



Case report

Anemia with elevation of growth differentiation factor-15 level in linezolid treated multidrug-resistant tuberculosis: Case series of three patients

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ABSTRACT

Linezolid is now recommended as a first line drug for Multidrug Resistant Tuberculosis (MDR-TB). Previous studies reported hematologic toxicity as one of the main side effects. The mechanism of this toxicity is mitochondrial dysfunction, for which a biomarker is Growth differentiation factor-15 (GDF-15). There is no previous report about GDF-15 and its association with hematologic toxicity from Linezolid in the treatment of MDR-TB. We present three cases of MDR-TB involving severe anemia associated with linezolid who had GDF-15 elevation. These cases highlight the need for more research into the relationship between GDF-15 and hematologic toxicity in MDR-TB patients treated with linezolid.

Introduction

In 2018, WHO re-classified linezolid as a group A drug for treatment of Multidrug Resistant Tuberculosis (MDR-TB), meaning that Linezolid should be part of the initial regimen for MDR/ Extensively Drug Resistant Tuberculosis (XDR-TB) in longer treatment [1]. Linezolid has good efficacy, but can cause severe adverse events, especially hematologic toxicity [2]. A previous study showed that linezolid blocks mitochondrial protein biosynthesis and decreases adenosine three phosphate (ATP) production in bone marrow precursor cell, leading to hematologic toxicity [3].

Mitochondrial dysfunction can be detected by measuring concentration of growth differentiation factor-15 (GDF-15). The measurement of GDF-15 level is beginning to enter clinical practice as a diagnostic biomarker in mitochondrial diseases and as a prognostic marker in conditions such as heart failure and certain cancers [4,5].

Anemia caused by linezolid is common, with a reported incidence as high as 38.1 % in MDR and XDR -TB patient treated with linezolid for median 300 (140–690) days [6]. However, the possible association

between anemia and GDF-15 level in MDR-TB has not been reported. In this case series, we described three patients with MDR-TB who developed anemia during treatment with linezolid-containing regimen. We measured GDF-15 level to explore the possibility of mitochondrial toxicity as the cause of severe anemia.

Patient 1

A 20-year-old woman, with body weight 46 kg was diagnosed as having MDR-TB plus resistance to fluoroquinolone and was treated with linezolid-containing regimen (cycloserine 750 mg, clofazimine 100 mg, linezolid 600 mg (13 mg/kg body weight), bedaquiline 200 mg). She had no other disease and was not taking any other medication. Before starting MDR-TB treatment, her hemoglobin (Hb) level was 12.1 gr/dL, neutrophil and platelet count were 3450/ml and 298,000/ml respectively. She developed severe anemia (Hb level 5.5 g/dL) at 12th week of treatment, accompanied with declining neutrophil and platelet count (1570/ml and 254,000/ml, respectively). No other causes of anemia were found. She was hospitalized and had 4 packed red cell transfusion.

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Linezolid was stopped and delamanid 100 mg bid was started. Four weeks after linezolid was stopped, her Hb level was 12.7 gr/dL, neutrophil and platelet count were 2180 /ml and 280,000/ml, respectively. GDF-15 level was normal at baseline: 1189.52 pg/ml (normal 200 –1200 pg/ml), and markedly elevated to 4108.88 pg/ml at 2nd week of linezolid treatment.

Patient 2

A 66-year-old man, with body weight 40 kg was started on treatment for MDR-TB with the WHO recommended initial regimen (cycloserine 750 mg, levofloxacin 750 mg, clofazimine 100 mg, linezolid 450 mg (11 mg/kg body weight), bedaquiline 200 mg). He had diabetes mellitus treated with insulin. He had not taken any other medication. Before starting TB treatment, he already had low hemoglobin level (10 g/dL), normal neutrophil (6190/ml) and platelet (447,000/ml) count. He developed severe anemia (Hb level 5.7 g/dL) at the 8th week of treatment with neutrophil 1980/ml and platelet 241,000/ml. No other causes of anemia were found. He was hospitalized and received 3 packed red cell transfusion. Linezolid was stopped and changed with delamanid 100 mg bid. Four weeks after linezolid was stopped, his Hb level was 8.8 gr/dL, neutrophil and platelet count were 3030 /ml and 147,000/ml, respectively. Elevated GDF-15 level was detected before treatment: 11,634.4 pg/ml (normal 200–1200 pg/ml).

Patient 3

A 51-year-old man, with body weight 69 kg was diagnosed as having MDR-TB and was treated with linezolid-containing regimen (cycloserine 750 mg, levofloxacin 750 mg, clofazimine 100 mg, linezolid 600 mg (8 mg/kg body weight), bedaquiline 200 mg). He had diabetes mellitus, chronic kidney disease, and mass in urinary bladder. His blood sugar level was controlled by diet, he had not taken any other medication. Before starting TB treatment, his Hb level was 13 g/dL, neutrophil and platelet count was 7690 /ml and 490,000/ml, respectively. He developed moderate anemia (Hb level 9.6 g/dL) at 4th week of treatment with neutrophil 6560 /ml and platelet 543,000/ml. There was no other cause of anemia found. He was hospitalized and received 2 packed red cell transfusion. Linezolid was continued. Four week later, his Hb level was 11.4 gr/dL, neutrophil 7390 /ml and platelet 434,000/ml. GDF-15 level was markedly high at baseline: 12,105.28 pg/ml (normal 200–1200 pg/ml) and further increased to 27,450.88 pg/ml at the 2nd week of linezolid treatment.

Hematology parameters over time after starting MDR-TB treatment in all three patients are depicted in Fig. 1.

Discussion

The three patients presented all developed anemia at some point after starting linezolid treatment. The hemoglobin level returned to

normal values in the two patients in whom Linezolid was stopped. Interestingly, all three cases had increasing GDF-15 level prior the occurrence or worsening of anemia with two patterns of GDF-15 kinetics. In case 1: levels were normal before treatment but increased 3.5-fold after 2 weeks of linezolid treatment. In case 2 and 3, the levels of GDF-15 were elevated before the start of Linezolid treatment and increased 2.3fold in one, but was not measured in the other.

GDF-15 is a cytokine related to the superfamily of transforming growth factor- β (TGF- β), and is weakly expressed under physiological conditions [7], but increases with hypoxia, inflammation, or oxidative stress [8]. Previous studies reported conflicting results about mitochondrial dysfunction and GDF-15 in TB infection and TB disease. In-vitro TB infection caused mitochondrial dysfunction in *Mycobacterium tuberculosis* (Mtb) infected human alveolar epithelial cell [16]. Infection with *M. tuberculosis* could cause damage in macrophage's mitochondria membrane, increase cytochrome C release from inter-membrane space and cause further necrosis in invitro experiment using mononuclear cells from peripheral blood of healthy donors [12] Liu et al. reported that the mean GDF-15 levels in patients with TB disease and Tuberculosis Infection (TBI) before treatment with antituberculosis were 350 + 10 pg/ml, and 280 + 10 pg/ml, respectively, compared to 310 + 20 pg/ml in healthy controls [9]. On the other hand, Mann et al. reported higher GDF-15 levels in patients with spinal TB before treatment with anti-tuberculosis compared to patients with mechanical back pain (1054 [491 – 1396] vs 653 [416 – 853] pg/ml)[17]. To our knowledge GDF-15 level in patients treated for MDR-TB have not been reported.

Linezolid had been reported to disrupt mitochondrial function [10, 11]. Leach et al. conducted in vitro experiment using *Staphylococcus aureus* bacteria and mitochondrial ribosomes of human cells incubated with linezolid. Bacterial ribosomes and mitochondrial ribosomes are both of prokaryotic origin and share similarities, which provides the theoretical basis of why linezolid binds to these mitochondrial ribosomes and thus causes loss of mitochondrial function in haemopoietic cells [13].

We found 2 distinct phenomena of GDF-15 level. The first one was GDF-15 already elevated at baseline probably due to diabetes and renal failure (patient 2 and 3). Elevated GDF-15 level had been reported in cancer, diabetes and renal failure [14,15].

The second one was elevation after linezolid treatment (patient 1). In our cases, higher baseline GDF-15 levels or an increasing GDF-15 levels during follow-up were preceding the development of anemia after linezolid treatment. The changes of GDF-15 level after starting treatment with linezolid could be represent of mitochondrial dysfunction caused by linezolid. These changes were observed at 2nd week of treatment, preceding the development of anemia (at the 4th to 12th week). Correlation of high level of GDF-15 with low hemoglobin level had been reported in patients with anemia of chronic disease, ineffective erythropoiesis, multiple myeloma, heart failure and chronic kidney disease [Theurl, 2010 #63][Tanno, 2010 #64][Banaszkiewicz, 2020

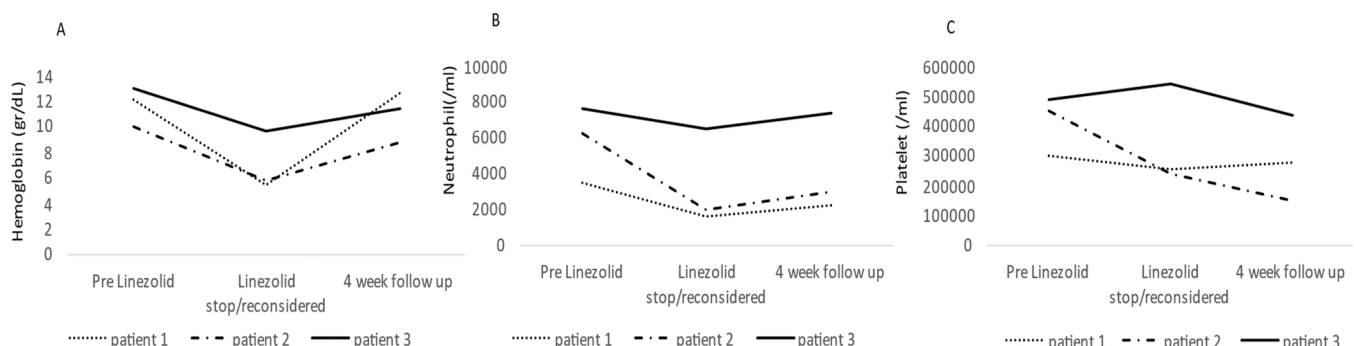


Fig. 1. Hematology parameter changes over time after starting MDR-TB treatment, A. Hemoglobin level, B. Neutrophil count, C. Platelet count.

#65][Przybyłowski, 2014 #66][Ueland, 2021 #67][Nalado, 2020 #68][18–23].

There are some limitations in this case report. We did not measure GDF-15 level in healthy controls, and there was substantial variability of GDF-15 at baseline (normal in patient 1, elevated in patient 2 and 3), plus linezolid was not stopped in patient 3. Bone marrow examination was not performed to exclude possible other causes of anemia. We have only one examination of GDF-15 level in patient 2 because the patient did not come to clinic at 2nd week of treatment.

Conclusion

Our cases indicated that there may be an association of GDF-15 and anemia in MDR-TB patients treated with linezolid. MDR-TB patients with markedly elevated GDF-15 level at 2nd week or if already had high GDF-15 level at baseline warrant close monitoring of hemoglobin level during treatment with linezolid-containing regimen. The role of GDF-15 levels as a predictor of hematologic toxicity in linezolid treated MDR-TB need further studies.

Ethical approval

Research Ethical Committee Faculty of Medicine Universitas Padjadjaran number 559/UN6.KEP/EC/2021.

Consent

Written informed consent was obtained from the patients for publication. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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CRediT authorship contribution statement

Conceptualization: **Oehadian A, Santoso P, Menzies D, Ruslami R** Writing-original draft preparation: **Oehadian A, Santoso P** Writing-review and editing: **Menzies D, Ruslami R**, Approval of final manuscript: All authors.

Conflict of interest

The authors report no conflicts of interest.

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