Changes in nitric oxide synthase and nitrite and nitrate serum levels in patients with or without MDR-TB undergoing the intensive phase of anti-tuberculosis therapy

Dmytro O. Butov a,*, Mikhail M. Kuzhko b, Irina M. Kalmykova c, Irina M. Kuznetsova d, Tatyana S. Butova a, Olena O. Grinishina e, Olga A. Maksimenko f

a Kharkiv National Medical University, Kharkiv, Ukraine
b F.G. Yanovsky National Institute of Phsyiatry & Pulmonology, National Academy of Medical Sciences, Kiev, Ukraine
c Regional TB Dispensary No. 1, Kharkiv, Ukraine
d Regional TB Hospital No. 1, Kharkiv, Ukraine
e Regional TB Dispensary No. 3, Zmeyev, Ukraine
f Regional TB Dispensary No. 4, Izyum, Ukraine

ABSTRACT

Background: There is a paucity of published data on the effect of TB chemotherapy on nitric oxide (NO) synthesis and metabolism in newly diagnosed and relapsed patients with or without multi-drug resistant tuberculosis (MDR TB).

Methods: The pattern of NO response in 140 patients with pulmonary TB, including 74 with MDR-TB and 66 without MDR-TB has been studied and compared to the NO status of 30 healthy donors. Patients comprised those with newly diagnosed TB and recurrent or relapsed TB. The NO status was assessed by measuring inducible NO synthase (iNOS) and nitrates and nitrites levels. This was measured prior to treatment initiation and two months after the prescribed chemotherapy.

Results: Increased levels of NO indices were found in patients with tuberculosis when compared to healthy controls. After two months of chemotherapy a significant decrease in NO indicators was observed in the patients with TB, particularly in those without MDR-TB and newly diagnosed TB. The NO status was assessed by measuring inducible NO synthase (iNOS) and nitrates and nitrites levels. This was measured prior to treatment initiation and two months after the prescribed chemotherapy.

Conclusion: Changes in serum levels of nitrites and nitrates as well as iNOS activity in neutrophils may serve as diagnostic criteria to differentiate various clinical forms of TB and help as prognostic tool to predict treatment outcome.

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**Introduction**

About one third of the world’s population is infected with *Mycobacterium tuberculosis* (MTB) [1]. MTB is the leading cause of death, which kills about 2 million people every year. In 1993 the World Health Organization declared tuberculosis (TB) as a global epidemic [2]. Resistance of MTB to anti-mycobacterial drugs remains one of the urgent problems all over the world [3]. The spread of multi-drug resistant (MDR-TB) and recurrent TB sustains the TB epidemics; therefore, this disease remains one of the main threats to global health [1]. In some countries it is even considered to be a national security threat. The issuance of numerous guidelines and recommendations to control MDR-TB is indicative of this [4–9].

According to consensus opinion, unfavorable socio-economic conditions are considered to be one of main causes for TB recurrence [10–12]. However, it is also clear that changes in the immune system and metabolite controllers of the human body play an important role. One of these metabolite controllers is nitric oxide (NO). NO, the product of the arginine dependent pathway of human mononuclear phagocytes, regulates a host of physiological functions and pathologic processes. The discovery of the importance of NO by Furchgott, Murad and Ignarro brought them the Nobel Prize award. There is an evidence that NO and its metabolites, e.g., nitrates and nitrites, can mobilize the anti-mycobacterial defense in the host [13]. NO is synthesized in immune cells under the influence of NO inducible synthase (iNOS), the activity of which is influenced by various cell damage factors [14,15]. The NO-producing phagocytic cells appear to act as one of the critical effectors of cellular immunity to produce the direct anti-mycobacterial effect [16,17]. Nevertheless, the excessive production of NO can also enhance the damaging effect, negatively affecting effector cells. This results in apoptosis and necrosis. Thus, different concentrations of NO can either stimulate or slow down apoptosis [17].

Very few studies exist that are devoted to the evaluation of the levels and role of NO in patients with pulmonary TB. A consistent decrease in the level of NO in TB was noted in some publications [13]. But in other studies, the rise of NO levels in patients with pulmonary TB was noted [18].

The significance of the problem and contradictory observations led to this study. The aim of this study was to evaluate the effect of NO synthesis and respective metabolites in patients with various forms of TB during the intensive phase of anti-tuberculosis therapy.

**Materials and methods**

**Patients**

This study involved 140 patients who were divided as follows: Group 1 comprised of 74 patients with MDR-TB; Group 2 comprised of 66 patients with TB without MDR; and Group 3 comprised of 30 healthy individuals as controls; all subjects were in the 20–70 years age range. Group 1 was further divided into two subgroups; Group 1A with 41 patients who had recurrent TB with MDR-TB (rMDR-TB) and Group 1B with 33 patients with newly diagnosed MDR-TB (ndMDR-TB). Group 2 was also subdivided into Group 2A consisting of 15 patients with rTB without MDR-TB and Group 2B which had 51 patients with newly diagnosed TB without MDR (Fig. 1). All patients had an infiltrating form of pulmonary TB. The first-line drugs were used as standard chemotherapy: isoniazid (0.3 g), rifampicin (0.6 g), pyrazinamide (2 g), ethambutol (1.2 g) and/or streptomycin (1 g) with dose reduction after the intensive phase of the therapy. The patients were observed and treated at the Regional TB Hospital No. 1 and Regional TB Dispensary No. 1 – both in Kharkiv city; and Regional TB Dispensaries Nos. 3 and 4 in Zmeyev and Izyum cities of Kharkiv region, respectively. This study has received the ethics approval of the committee at Kharkiv TB Hospital, and all patients have given informed consent prior to study initiation.

**Nitrites, nitrates and iNOS measurement**

Taking into consideration the fact that NO is an unstable molecule with the half-life of less than 5 s, the study was aimed to measure the content of stable serum metabolites of the NO (nitrites, nitrates) and iNOS activity in neutrophils according to previously published spectrophotometric methods [19,20]. Measurements of serum samples of patients were conducted during the first days after the admission to the hospital and 2 months after the standard anti-mycobacterial therapy. As NO metabolites can be influenced by exogenous factors like diet, blood samples were collected prior to the morning meal.

**Statistical evaluation**

The obtained data were evaluated by the standard Student t-test [21]. The difference was considered to be significant at p < 0.05.

**Results and discussion**

Table 1 shows that the initial activity of iNOS in the neutrophils and the levels of NO metabolites (nitrites and nitrates) in the venous blood of all patients with pulmonary TB was significantly greater – almost twice that as in the healthy donors (p < 0.001). This suggests that prior to treatment there is an intensive formation of NO in the patients with TB. Similarly, there is a significant difference in these indices (p < 0.001) in patients with all forms of TB before treatment initiation as compared with the status after two months of anti-tuberculosis therapy. The indicators of NO synthesis and metabolism appeared to be higher in patients without MDR than in patients with MDR-TB. After two months of treatment the patients with MDR had indicators higher than those without MDR-TB, which suggests a more intensive reparative process correlating with a significant reduction of MTB in patients without MDR as compared with those with MDR. The NO indices were higher in patients with newly diagnosed pulmonary tuberculosis (NDPTB) than in the patients with recurrent pulmonary tuberculosis (RPTB). However, after two months the same indices were higher in patients with RPTB than in those with NDPTB. Therefore, the changes in NO indices are more profound in patients with RPTB than in...
patients with NDPTB. When RPTB patients with MDR are compared to patients with relapsed TB without MDR, the more reliable indicators of NO were observed in TB patients without MDR than in those with MDR before treatment ($p < 0.05$). After two months of chemotherapy the indices were higher in TB patients with MDR than in TB patients without MDR. The same outcome was observed in patients with NDPTB with or without MDR. The levels of nitrites after the two months in the RPTB patients with MDR and without MDR were not affected in a significant manner ($p > 0.05$). The comparison of indices of RPTB with MDR and those of NDPTB without MDR reveals a significant difference between these subgroups ($p < 0.05$). Moreover, when RPTB without MDR is compared with NDPTB with MDR, the differences were insignificant ($p > 0.05$) both prior to treatment as well as after the two months, which suggests that the clinical course is more severe in patients with relapsed TB.

Table 1 – Nitric oxide synthase (iNOS) activity and levels of nitrates and nitrites in patients with pulmonary tuberculosis prior to and after 2-month TB chemotherapy as compared with healthy controls (Mean ± SD).

<table>
<thead>
<tr>
<th>Groups and subgroups</th>
<th>Nitrates (µmol/L)</th>
<th>Nitrites (µmol/L)</th>
<th>iNOS (pmole/min/mgB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After 2 months of treatment</td>
<td>Before treatment</td>
</tr>
<tr>
<td>1 MDR-TB (N = 74)</td>
<td>62.89 ± 1.42 $a,b$</td>
<td>46.65 ± 1.04 $a,d$</td>
<td>5.626 ± 0.15 $a,b$</td>
</tr>
<tr>
<td>1A recurrent MDR-TB (N = 41)</td>
<td>59.29 ± 1.79 $a,h$</td>
<td>49.38 ± 1.3 $a,d,h$</td>
<td>5.027 ± 0.17 $a,b,h$</td>
</tr>
<tr>
<td>1B new Dx MDR-TB (N = 33)</td>
<td>67.36 ± 2.03 $b,g,k$</td>
<td>43.26 ± 1.5 $b,d,g,k$</td>
<td>6.371 ± 0.19 $b,g,l$</td>
</tr>
<tr>
<td>2 TB (N = 66)</td>
<td>72.02 ± 1.43 $b$</td>
<td>35.65 ± 1.06 $c,d$</td>
<td>6.747 ± 0.17 $b$</td>
</tr>
<tr>
<td>2A recurrent TB (N = 15)</td>
<td>66.26 ± 1.89 $c,d,i$</td>
<td>40.53 ± 1.83 $c,d,i$</td>
<td>5.686 ± 0.2 $c,d,i$</td>
</tr>
<tr>
<td>2B new Dx TB (N = 51)</td>
<td>73.72 ± 1.71 $b,i$</td>
<td>34.22 ± 1.19 $b,h$</td>
<td>7.059 ± 0.19 $b,i$</td>
</tr>
<tr>
<td>3 Controls (N = 30)</td>
<td>37.98 ± 1.3</td>
<td>3.83 ± 0.093</td>
<td>81.03 ± 2.36</td>
</tr>
</tbody>
</table>

- a Discrepancy is significant ($p < 0.001$) when Groups 1 and 2 are compared.
- b Discrepancy is significant ($p < 0.05$) when compared with Group 3.
- c Discrepancy is not significant ($p > 0.05$) when compared with Group 3.
- d Discrepancy is significant ($p < 0.001$) when compared before treatment and after two months levels among Subgroups.
- e Discrepancy is significant ($p < 0.05$) when Subgroup 1A and 2A are compared.
- f Discrepancy is not significant ($p > 0.05$) between Subgroups 1A and 2A.
- g Discrepancy is significant ($p < 0.01$) between Subgroups 1B and 2B.
- h Discrepancy is significant ($p < 0.05$) between Subgroups 1A and 1B.
- i Discrepancy is significant ($p < 0.05$) between Subgroups 2A and 2B.
- j Discrepancy is significant ($p < 0.001$) between Subgroups 1A and 2B.
- k Discrepancy is not significant ($p > 0.05$) between Subgroups 2A and 1B.
- l Discrepancy is significant ($p < 0.05$) between Subgroups 2A and 1B.
and 2A. The nitrate levels were the same in Groups 2 and 2A and iNOS activity in Subgroup 2B. This suggests the normalization of the NO metabolism in the above Groups and Subgroups. In the remaining groups of patients the differences in NO indices were significant \( (p < 0.05) \) as compared with healthy controls.

**Conclusions**

In patients with pulmonary TB, significantly higher levels of NO activity were observed as compared with the levels in healthy individuals. In patients with recurrent TB and MDR-TB, significantly lower levels of NO indicators were observed by comparison with patients with newly diagnosed pulmonary tuberculosis.

After two months on chemotherapy, a significant decrease in iNOS activity and NO metabolites was observed in patients with pulmonary TB, but the decrease of NO indicators was manifested mostly in the NDTPT patients and patients without MDR as opposed to patients with recurrent TB and MDR-TB, which suggests lower levels of immunologic and reparative processes in such patients.

Therefore, the levels of nitrates and nitrates as well as iNOS activity may serve as additional diagnostic criteria to differentiate MDR-TB from non-resistant TB in patients with relapsed and newly diagnosed TB. Easily assessed NO-related markers can also serve as predictors of treatment outcome since patients with drug-susceptible strains had lower NO output approaching levels found in controls. Further studies are warranted to elucidate the relationship between NO and resistance of mycobacteria to TB drugs.

**Author contributions**


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**Competing interests**

The authors have declared that no competing interests exist.

**Conflict of interest**

We have no conflict of interest to declare.

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**References**


