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

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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 Immunoxel (Dzherelo), Svitanok 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 7.3×10^9 cells L^{-1} ($p = 0.003$). Conversely, in patients on ATT leukocyte counts rose 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 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

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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 Immunoxel (Dzherelo), Svitanok 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; Chkhetiany *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 Svitanok 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 (Chkhetiany *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, Svitanok 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 Svitanok were generously supplied by Ekomed LLC. 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 Immunoxel, a slightly slightly modified form of Dzherelo. The same dose, 30 drops, of Lizorm and Svitanok 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-predetermined 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 $p \leq 0.05$ 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 immunatherapy 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 ($p = 0.0001$) m-dash 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} em-dash 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}) e_m-dash 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 em-dash 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 (Immunoxel), Svitanok and Lizorm

Sex	Age	Type of TB infection at baseline	Resistance to TB drugs[a]	Prescribed TB drugs regimen	Days until discharge	Days to negative culture
M	47	Relapse	H/R/S/K/L	H/R/Z/S/E Proth	Died after 9.5 months	No conversion
M	52	Relapse	H/R/Z/S/K/O	H/R/Z/S/E PAS/L/A/Cs	Still treated 12 months	No conversion
M	32	Relapse	H/R/Z/S/K/L	H/R/Z/S/E PAS/A	Still treated 10 months	No conversion
M	46	Relapse	H/R/Z/E/S/K/L	H/R/Z/S/E/PAS/ Cs/RFB	Still treated 9 months	No conversion
M	67	Relapse	H/R/E/K/L	H/R/Z/S/E Proth/PAS/RFB	Still treated 9 months	No conversion
M	42	First Rx	H/R/E/K/O/PAS	H/R/Z/S/E/Eth/RFB +Dzh/Sv/Li	74	23
M	44	First Rx	H/R/K/L/Eth/PAS	H/R/Z/S/E/Eth +Dzh/Sv/Li	143	34
M	35	First Rx	H/R/K/A/C/P	H/R/Z/S/E/Proth +Dzh/Sv/Li	93	20
M	47	First Rx	H/R/S/K/L/P	H/R/Z/S/E/Proth/PAS +Dzh/Sv/Li	133	22
M	25	Relapse	H/R/Z/O/K/A/PAS	H/R/Z/S/E/Proth/RFB +Dzh/Sv/Li	89	25
M	52	First Rx	H/R/A/P/PAS	H/R/Z/S/E/Proth +Dzh/Sv/Li	117	37
M	48	Relapse	H/R/K/OA/PAS	H/R/Z/S/E/Proth/RFB +Dzh/Sv/Li	122	35
	41.9±9.2				110.1±25.3	28±7.1

		Weight change (kg)		Leukocyte ($\times 10^9$ L)		Hb (g L^{-1})		Total bilirubin (μmol L^{-1})		ALT (IU L^{-1})	
Sex	Age	Before	After	Before	After	Before	After	Before	After	Before	After
M	47	67	55	8.9	4.0	115	90	10	12	37	12
M	52	66	68	10.9	21	100	101	10	11	12	12
M	32	70	68	8.5	13.4	162	102	13	14	37	12
M	46	65	63	10.5	11.4	119	95	14	14	37	12
M	67	73	75	4.8	19.4	86	95	18	14	25	12
	48.8±12.6	68.2±3.3	66.8±7.4	8.7±2.4	13.8±6.8	116.4±28.6	96.6±4.9	13.0±3.3	13.0±1.4	29.6±11.1	12.0±0
		Mean loss = 1.4 kg p = 0.40		Mean gain = 5.1×10^9 L p = 0.21		Mean loss = 19.8 g L^{-1} p = 0.18		Mean loss = 0 μmol L^{-1} p = 1.0		Mean loss = 17.6 IU L^{-1} p = 0.02	
M	42	59	68	11.6	8.1	122	114	10.5	11.7	25	50
M	44	63	69	4.5	6.8	120	118	18.6	10.5	12	50
M	35	50	63	9	10	88	118	32.4	10.5	25	12
M	47	52	62	9.1	9.1	108	116	11.7	10.7	62	12
M	25	65	78	8.2	6	109	120	10.5	10.5	62	12
M	52	64	74	11	6	100	118	11.7	10.4	75	12
M	48	72	78	8.8	5.3	82	122	14	10.5	37	12
		60.7±7.7	70.3±6.6	8.9±2.3	7.3±1.8	104.1±15.1	118.0±2.6	15.6±7.9	10.7±0.5	42.6±23.8	229±185
		Mean gain = 9.6 kg p = 0.0001		Mean loss = 1.6×10^9 L p = 0.18		Mean gain = 13.9 g L^{-1} p = 0.07		Mean loss = 4.9 μmol L^{-1} p = 0.16		Mean loss = 19.7 IU L^{-1} p = 0.23	

[a]Criteria for definition of XDR are as per the WHO recommendations. TB drugs are H: Isoniazid, R: Rifampicin, Z: Pyrazinamide, E: Ethambutol, S: Streptomycin, L: Levofloxacin, O: Ofloxacin, C: Ciprofloxacin, P: Pefloxacin, K: Kanamycin, A: Amikacin, Cs (Cycloserine), PAS: Para-aminosalicylic acid, Eth: Ethionamide, Proth: Prothionamide, RFB: Rifabutin, Dzherelo: Dzh, Svitanok: Sv, Lizorm: Li

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 to 118 g L^{-1} ($p = 0.07$), whereas leukocyte counts descended to quasi-normal levels from

8.9 to 7.3×10^9 cells L^{-1} ($p = 0.003$). 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; Chkhetiany *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 Keshavjee *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.

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