Adjuvant Immunotherapy of Extensively Drug-Resistant Tuberculosis (XDR-TB) in Ukraine

Natalia D. Prihoda, Olga V. Arjanova, Larisa V. Yurchenko,
Nina I. Sokolenko, Lyudmila A. Vihrova,
Volodymyr S. Pylypchuk, Valery M. Frolkov and Galyna A. Kutsyna
Lisichans Regional Tuberculosis Dispensary, Lisichansk, Ukraine
Lisichansk Regional Hospital, Lisichansk, Ukraine
Ekomed LLC, Prospect Prawdy 80-A, Kiev, Ukraine
Luhansk State Medical University, Luhansk, Ukraine

Abstract: Conventional TB chemotherapy success rates are very low in patients with Extensively Drug-Resistant Tuberculosis (XDR-TB). We treated twelve XDR-TB individuals, seven of which in addition to standard Anti-TB Therapy (ATT) received Immunoceol (Dzherelo), Svitanox and Lisorm over-the-counter herbal immunomodulators. All seven patients who received adjunct immunotherapy improved clinically and radiologically and were discharged after 3.7 ± 0.8 months, with average/median time to mycobacterial clearance 28/25 days. None of five patients on TB drugs alone improved and one died. Patients on immune intervention gained 9.6 kg (p = 0.0001) while those on ATT lost 1.4 kg. The levels of total bilirubin decreased from 15.6 to 10.7 μmol L⁻¹, similarly, the values of alanine transaminase (ALT) declined from abnormally high 42.6 IU L⁻¹ to normal levels 22.9 IU L⁻¹ (p = 0.23). Patients on ATT had unchanged levels of bilirubin, but their ALT declined from 29.6 to 12 IU L⁻¹ (p = 0.02). The levels of hemoglobin rose from 104.1 to 118 g L⁻¹ (p = 0.07) whereas leukocyte counts descended to normal levels from 8.9 to 7.3 × 10⁹ cells L⁻¹ (p = 0.003). Conversely, in patients on ATT leukocyte counts rose from 8.7 to 13.8 × 10⁹ cells L⁻¹ (p = 0.21), whereas hemoglobin declined to below normal levels from 116.4 to 96.6 g L⁻¹ (p = 0.18). These results show that immune-modulating interventions can favorably influence the effect of TB drugs. The difference between two treatment outcomes was highly significant (Mantel Haenszel odds ratio = 11, p = 0.0009 at 95% CI). Thus, adjunct herbal immunotherapy is safe, shortens treatment duration and can overcome drug resistance even in patients with XDR-TB.

Key words: Immunomodulator, MDR-TB, XDR-TB, phytoconcentrates, Mycobacterium

INTRODUCTION

The extensively resistant TB (XDR-TB) is diagnosed when M. tuberculosis bacilli in addition to lack of sensitivity to isoniazid (H) and rifampicin (R), two most commonly-used, first-line TB drugs, are also resistant to any one of fluoroquinolones and of second-line injectable drugs, e.g., capreomycin, kanamycin and amikacin (Migliori et al., 2008). This emerging form of TB caused worldwide concern after outbreak in KwaZulu Natal Province of South Africa where 52 of 53 patients with XDR tuberculosis and HIV co-infection died within 2 weeks of the time of diagnosis (Gandhi et al., 2006). Success rates in treating XDR-TB are significantly lower than among drug-sensitive cases ranging between 29 and 67%. In addition, it takes much longer (18-24 months) to
achieve a cure and concerns over adverse effects of drugs became more prominent since second-line drugs are more toxic. The cost is another factor as the deployment of second-line drugs increases treatment cost by about hundred-fold. Clearly, there is an urgent need to find additional therapeutic interventions that could overcome these problems.

Immunomodulators Immunocel (Dzherelo), Svitanka and Lizorm are made from a proprietary combination of medicinal plants and are commonly used in Ukraine for the management of TB and HIV infections, including patients with dual infection (Arjanova et al., 2009; Chechitan et al., 2007; Melnik et al., 1999; Nikolaeva et al., 2008; Prihoda et al., 2007; Zaitzeva et al., 2008). They have been approved in 1997 by the Ministry of Health of Ukraine as functional supplements with therapeutic indications. Dzherelo and Svitanka were specifically recommended as immune adjuvants to the therapy of pulmonary tuberculosis (Melnik et al., 1999). So far, the phytoconcentrates we have decided to use in this study have been taken safely by several hundred thousand individuals for various indications including chronic bacterial and viral infections such as TB and HIV, autoimmune diseases and malignancy (Chkhettiani et al., 2007). In this retrospective study, conducted at Lisichansk TB Dispensary, we have compared the adjunct effect of herbal immunomodulators to outcome of treatment with conventional TB therapy.

MATERIALS AND METHODS

Patients

Lisichansk TB Dispensary is within Luhansk administrative region of the Eastern Ukraine with total population 2.5 million. Approximate population of registered TB patients in this region is 2000. Lisichansk TB dispensary has turnover of about 600-800 patients per year. The dispensary has six medical doctors and approximately 15 medical nurses and lab technicians who care for hospitalized patients and perform the lab work.

Twelve patients with pulmonary XDR-TB were identified retrospectively, five who received individualized TB drugs regimen and seven who received in addition to ATT a combination of immunomodulating phytopreparations Dzherelo, Svitanka and Lizorm. All patients were males with age range between 25 and 67 years. Five presented with first-diagnosed TB and the rest were previously treated, relapsed cases of TB. All study patients presented with acute symptoms of pulmonary TB that required hospitalization. Most common symptoms were prolonged heavy cough, pain in the chest, high fever, profuse night sweats, fatigue and loss of weight and appetite. Active pulmonary tuberculosis was certified by a medical history and clinical findings compatible with tuberculosis, a chest X-ray showing lung involvement and positive sputum smear for Acid-Fast Bacilli (AFB) and the culture of M. tuberculosis. The conduct of the study was approved by the Internal Review Board (IRB) of Lisichansk TB dispensary in accordance with the Helsinki Declaration.

Treatment Regimen

All anti-TB drugs were procured free-of-charge through the centralized national supply system of Ukraine. The over-the-counter phytoconcentrates, Dzherelo, Lizorm and Svitanka were generously supplied by Ekomed ILC. Individualized, first- and second-line anti-TB drugs were administered to all hospitalized patients based on physician’s decision prior to or after results of drug susceptibility tests. In the immunotherapy group, in addition to ATT, patients received a daily dose of Dzherelo which was given as 30 drops diluted in a half-glass of water 30 min before breakfast. Some patients received Immunocel, a slightly modified form of Dzherelo. The same dose, 30 drops, of Lizorm and Svitanka were given before lunch and supper, respectively. The exact formula of phytoconcentrates has been described by Prihoda et al. (2008). Sputum smear and culture examinations for AFB were
performed at monthly intervals. The decision to discharge was based on at least twice-repeated negative culture outcome and satisfactory clinical and radiological findings.

**TB Drug Resistance Testing**

The drug resistance to first- and second-line TB drugs was tested with commercially supplied kits (Tulip Diagnostics, Goa, India). The cultures of *M. tuberculosis* derived from sputum of each patient were inoculated into ready-to-use tubes containing TB drugs incorporated at manufacturer-predicted concentrations into standard Löwenstein-Jensen agar slants. The cultures were incubated at 37°C and checked periodically until appearance of colonies in control tubes without drugs. The calculation of the proportion of resistant bacilli was done by comparing counts on drug-free and drug-containing Löwenstein-Jensen medium.

**Statistical Analysis**

The obtained results were analyzed with available online statistical software (GraphPad Software, Inc., La Jolla, CA). All statistical analysis were done on intent-to-treat basis, involving the total number of patients without subgrouping them into responders and non-responders. The stratification analysis of patients was conducted to reveal the difference between distinct treatment categories. Parametric baseline values relative to the end of study values were evaluated by paired or unpaired Student t-test. The categorical test was done by Mantel Haenszel’s odds ratio calculation. The probability values were considered as significant at p<0.05 cut-off value.

**RESULTS AND DISCUSSION**

None of five patients on conventional TB drugs regimen had positive outcome after 9 months of treatment and one patient died after 9.5 months. The duration of treatment in the immunotherapy group ranged between 10.6-20.4 weeks with average/median 15.7/16.7 weeks (Table 1). The treatment lasted until patients were discharged from the dispensary upon twice-repeated negative culture findings and clinical and radiological improvements. The time to negative culture conversion ranged between 20-37 days with mean/median 28/25 days. Mycobacterial clearance was confirmed by repeated cultures at monthly intervals.

There appears to be no difference between first-diagnosed TB cases versus chronic, previously treated TB in terms of median days to discharge, i.e., 117 vs. 105.6, or days to mycobacterial clearance, 23 vs. 30. However, sample size was too small to reveal statistically significant difference.

At the end of the study every patient in the immunotherapy group had gained substantial lean body mass ranging between 6 and 13 kg. The average accrual in lean body mass was 9.6 kg, which was statistically highly significant as evidenced by a paired Student’s t-test (*p = 0.0001*) - dashed an effect that was evident as early as one month from initiation of the therapy. In contrast, patients on ATT had lost on average 1.4 kg (*p = 0.4*).

The potential hepatotoxicity of ATT combination with herbal preparations was monitored by quantitative liver function tests. Surprisingly, despite intensive chemotherapy, patients have shown signs of better liver function. The level of total bilirubin had decreased from mean 15.6 to 10.7 µmol L⁻¹ - dashed favorable change that was not statistically significant (*p = 0.16*). Similarly, the values of alanine transaminase (ALT), another marker of liver damage, declined from abnormally high (42.6 IU L⁻¹) to normal levels (22. IU L⁻¹) - dashed change that was not statistically significant (*p = 0.23*). Patients on ATT had same levels of bilirubin but their ALT declined from 29.6 to 12 IU L⁻¹ (*p = 0.02*).

Another phenomenon observed during therapy is a reversal of baseline anemic state and pro-inflammatory condition symptoms very common in TB. Most patients at study entry
Table 1: Baseline and outcome characteristics of XDR-TB patients treated with TB drugs without or in combination with Dzherelo (Immanoxid), Svitnakol and Lizorm

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Type of TB infection at baseline</th>
<th>Resistance to TB drugs</th>
<th>Prescribed TB drugs regimen</th>
<th>Days until discharge</th>
<th>Days to negative culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>47</td>
<td>Relapse</td>
<td>H/R/S/K/L</td>
<td>H/R/Z/S/E Proth</td>
<td>Died after 9.5 months</td>
<td>No conversion</td>
</tr>
<tr>
<td>M</td>
<td>52</td>
<td>Relapse</td>
<td>H/R/Z/S/K/O</td>
<td>H/R/Z/S/E</td>
<td>Still treated 12 months</td>
<td>No conversion</td>
</tr>
<tr>
<td>M</td>
<td>32</td>
<td>Relapse</td>
<td>H/R/S/K/L</td>
<td>H/R/Z/S/E PAS/A</td>
<td>Still treated 10 months</td>
<td>No conversion</td>
</tr>
<tr>
<td>M</td>
<td>46</td>
<td>Relapse</td>
<td>H/R/Z/E/S/K/L</td>
<td>H/R/Z/S/E/PAS/Cs/RFB</td>
<td>Still treated 9 months</td>
<td>No conversion</td>
</tr>
<tr>
<td>M</td>
<td>67</td>
<td>Relapse</td>
<td>H/R/E/K/L</td>
<td>H/R/Z/S/E/PAS/RFB</td>
<td>Still treated 9 months</td>
<td>No conversion</td>
</tr>
<tr>
<td>M</td>
<td>42</td>
<td>First Rx</td>
<td>H/R/E/K/O/PAS</td>
<td>H/R/Z/S/E/Eth/RFB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>44</td>
<td>First Rx</td>
<td>H/R/K/L/Eth/PAS</td>
<td>H/R/Z/S/E/Eth/RFB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>35</td>
<td>First Rx</td>
<td>H/R/K/K/A/P/C</td>
<td>H/R/Z/S/E/Proth/RFB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>47</td>
<td>First Rx</td>
<td>H/R/S/K/L/P</td>
<td>H/R/Z/S/E/Proth/RFB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>25</td>
<td>Relapse</td>
<td>H/R/Z/O/K/A/PAS</td>
<td>H/R/Z/S/E/Proth/RFB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>52</td>
<td>First Rx</td>
<td>H/R/A/P/PAS</td>
<td>H/R/Z/S/E/Proth/RFB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>48</td>
<td>Relapse</td>
<td>H/R/K/O/A/PAS</td>
<td>H/R/Z/S/E/Proth/RFB</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

41.9±8.2 110.1±25.3 28±7.1

<table>
<thead>
<tr>
<th>Weight change (kg)</th>
<th>Leukocyte (×10^9 L⁻¹)</th>
<th>Hb (g L⁻¹)</th>
<th>Total bilirubin (μmol L⁻¹)</th>
<th>ALT (IU L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Age</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>--------</td>
<td>-----</td>
<td>--------</td>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>M</td>
<td>47</td>
<td>67</td>
<td>55</td>
<td>8.9</td>
</tr>
<tr>
<td>M</td>
<td>52</td>
<td>66</td>
<td>68</td>
<td>10.9</td>
</tr>
<tr>
<td>M</td>
<td>32</td>
<td>70</td>
<td>68</td>
<td>8.5</td>
</tr>
<tr>
<td>M</td>
<td>46</td>
<td>65</td>
<td>63</td>
<td>10.5</td>
</tr>
<tr>
<td>M</td>
<td>67</td>
<td>73</td>
<td>75</td>
<td>4.8</td>
</tr>
</tbody>
</table>

48.8±12.6 68.2±4.3 66.8±7.4 8.7±2.4 13.8±6.8 5.4±2.8 5.6±4.9 5.0±0.6 29.6±11.1 12.0±0.0

*p = 0.40  p = 0.21  p = 0.18  p = 0.10  p = 0.02  p = 0.01  p = 0.001  p = 0.05  p = 0.23


displayed signs of anemia and had abnormally elevated leukocyte counts. At the end of treatment these parameters were improved in a statistically significant manner. The levels of hemoglobin had risen from 104.1 g L⁻¹ to 118 g L⁻¹ (p = 0.97), whereas leukocyte counts descended to quasi-normal levels from
In patients on ATT the reverse trend was observed. Leukocyte counts had risen from 8.7 to 13.8×10^9 cells L^-1 (p = 0.21) whereas hemoglobin declined to below normal levels from 116.4 to 96.6 g L^-1 (p = 0.18).

These results show that immune-modulating interventions can favorably influence the efficacy of TB drugs (Arjanova et al., 2009; Chkhetiani et al., 2007; Nikolaeva et al., 2008; Prihoda et al., 2007; Zaitzeva, 2008). All seven patients who received ATT and immunotherapy improved clinically and radiologically and were discharged after 3.7±0.8 months, with average/median time to mycobacterial clearance 28.25 days. None of five patients on TB drugs alone improved and one had died. The difference between two treatment outcomes was statistically significant (Mantel Haenszel odds ratio = 11; p = 0.0009 at 95% CI).

Present results compare favorably to XDR-TB chemotherapy outcomes reported in several recent papers. According to study by Kim et al. (2008) only 29.3% of those with XDR-TB were cured. TB therapy success rate in Russian patients with XDR-TB as reported by Keshavye et al. (2008) was 48.3%. Earlier reported cure rates in Europe, USA, Peru and Korea were between 37.5-67% indicating that XDR-TB poses serious clinical challenge (Edward et al., 2008; Kwon et al., 2008; Migliori et al., 2008; Mitnick et al., 2008). In conclusion, adjunct herbal immunotherapy is safe, enhances significantly treatment outcome and can overcome drug resistance even in patients with extremely poor prognosis. Further studies are needed to confirm present findings.

ACKNOWLEDGMENTS

We thank all participants who volunteered in this study. The support of clinical staff and technicians who contributed to this study has been of tremendous help to us. We are grateful to other colleagues who shared their insight and provided helpful suggestions based on their own experience with phytoconcentrates used in present study.

REFERENCES


