.4 G/L; no significant abnormalities in other parameters - in reference range.Severe disturbance of hemostasis was registered – practically no coagulation of the blood (out of instrument range) 14h after admittance. Clinical chemistry and arterial blood gas analyses – the results are presented in Table 1. The X-ray study of lung and heart identified initial pulmonary edema. Abdominal sonography revealed an hypoechoic liver; spleen, gall bladder, and pancreas were without pathological changes. There was diffuse parenchymal process in liver and kidneys.

Clinical course. Gastric lavage, activated charcoal and supportive measures were implemented. The patient status rapidly deteriorated (some vital parameters are presented on Table 2). The patient was intubated and put on mechanical ventilation. The severe acidosis was not influenced by bicarbonate infusion and respiratory therapy.The arterial hypotension was not influenced by catecholamines (epinephrine, dopamine) and atropine, which contraindicated extracorporeal clearance. The patient died 22 hours after admittance.

Post-mortem examination. Autopsy findings revealed severe pulmonary edema, pleural effusions (about 300mL bilaterally) and ascites (about 200 mL). Initial focal congestive pneumonia with confluence was observed. Brain edema with wedging of the cerebellar tonsils into the foramen magnum was observed. The kidneys were anemic, with punctate hemorrhages in the renal pelvis. Heart, liver, pancreas and spleen were without macroscopic specifics. Stomach and initial part of the small intestine were filled with black content (activated charcoal). Small intestine and colon were dilated. Blood stasis in the walls of the small intestine was found. The histology findings revealed as follows brain – perivascular and pericellular edema; lung-haevyedema, blood stasis, focal congestive pneumonia, with confluence; myocardium-fragmentation of myofibres; kidneys-anemia; liver- without specifics.

### *TOXICOLOGICAL ANALYSIS*

Materials and methods. Blood, urine and gastric content were collected ante-mortem and blood, tissue samples (liver, kidney) were taken during the autopsy. All samples were frozen (- 10°C) and transported in fridge bag to the Toxicology Lab. HS-GC-FID analysis of alcohol was performed (Agilent HS 7697 A; Agilent GC 7890 B; HP-Innowax capillary column). The immunoassays were accomplished using Innovacon® (USA) multidrug cassette. The salicylate assay was done using Trinder' reagent. The general unknown screening of blood and urine was performed after basic liquid-liquid extraction with ethylacetate and GC-MS analysis on HP-5ms column (Agilent GC 7890 B; Agilent MSD 5977 A) using library (NIST, PMW, Willey) identification of mass-spectra obtained. The screening and measurement of drugs in liver sample was performed after deproteinization in oxalic acid, lipids removal (cold chloroform) and alkaline liquid-liquid extraction using methyl-tert-butyl ether. The quantitative analysis of clonidine was done using HPLC-UV (Perkin Elmer series 410 pump/LC 95 UV-Vis) on ODS column (Luna®, Phenomenex) and mobile phase consisting of 10 mM triethylamine/phosphoric acid (pH 2.7) and acetonitrile (80:20). The metformin was measured after direct protein precipitation with acetonitrile and using sodium 1-octanesulfonate as ion-pairing agent in the mobile phase (10 mM triethylamine/phosphoric acid, pH 2.7/acetonitrile; 85:15) on Agilent 1260 Infinity HPLC-DAD system equipped with ODS column (Zorbax®, Agilent).

***Results.*** The results of toxicology testing are presented on Table 3.

## *DISCUSSION*

Based on preliminary anamnestic data a suicide attempt using drug overdose is suspected. The patient announced drugs as Chlohadon® (clonidine/chlorthalidone), Atarax® (hydroxyzine), Metfogamma® (metformin), Antistenocardin® (dipyridamole), but woman did not specify the amount. On the next day, members of her family found blister-packs of Aspirin-protect® (acetylsalicylic acid) and Yasmin® (drospirenone/ethinylestradiol). The toxicological profiles of the listed drugs determine three medicines as significant for acute poisoning – clonidine, metformin and acetylsalicylic acid.

Based on medical data the acute poisoning with rapid onset is presented. There is a direct influencing of glucose metabolism (metformin) and acid-base homeostasis (salicylates, MALA). The enhanced enzyme activities (Table 1)

and increased amount of creatinine implies acute liver and kidney process. An interesting down-up deviation in blood glucose is observed. The acidosis is rapidly developed without reaction on respiratory therapy and bicarbonate infusion (typical for MALA) and the last measured value (pH 6.63) is life-incompatible.

The toxicological tests are negative for drugs in antemortem stomach content which suggests a time of ingestion more than 2 hours before hospital admittance. Therefore a high degree of resorption is expected and progress of intoxication dynamics. The measured initial blood concentrations of metformin and clonidine are ranged as comatose-lethal, which confirms overdosage.

Hydroxizine was not found as a parent drug in the samples, only its metabolite was identified in the urine. Salicylates were not identified in all samples. Acetylsalicylic acid was detected in general unknown urine screening but insignificant as amount (traces) for the case.

Based on results obtained it was concluded that an acute poisoning with metformin and clonidine is presented. Metformin when taken at dosage higher than 3g/24h can initiate MALA. The severe MALA is obvious in the present case – extremely high acidemia and free lactic acid were found in the urine screening which is not typical for the described sample-prep protocol. However, the level of lactate in blood was so high (no data from the hospital stay) that free lactate is excreted in the urine.

The deviations in glucose level are typical for metformin overdosage and also in cases of MALA. Metformin does not usually cause hypoglycemia when administered as monotherapy, but hypoglycemia can occur in setting of MALA (14,15). In cases of MALA hypoglycemia is more often cited (glucose lower than 1 mmol/L) [2,9]. However, there is a reported case of hyperglycemia (glucose higher than 30 mmol/L) [16-18]. To the best of authors' knowledge it is a unique case in metformin poisoning where metformin-induced hypoglycemia is followed by extreme hyperglycemia.

The severity of acidosis is also characteristic sign of acute MALA. The pH of arterial blood is often lower than 6.8 [9,10]. The bicarbonate alone is not enough to compensate the acidosis, which was described in the current case. Another complication is the effectiveness loss of catecholamines at pH lower than 6.9 (some authors recommend vasopressin administration) [10].

The combination of metformin and clonidine realizes effects which additionally intensify the poisoning. At the same time clonidine effects (bradycardia, hypotension) does not allow effective counteraction of MALA as hemodialysis performance. The other aggravating fact is the complete resorption of the drugs.

In the case presented, the initial dose of clonidine was estimated of approx. 20-30 tablets Chlophadon (containing 0.15 mg clonidine; b.w. 60 kg, Vd 0.9 L/kg) and approx. 10-13 tablets of Metfogamma (containing 1000 mg metformin; b.w. 60 kg, Vd3 L/kg). The estimated amount of tablets corresponds to the found empty blister-packs of these two drugs.

The anamnestic data of 8 g acetylsalicylic acid is initially misleading. The hydroxyzine and dipyridamol are probably taken in different time period or not relate to the case.

***Table 1.*** *Clinical chemistry and ABGs results.*

| Lab. test | Units | Initial value | Clinical course | Last value |
|---|---|---|---|---|
| Glucose | [mmol/L] | 3.12 | 3.87..0.21..31.05..28.7 | 20.5 |
| Urea | [mmol/L] | 5.6 | 5.0 | 6.7 |
| Creatinine | [µmol/L] | 162 | 286 | 323 |
| Uric acid | [µmol/L] | 119 | 222 | 255 |
| Total Protein | [g/L] | 68.4 | 46.9 | 34.9 |
| Albumin | [g/L] | 42.6 | 27.1 | 23.8 |
| ASAT | [U/L] | 50.0 | 127.2 | 377 |
| ALAT | [U/L] | 10.6 | 114.9 | 435.1 |
| GGT | [U/L] | 16 | 24 | 34 |
| HBDH | [U/L] | 85 | 248 | 426 |
| CK/CK-MB | [U/L] | 66/17 | 913/161 | 1077/234 |
| LDH | [U/L] | 284 | 911 | 1505 |
| Total Bilirubine / conjugated (direct) | [µmol/L] | 11.4/4.4 | 9.99/6.3 | 15.98/8.5 |
| $K^+$ | [mmol/L] | 4.33 | 4.52 | 4.13 |
| $Na^+$ | [mmol/L] | 133.5 | 123.3 | 130.4 |
| pH | | 7.29 | 6.51..6.58..6.62..6.58 | 6.63 |
| $P_a CO_2$ | mm Hg | 26.2 | 54.6..56.6..68.6..83.4 | 95.2 |
| $P_a O_2$ | mm Hg | 119 | 70.3..88.8..60.4..46.6 | 41.7 |
| $HCO_3$- | [mEq/L] | 12.4 | 2.8..3.6..4.1..3.7 | 4.7 |
| BE | [mmol/L] | -12.9 | -29.4..-28.0..-26.5..-26.4 | -24.3 |
| t $CO_2$ | [mmol/L] | 25.1 | 12.3..14.3..18.5..21.0..26.3 | 28.5 |
| Sat $O_2$ | [%] | 99.1 | 72.3..84.7..71.2..54.6..54.9 | 37.5 |

***Table 2.*** *Some vital parameters of the patient.*

| Parameter | Units | Initial value | Clinical course | Last value |
|---|---|---|---|---|
| Heart rate | [b/min] | 60 | 66..63..66..90..78..87..72..62 | 30 |
| Blood pressure | [mm Hg] | 80/60 | 70/50..80/45..81/40..70/30..60/30..40/20 | 42/30 |
| Body temp. | [°C] | 36.0 | 36.7..36.2..38.4 | |

## *CONCLUSION*

As a conclusion of the forensic toxicology report in the current case a suicide with metformin and clonidine overdose was found. Based on clinical report interpretation and on toxicology testing results, an explanation of

***Table 3**.Toxicology results.*

| Sample | Blood | Urine | Stomach content | Blood | Liver |
|---|---|---|---|---|---|
| Test | *Antemortem* | | | *Postmortem* | |
| Alcohol | ( - ) | ( - ) | | ( - ) | |
| Immunoassay[1] | | ( - ) | | | |
| Screeningnot (GC-MS) | | lactic acid, metformin, hydroxyzine-M, piracetam, metoclopramide, acetylsal. acid | Drugs are detected. ( - ) | metformin, piracetam, metoclopramide, atropine | |
| Salicylates | ( - ) | ( - ) | ( - ) | ( - ) | ( - ) |
| Clonidine[2] | 0.1 µg/mL | | | 0.02 µg/mL | ( + ) |
| Metformin[3] | 66 µg/mL | | | 20 µg/mL | ( + ) |

1 BAR, BZD, TCA, OPI, COC, AMP, MET, THC, MTD, MDMA.
2 Clonidine: therapeutic 0.001-0.002 µg/mL toxic 0.025-0.5 µg/mL.
3 Metformin: therapeutic 0.1-1.0 µg/mL; toxic 5-10 µg/mL.

the poisoning mechanism and all effects of toxicity observed as well as their forensic significance, are presented. The case reported illustrates a unique alteration of blood glucose level in metformin poisoning – hypoglycemia followed by extreme hyperglycemia.

*Acknowledgement*
Dr. V. N. Atanasov thanks the European Social Fund and Republic of Bulgaria, Operational Programme "Human Resources Development" 2007-2013 framework, Grant No BG051PO001-3.3.06-0048 / 04.10.2012.

## *Sažetak*

Devojka starosti 19 godina, primljena je u Urgentni centar. Prema anamnestičkim podacima uzela je različite lekove (prazna blister pakovanja): 15 g metformina, 625 mg hidroksizina, 750 mg dipiridamola, 3 mg klonidina i 400 mg hlortalidona (kombinovani antihipertenziv), 8 g acetilsalicilne kiseline, 63 mg drospirenona / 0,63 mg ethinil estradiola. Inicijalno, bolesnica je bila izmenjenog mentalnog statusa uz normalne vitalne znakove, ali vrlo brzo dolazi do pogoršanja opšteg stanja. Zabeležena je ozbiljna acidoza (pH 6,51, BE – 29,4 mmol/L). Ovo je prvi slučaj trovanja metforminom sa ekstremnom hipoglikemijom (0,21 mmol/L) koja je preraska u hiperglikemiju (31,05 mmol/L). Zabeležen je pad srčanog pulsa (30/min) i 22 h od prijema bolesnica je preminula. Analizirani su za životni i *post mortem* uzorci (krv, urin, sadržaj želuca/krv, uzorci tkiva organa). U radu su prikazani rezultati forenzičke toksikološke analize koji ukazuju na letalno predoziranje metforminom/klonidinom, kao i interpretacija i diskusija rezultata.

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■ The paper was received on 04.08.2014. Accepted on 15.08.2014.