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Association of Nutritional Status with Drug Sensitive and Drug Resistant Outcomes Among Pulmonary Tuberculosis Patients

Harshita Gupta[1], Surya Kant[2], Amita Jain[3], S.M. Natu[4], Savita Ahluwalia[5]
[1,2]*Department of Pulmonary Medicine, King George's Medical University, Lucknow, Uttar Pradesh, India*
[3]*Department of Microbiology, King George's Medical University, Lucknow, Uttar Pradesh, India*
[4]*Department of Pathology, King George's Medical University, Lucknow, Uttar Pradesh, India*
[5]*Department of Home Science, M.V.PG University, Lucknow, Uttar Pradesh, India*

Abstract: *The study established the association of nutritional status with drug sensitive and drug resistant outcomes among pulmonary tuberculosis (TB) patients. A hospital based prospective follow-up cohort study design. Two urban Directly Observed Treatment Short-course (DOTS) centers in Lucknow District of Uttar Pradesh, India. The newly diagnosed Acid-fast Bacilli (AFB) positive cases between the age group of 12 to 65 years were enrolled. The drug-susceptibility outcomes were assessed by mycobacterial culture and drug-susceptibility test. The assessment of nutritional status was performed by Body Mass Index (BMI), Mid-upper-arm circumference (MUAC), 24-hour dietary recall method and measurement of serum concentration of retinol-binding protein (RBP) and zinc. A total of 185 newly diagnosed patients were recruited. Out of these, 14 patients were lost to follow up after treatment. The mean (±SD) age of the remaining 171 patients was 29 (±12) years. In the culture of 171 patients, 156 (91.2%) isolates were culture positive and subjected to drug-susceptibility test. Of these, 129 (82.6%) isolates were sensitive to all the drugs and 27 (17.3%) isolates were resistant to any drug. A significant difference was observed in serum RBP ($p<0.0001$) and zinc ($p=0.005$) at six month in drug sensitive and resistant patients. Among all the nutritional status parameters serum zinc ($p=0.01$) was significantly associated with drug resistant patients. Inadequate level of serum zinc was probably associated with drug resistance among new pulmonary TB patients.*

Key Words: *Tuberculosis, drug-susceptibility testing, nutritional status*

I. Introduction

TB is a contagious disease caused by the bacillus Mycobacterium tuberculosis *(M.tb)* [1]. It is one of the major public health problem in India. India is the highest TB burden country throughout the world, accounting for approximately one-fifth of the global incidence- an estimated 2 million cases annually [2]. In India, more new TB cases are seemed annually than any other country. It is estimated that about 40% of Indian population is infected with TB bacillus (but who are not infected with human immunodeficiency virus) [1]. In all the infected cases, active TB does not develop because of the infected person's immune system halts growth of the bacteria [2]. Drug resistance may raise a situation where patients have nothing but their immune system helps to fight off the disease [2]. Micronutrient deficiencies especially zinc and vitamin A weakens the immune response which is responsible for host resistance to infections and leads to develop active TB. It was found that low serum zinc and serum RBP levels to be associated with more extensive or severe disease [3]. Zinc has been shown to be essential in vitamin A metabolism as it is required for mobilization of vitamin A from the liver. Serum zinc and serum RBP status has been determined to be an important determinant of TB outcomes [4].

Thus, the effective management of TB, therefore requires detailed evaluation of the nutritional status since this can help to prevent poor outcomes responsible for the TB and also help in making projection of the association of nutritional status parameters with drug-susceptible outcomes of TB. To the best of my knowledge, none of the study is available to determine the association of nutritional status with drug resistant and drug sensitive outcomes in newly diagnosed pulmonary TB patients.

II. Material And Methods

2.1 Study design and setting

A prospective follow-up cohort study was conducted between January 2010 to March 2011 at the two urban Directly Observed Treatment Short-course (DOTS) centers of Lucknow Medical University and a TB Hospital located near to Medical University, Lucknow District of Uttar Pradesh, India.

2.2 Study Population

The study consisted of 185 newly diagnosed sputum smear positive for AFB in pulmonary TB patients of both sexes and between the age group of 12 to 65 years at the time of interview and were about to be

registered for treatment. Patients were excluded if they fulfilled any of the following exclusion criteria: previous history of anti-tuberculosis treatment (ATT); pregnant and lactating women; subjects known to be HIV positive/ or suffering from any immuno-deficient state; and use of corticosteroids or supplements containing vitamin A and zinc etc. during the earlier month. All subjects were free from alcoholism.

2.3 Ethical considerations

The study was ethically approved by the institutional ethics committee of a medical university, Lucknow, U.P., India. All eligible patients were informed about the study and signed an informed consent form from each subject before the beginning of the study.

2.4 Data collection

2.4.1 Personal interview and clinical examination

A structured questionnaire was used to collect the information regarding socio-demographic background and data about family history. Socio-economic status was assessed by Kuppuswamy's socio-economic status (SES) scale [5]. Subsequently, patients were thoroughly examined by medical doctors at both the hospitals.

2.4.2 Assessment of clinical outcomes

Clinical outcomes were assessed at baseline and after six month of ATT. Following symptoms were clinically assessed included fever, cough, expectoration, chest pain breathlessness, wheezing, hemoptysis, loss or improve of appetite and weight loss or gain. Chest radiographs (CXR) were made of all the patients at the time of diagnosis. Patients were evaluated by judging the site of lesions, zone of involvement, the nature of the lesion (visible cavitary and non-cavitary area) in both lungs as well as classified as the extent of the lesion having mild, moderate and far advanced lesion as per American Thoracic Society classification [6].

2.4.3 Assessment of bacteriological outcomes

Bacteriological outcomes were assessed by RNTCP guidelines, 2006 [7] included AFB-smear examination and grading, AFB-culture and drug-susceptibility test. All specimens were carried to the accredited Intermediate Reference Laboratory (IRL) at the Department of Microbiology, Medical University, Lucknow where further processing was done.

2.4.3.1 Specimen collection

The diagnosis of TB was done in accordance to the RNTCP guidelines, 2006. At the time of enrolment; three sputum specimens on two consecutive days from each patient were collected in properly labeled screw capped, sterile disposable plastic bottles after oral gargling with normal water. Thus, there were three samples: SPOT - EARLY MORNING - SPOT. Specimen contained mucoid or mucopurulent material with minimum amounts of oral or nasal material into the McCartney bottles, and volume was of approximately 5 ml.

2.4.3.2 AFB-smear examination and grading

An AFB smear examination was carried out by direct microscopy using the Ziehl- Neelsen Method. Sputum smear result was examined and interpreted according to the AFB grading [8].

1.4.3.3 AFB-culture and drug-susceptibility test

Culture examinations were done on all diagnostic specimens, regardless of AFB smear positive. Sputum specimens from each patient were processed with Sodium Hydroxide (NaOH) Method- Modified Petroff's procedure and cultured on Lowenstein-Jensen (L-J) slopes [8]. All inoculated L-J drug and control media were incubated at 37ºC. All cultures were examined 48-72 hours after inoculation to detect gross contaminants. Thereafter, cultures were examined weekly, up to eight weeks on a specified day through the week. Typical colonies of *M.tb* were rough, crumbly, waxy, non-pigmented (buff-colored) and slow-growers, i.e., only appeared two to three weeks after inoculation [8]. The colony was confirmed by Z-N staining. Drug resistance was expressed in proportion method, where a strain was considered to be drug resistance if the number of colonies that grew on a drug containing media was 1% or more of the colony that grew on a control drug free media. The drug containing media and media concentration for streptomycin, isoniazid, rifampicin, ethambutol were 4 µg/ml, 0.2 µg/ml, 40 µg/ml and 2 µg/ml respectively [9]. The control (drug free) media showed good growth at least 50 to 100 colonies [8].

2.4.4 Dietary and anthropometric assessment

2.4.4.1 Dietary assessment

24-hour dietary recall method was used to collect the data regarding nutrient intake of an individual patient. It was based on the foods and amounts actually consumed by the patient on three specific days. The patients were asked to recall as much detail as possible regarding the food intake for the past 24- hour (recorded in the questionnaire). Special efforts were made to include all foods eaten in the preceding 24-hour by asking what the patient had eaten in the morning, at lunch, during the afternoon, in the evening, at dinner, and during the night. Portion size was estimated by using the proportion size booklet of National Health and Nutrition Examination Survey [10]. It was assessed by asking the patients with the help of digital photographs to facilitate more accurate portion size. The portion sizes were converted into food exchanges and then the nutrient intake of an individual was calculated using standard food exchange table [11]. In order to translate foods into the nutrients were compared with the Recommended Dietary Allowances (RDA) of respective age, sex and occupation of an individual patient in the nutrition database of the Medical Research Council [12]. The percent nutrient intake of the RDA was calculated. The association of nutrient intake with drug-susceptible outcomes was then assessed.

2.4.4.2 Anthropometric measurements

Anthropometric measurements were taken before and after ATT. Patients were assessed by anthropometric parameters such as height, weight, BMI and MUAC. Standard protocol for anthropometric measurement was followed by the National Centre for Health Statistics (National Health and Nutrition Examination Survey III Anthropometric Procedures) for various age groups. Measurements were taken three times consecutively and mean values were observed. The same instruments were used to take the measurements and were calibrated each morning to ensure validity of the results. Weight was measured to the nearest 0.1 kg and height to the nearest 1 mm and Mid-upper-arm circumference (MUAC) was measured to the nearest 1 mm [13]. To eliminate the inter-examiner error, only one person took all the anthropometric measurements.

2.4.5 Blood collection and analysis

Fasting blood samples were collected between 08:00 a.m. to 10:00 a.m. at the time of enrollment and at the end of treatment i.e. after six month. Approximately 5 mL of whole blood was taken by vein puncture into vacutainer. Serum was separated after centrifugation of blood at 3000 rpm for 10-15 minutes and then stored in -80 ºC until analysis of biochemical parameter.

2.4.6 Biochemical Assessment

Serum zinc concentration was measured by Colorimetric Method (QuantiChrom^{TM} Zinc Assay Kit (DIZN-250), at 425 nm with the lowest cut-off value of serum zinc i.e. 11 mmol/L [14]. Serum retinol concentration was measured by (RBP)4 Enzyme-Linked Immunosorbent Assay (ELISA) kit (Adipogen, Inc., Korea, Cat. No. AG-45A-0060EK-KI01). The lowest level of RBP4 that can be detected by this assay was 1 ng/ml.

III. Statistical Analysis

The data collected was entered into Microsoft Excel computer program and checked for any inconsistency. The descriptive statistics such as percentage, and mean (±SD) were calculated. The unpaired t-test was used to test the significance between the two means. The paired student t-test was used to compare the changes in nutritional status and nutrient intake from baseline to six- month treatment. Multivariate logistic regression analysis was used to determine the factors associated with treatment outcomes. The p-value less than 0.05 was considered as significant. All the analysis was carried out by using SPSS 15.0 version.

IV. Results

A total of 185 newly diagnosed patients with pulmonary TB were recruited. Out of these, 14 (7.6%) patients were lost to follow up at the end of the treatment. Remaining 171 (92.4%) patients, 57.3% were males and 42.6% were females. The most frequent age group in the present study was 21-30 years consisted of 38% patients and 26.9% patients were below 21 years. The mean (±SD) age of the study population was 29 (±12) years. Sputum positivity grade +3 was most prevalent (35.1%). The majority of the patients were Muslims (52.6%). Most of the patients (77.7%) belonged to the upper lower income group. Majority (74.8%) of the patients were non-vegetarian and 65.5% were smokers (Table 1).

Table 1. Characteristics and radiographic presentation of the TB patients

Variables	n=171
Age (years)*	29 (±12)
Gender (n, %)	
Male	98 (57.3)
Female	73 (42.6)
AFB positivity grade (n, %)	
+1	50 (29.2)
+2	44 (25.7)
+3	60 (35.1)
Scanty	17 (9.9)
Religion (n, %)	
Hindu	81 (47.3)
Muslims	90 (52.6)
Socio-economic status (n, %)	
Upper	1 (0.5)
Upper Middle	2 (1.2)
Lower Middle	34 (19.8)
Upper Lower	133 (77.7)
Lower	1 (0.5)
Eating Habits (n, %)	
Vegetarian	32 (18.7)
Non-vegetarian	128 (74.8)
Eggarian	11 (6.43)
Family History of TB (n, %)	
Yes	41 (23.9)
No	130 (76.0)
Smoking (n, %)	
Yes	112 (65.5)
No	59 (34.5)
Chest Radiographic (nature of lesion) (n, %)	
Cavitary	57 (33.3)
Non-cavitary	114 (66.6)
Type of Lesions	
Mild	15 (8.8)
Moderate	115 (67.2)
Far Advanced	55 (32.7)

Data were expressed as *mean ± standard deviation

During clinical assessment, most of the patients had persisting fever (99.4%), chronic cough (93.5%), weight loss (92.4%), and appetite loss (99.4%); other frequent symptoms were chest pain (76.0%), breathlessness (73.6%) and hemoptysis (28.0%). The prevalence of cavitary nature of lesion in the chest x-ray was observed in 33.3% patients. The BMI was significantly ($p<0.0001$) increased from baseline to 6 month in both drug sensitive (Baseline=16.45±2.52, 6 month=18.68±3.01) and resistant (Baseline=16.17±2.04, 6 month=17.14±2.59) patients. Almost similar findings were observed for MUAC. There was no significant ($p>0.05$) difference in BMI and MUAC at 6 month in drug sensitive and drug resistant patients (Table 2).

Table 2. Association of drug-susceptibility outcomes with nutrient intake, anthropometric status and biochemical parameters

	Nutritional status	Drug-susceptibility outcome (n=156)	Mean ± sd		p-value[1]
			Baseline	6 month	
Nutrient intake	Zinc (mg/day)	Sensitive (n=129)	3.63±1.29	5.97±1.57	0.001*
		Resistant (n=27)	3.43±1.23	5.36±1.87	<0.0001*
		p-value[2]	0.46	0.08	
	Retinol (µgm)	Sensitive (n=129)	122.34±87.02	188.42±101.84	0.001*
		Resistant (n=27)	113.57±80.92	181.81±113.08	<0.0001*
		p-value[2]	0.63	0.76	
Anthropometric status	BMI (kg/m^2)	Sensitive (n=129)	16.45±2.52	18.68±3.01	<0.0001*
		Resistant (n=27)	16.17±2.04	17.14±2.59	<0.0001*
		p-value[2]	0.60	0.20	
	MUAC (cm)	Sensitive (n=129)	7.66±0.87	8.51±1.06	0.001*
		Resistant (n=27)	7.59±0.83	8.13±0.81	<0.0001*
		p-value[2]	0.68	0.08	
Biochemical status	Serum zinc (µmol/l)	Sensitive (n=129)	6.87±1.14	8.75±1.42	0.001*
		Resistant (n=27)	5.77±0.94	7.84±1.86	0.001*
		p-value[2]	<0.0001*	0.005*	
	Serum RBP (µmol/l)	Sensitive (n=129)	0.54±0.05	0.84±0.09	0.001*
		Resistant (n=27)	0.50±0.06	0.76±0.11	0.001*
		p-value[2]	0.001*	<0.0001*	

[1]Paired t-test, [2]Unpaired t-test, *Statistically significant

In nutritional assessment, significant ($p<0.0001$) increase was observed in zinc and retinol intake at six month. It was found that the percent intake of zinc and retinol was inadequate when compared to RDA in all the patients from baseline to six month (Fig 1).

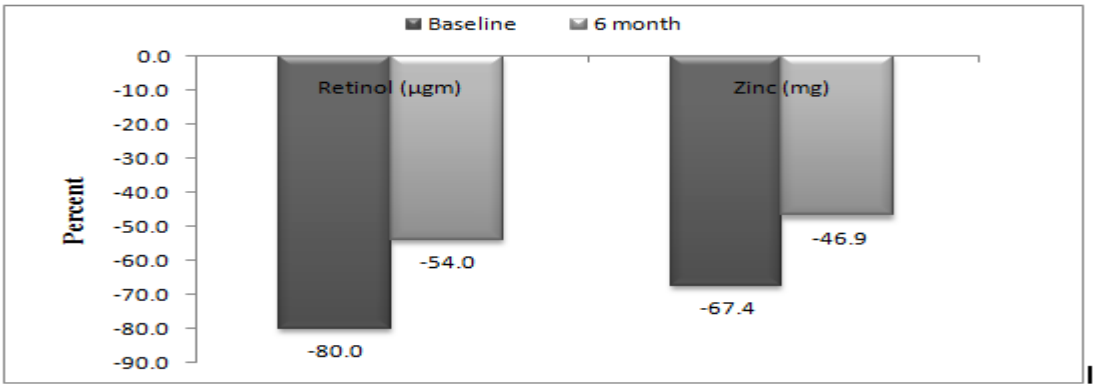

Figure 1. Percent retinol and zinc intake of RDA from baseline to 6 month

Out of 171 sputum smear positive patients for AFB, 156 (91.2%) isolates of patients were culture positive and subjected to drug-susceptibility test. Of these, 129 (82.6%) isolates were sensitive to all the drugs and 27 (17.3%) isolates were resistant to any drug. A significant ($p<0.01$) increase was established in the intake of zinc and retinol in both drug sensitive and drug resistant isolates of patients from baseline to six month. However, no significant ($p>0.05$) difference was observed in zinc and retinol intake between drug sensitive and resistant outcomes at six month (Table 2). In bio-chemical assessment, significant difference was observed in serum zinc ($p=0.005$) and serum RBP ($p<0.0001$) between drug sensitive and drug resistant outcomes (Table 2). Multivariate logistic regression analysis revealed that the serum zinc was significantly associated with drug resistance ($p=0.01$) (Table 3).

Table 3. Association of biochemical parameter with the drug resistance (result of multivariate logistic regression)

Biochemical parameter	Adjusted OR (95%CI)	p-value
Serum zinc (µmol/l)	2.29 (1.21-4.37)	0.01*

OR-Odds Ratio, CI-Confidence interval, *Statistically significant

V. Discussion

The results demonstrated a significant level of micronutrient malnutrition in tuberculosis patients, as evident from their lower serum zinc and RBP concentration after six months treatment in drug resistant patients. Malnutrition in patients with tuberculosis has been documented in many studies [15-17] and is once again reflected in the above results. The present study demonstrated that BMI and MUAC were significantly lower before treatment and improved after treatment in both drug sensitive as well as drug resistant patients. Malnutrition, as evidenced by a decreased BMI and MUAC, may be attributed to an increase in energy expenditure as a result of the acute phase response (APR) and reduced nutrient intake, as well as fatigue and shortness of breath in the case of severe infections. The latter may make it difficult to ingest food [18]. In the initial phase of the disease, the APR was responsible for the increase in energy expenditure in patients, but once they are on treatment, the APR declines and nutritional status may improve Serum zinc and serum RBP concentration were statistically significantly lower in drug resistant patients as compared to drug sensitive patients at six month. In drug resistant patients, serum vitamin A may also be affected by the APR, with a consequent decrease in RBP which is responsible for transport of retinol to various tissues [19-20]. Increased APR may increases the urinary losses of vitamin A associated with fever and infection which leads to decrease the RBP concentration [19-20]. A zinc deficiency may cause a secondary vitamin A deficiency by impairing the production of RBP. During the APR, serum zinc concentration also decreases due to hepatic sequestration. Decreased retinol concentration may be caused by reduced mobilization of RBP from the liver due to zinc deficiency, as RBP is a zinc-dependent protein [21]. Among all the nutritional status parameters serum zinc was probably associated with drug resistant outcome of patients. We observed a decreased serum zinc concentration,

increases the susceptibility to the patients with drug resistance. Decreased serum zinc concentration indicated the insufficiency of zinc intake in the diet. Daily zinc supplementation may represent a novel approach and add benefit among drug resistant patients. Zinc supplementation along with ATT may strengthen the immune system which decreases the burden of *Mycobacterium* and letting them for faster recovery. Thus, zinc supplementation and regular monitoring of the nutritional status by a dietician are essential for the successful management of drug resistance among newly diagnosed pulmonary TB patients and should be an essential part of tuberculosis control programmes. One of the limitation of this study might be perception of patients towards no previous history of ATT because sometime patients did not recognize their previous history of ATT due to social phenomenon.

VI. Conclusion

Findings of this study indicated that serum zinc was closely associated with drug resistant patients. Adjunctive zinc supplementation may accelerate the beneficial therapeutic effect of TB chemotherapy and allowing them for faster recovery of drug resistance among newly diagnosed pulmonary TB patients.

VII. Acknowledgement

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