UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA
DOCTORAL SCHOOL

DOCTORAL THESIS ABSTRACT

Pulmonary tuberculosis and diabetes mellitus

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INTRODUCTION

Diabetes mellitus and tuberculosis are two chronic diseases with major impact on morbidity and mortality worldwide.

Currently, according to the latest published data, over 400 million people with diabetes mellitus are reported globally. Moreover, over 2 billion people, the equivalent of one third of the world's population, are infected with Mycobacterium tuberculosis, and 10 million people have active tuberculosis. Millions of deaths occur annually due to both diabetes mellitus and tuberculosis.

The association diabetes mellitus – tuberculosis has been known for over 1000 years, but more recent data from the last decade demonstrate the importance of the association of the two diseases. Thus, it has been clearly established that diabetes mellitus increases the risk of progression of latent tuberculosis infection in active tuberculosis.

Numerous publications have mentioned that about one million cases of tuberculosis are attributed to diabetes mellitus, this number being almost equal to the number of people with HIV – tuberculosis coinfection.

Urbanization and lifestyle changes have increased the prevalence of diabetes mellitus, thus increasing the co-epidemic of diabetes – tuberculosis, which may jeopardize the measures implemented to control tuberculosis, especially in low-income countries.

The association diabetes mellitus – tuberculosis constitutes a threat to the health of the entire population, thus considering the bidirectional screening and careful monitoring of these patients.

STAGE OF KNOWLEDGE (THE THEORETICAL PART)

1.1. EPIDEMIOLOGICAL DATA

Diabetes mellitus (DM) is one of the most important health problems of this century, being a disease with major impact on morbidity and mortality worldwide. Chronic hyperglycemia causes vascular injury, thus DM being an independent risk factor for cardiovascular diseases, but also one of the major causes of blindness, chronic kidney disease and non-traumatic amputations [1, 2]. In addition, DM has

been associated with an increased risk of depression [1, 3], cancer [1, 4], physical and mental disability [1, 5, 6] and tuberculosis (TB) [1, 7, 8].

In 2017, the International Diabetes Federation (IDF) reported a prevalence of DM of 8.8%, worldwide there are 424.9 million people between 20 and 79 years old with this condition. By 2045, an increase in the number of persons with DM to 628.6 million is estimated, corresponding to a prevalence of 9.9%. Almost half (212.4 million) of the persons with DM are undiagnosed [1]. In Romania, according to the epidemiological study PREDATORR (PREvalence of DiAbeTes mellitus, prediabetes, overweight, Obesity, dyslipidemia, hyperuricemia and chRonic kidney disease in Romania), the prevalence of DM was 11.6% in adults, between 20-79 years old [9].

Tuberculosis (TB) is a major cause of morbidity and mortality worldwide, about 1.7 billion people, almost a quarter (23%) of the population being infected with Mycobacterium tuberculosis. This category of people has latent TB infection (LTBI). About 5 to 15% of them will develop active TB throughout life [10-12]. In 2017, WHO reported 10 million new cases of TB, equivalent to an incidence of 133/100,000 inhabitants/year, 90% being adults over 15 years of age [10]. In Romania, the global incidence of TB is the highest in the European Union and one of the largest in Europe, in 2017 being estimated 14,000 new cases of TB, corresponding to an incidence of 72/100,000 inhabitants/year [13, 14 , 15].

In 2017, worldwide, 0.79 million TB cases were attributed to DM [10]. It is estimated that LTBI-DM association will have implications in TB control worldwide, making TB eradication impossible by 2035 [16, 17]. In the context of the alarming increase in the prevalence of DM, especially in low and middle income countries, where the incidence of TB is also high, the association of the two diseases represent a threat to public health [18].

1.2. RISK FACTORS

DM-TB comorbidity is associated with numerous individual risk factors (age, sex, anthropometric indices, smoking, alcohol use, drug use, eating, sedentary lifestyle, family history of DM, TB, obesity, hypertension, the type of DM, the type of TB, personal history of hypertension), but also socio-economic determinants (educational level, occupation, income, living conditions, marital status, environment, ethnicity).

1.3. CLINICAL AND PARACLINICAL FEATURES

Patients with DM-TB co-epidemic have both symptomatology and atypical radiographic aspects, making it difficult to diagnose them. In addition, the persistence of the positive sputum examination significantly increases the risk of adverse therapeutic results.

TB screening should be considered in patients with DM who have fever, physical asthenia, apathy, cough, hemoptysis and chest pain, which often present weight loss and night sweats that cannot be justified by the metabolic imbalance [19, 20, 21].

1.4. TREATMENT

TB associated to DM is multi drug resistant, also causing problems regarding drug interaction. More careful monitoring of anti TB treatment in DM patients is needed in order to evaluate the response to treatment, to determine the duration of treatment, early detection of any adverse effects or drug interactions, and to detect early relapse or failure, thus being necessary for dose or therapeutic regimen adjustment [20, 22].

1.5. PROGNOSIS

DM-TB comorbidity is associated with an increased risk of death, failure of therapy and relapse of the disease after treatment compared to patients with normoglycemia, thus glycemic balance being extremely important in this category of patients.

PERSONAL CONTRIBUTION (THE PRACTICAL PART)

2.1. THE IMPORTANCE OF THE THEME

Despite all the measures taken by the authorities, the steadily increasing prevalence of DM will increase TB prevalence in developing countries over the next decades, with DM-TB comorbidity being one of the most important challenges of TB eradication programs.

Romania is the country with the highest incidence of TB in the European Union and one of the largest in Europe [13, 15], also ranking second in the European Union in terms of TB mortality rate [24], most deaths being recorded in the South-

West Oltenia region [14]. Dolj County is currently ranked third in terms of global TB incidence in our country [14, 25].

Moreover, the prevalence of DM in Romania is one of the highest in Europe [1, 9], in the context of rapid urbanization, but also of unhealthy lifestyle.

According to the aforementioned data, it is necessary to implement bidirectional screening and monitoring programs for the DM-TB co-epidemic, with the unconditional support of national and international authorities, aiming to prevent, early diagnose and effectively treat these patients.

2.2. THE OBJECTIVES OF THE STUDY

The current study presents the following objectives:

- Primary objectives:
- ✓ Analysis of the prevalence of MTB infection in a group of patients with DM, not selected, from Dolj county;
- ✓ Analysis of personal history of TB and other risk factors for TB in patients with DM and MTB infection, compared to the control group (patients with DM, without MTB infection).

- Secondary objectives:
- ✓ Analysis of pulmonary changes revealed by chest x-ray in patients with DM and MTB infection (GROUP 1), compared to the control group (GROUP 2);
- ✓ Analysis of the clinical symptoms suggestive of active TB in patients with DM and MTB, compared to the control group.

2.3. MATERIAL AND METHODS

2.3.1. STUDY DESIGN

General dates:

- Study design: cross-sectional;
- The period of the study;
- The clinic where the study was conducted.

The study is part of a complex international project – TANDEM (Concurrent Tuberculosis and Diabetes Mellitus: Unraveling the causal link and improving care). The multidisciplinary consortium has partners in Romania, Peru, South Africa and

Indonesia and also laboratories, researchers and clinicians in Germany, the UK and the Netherlands. The TANDEM project was carried out in Romania between 2013-2017, in six Diabetes and Pneumology Centers in Dolj and Gorj counties.

The present study has a cross-sectional design and was conducted between February 2014 - February 2016 within the Diabetes, Nutrition and Metabolic Diseases Clinic of the Clinical Emergency County Hospital Craiova and the Diabetes Department of the Medical Clinic II of the Municipal Clinical Hospital Philanthropy Craiova. The study included the screening of subjects with DM for TB, randomly enrolled and inclusion of subjects confirmed to be eligible after screening. The study enrolled 603 adult subjects diagnosed with DM, their participation being voluntary.

2.3.2. ETHICAL CONSIDERATIONS

The TANDEM project was approved by the London School of Hygiene and Tropical Medicine – Interventions/Observational Research Ethics Committee and the Institutional Review Boards in each country member of the consortium (Indonesia, Romania, Peru and South Africa).

The present study was conducted in accordance with the Guidelines for Good Epidemiological Practice developed by the International Society for Pharmacoepidemiology (ISPE), the Good Clinical Practice (GCP) and the Helsinki Declaration - updated, as well as by obtaining the informed consent of the subject. The personal data of the subjects were recorded on the computer, in a secure electronic database, password protected, centrally managed (REDCap).

2.3.3. INCLUSION CRITERIA

- Adult subjects, over 18 years of age;
- Subjects with type 1 DM or type 2 DM;
- Subjects with permanent residence in Romania;
- The signing of the informed consent, in full knowledge of the facts.

2.3.4. EXCLUSION CRITERIA

- Subjects under the age of 18;
- Subjects with gestational DM or other forms of DM;
- Subjects under anti-TB treatment in the moment of the enrollment in the study;

- Subjects who refused to sign the informed consent or could not sign it in full knowledge of the facts.

After establishing the inclusion and exclusion criteria, all eligible subjects participated in an interview, in which socio-economic, anamnestic, clinical and paraclinical data were recorded.

2.3.5. DATA COLLECTION

- Demographic data: age, gender, ethnicity, environment, marital status, educational level.

According to *age*, the subjects were classified in several categories: <40 years; 40-59 years; 60-79 years; ≥ 80 years.

The *ethnicity* was classified as: romanian; roma.

Regarding *marital status*, the subjects were divided as follows: married; unmarried.

The *educational level* was defined according to the level of education completed by the subjects, as follows: no formal education; less than primary school; primary school; secondary school; high school; college/university; postgraduate studies.

- Socio-economic data: occupation, economic conditions, living conditions.

Regarding *occupation*, the subjects were classified according to the labor market status in the last 12 months, as follows: budgetary; employee in the private sectort; independent; unpaid; student; household; retired; unemployed (able to work); unemployed (unable to work).

We used Principal Component Analysis, a statistical procedure, in order to obtain an index of the *economic status* according to the assets held by each subject [23]:

- possessing a bank account;
- type of toilet: tank toilet; traditional toilet; latrine with ventilation; bowl/bucket; without toilet;
- the main source of water for drinking and cooking: connection to the public network; private fountain; public source of the city network; public fountain; from neighbors; water vendor; spring; stream, river, lake, pond; rain water; bottled water;

- possessing the following salable goods: stove; refrigerator; microwave oven; washing machine; air conditioning; fan; computer; TV; DVD player; radio/CD player; camera; mobile phone; bicycle; motorcycle/scooter; car/truck.

Thus, the subjects were divided into four classes: poor, lower middle class, upper middle class, rich.

In order to evaluate *the living conditions*, the following data were recorded on each subject:

- whether he lives alone or not;
- how many people live with him;
- with whom he lives: partner; children; parents; other relatives; friends;
- where he lives: personal property; family accomodation; rented accomodation; rented room; no home; shelter (homeless).

- Anthropometric data: determination of height, weight, WC, hip circumference; calculation of BMI, WHR, WHtR.
- Vital signs: measurement of systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate, temperature.
- Pathological personal history: the illnesses were detected by medical documents prior to enrollment in the study and by anamnesis.

a) DZ: type of DM, DM duration, classification of current antidiabetic treatment, duration of treatment with metforminum, with other oral antidiabetics, respectively insulin.

Regarding *the type of DM*, the subjects were divided into type 1 DM and type 2 DM.

According to *the duration of DM*, the subjects were classified in the following categories: new case; <1 year; 1-5 years; 6-15 years; >15 years.

The current antidiabetic treatment has been classified as follows:

- subjects without treatment;
- treatment with metforminum;
- treatment with other oral antidiabetics, whether or not associated with metforminum: gliclazidum, glimepiridum, glipizidum, gliquidonum, repaglinidum, acarbosum, sitagliptinum, exenatidum once/week (QW), exenatidum twice/day (BID), lixixenatidum;
- treatment with insulin.

The duration of treatment with both metforminum and other oral antidiabetics, respectively insulin, was divided into the following categories: <1 year; 1-5 years; 6-15 years; >15 years.

b) Complications of DM and comorbidities: amputations; by-pass or stent in the lower limbs; unhealed wounds (lasting over 3 months); myocardial infarction; stroke; by-pass or cardiac stent or cardiac surgery; angina or heart failure; hypertension; hypercholesterolemia; hypertriglyceridemia; overweight/obesity; cataract or laser applications; glaucoma; blindness (non-traumatic); impaired vision; chronic kidney disease (CKD).

The presence of antihypertensive and statin treatment was also analyzed.

c) Personal pathological history of TB:

- the subjects were asked how many times they were diagnosed with TB, how much time elapsed since the diagnosis of TB (<6 months prior; 6-12 prior;>1 year prior);
- the duration of the anti-TB treatment performed in the antecedents was analyzed, being divided into three categories: <1 month; 1-5 months; > 5 months.

- Exposure to TB:

- family history of TB - 1st degree relatives: parents, children, brothers, sisters;
- proximity to patients with TB: the subject was asked if he had prolonged contact, outside the house, with people with active TB; if he lived with someone with active TB: in the same house, in the same room, in the same bed; if he lived, worked or volunteered in shelters for homeless, prison, detoxifying unit, hospitals or other medical units.

- Clinical symptoms suggestive of TB: weight loss, cough, fever/subfebrility, dyspnoea, chest pain, night sweats.

The unintentional weight change (in the last three months) was classified as follows:

- weight loss <5 kg;
- weight loss 5-10 kg;
- weight loss ≥10 kg;
- without changes;
- weight increase <5 kg;
- weight increase ≥5 kg.

Regarding cough, the subjects were asked about the duration of the cough, the productive character of the cough, the presence of blood in the sputum.

- Screening of TB infection: TST.

The TST was considered positive at a value ≥ 10 mm.

- Active TB screening: chest x-ray.

The chest X-ray results were classified as follows:

- normal;
- abnormal – possibly active TB;
- abnormal – possibly inactive TB;
- abnormal – does not suggest TB.

- Data regarding BCG vaccination: the presence of BCG scar.
- Data regarding the behavioral factors: smoking, alcohol consumption.

Regarding *smoking*, the subjects were divided into the following categories: smokers, former smokers and non-smokers.

Smokers were considerend the subjects who smoked, daily or occasionally, more than one cigarette per day and the subjects who quit smoking for less than one year.

Former smokers were considered the subjects who had given up smoking for more than a year.

Subjects who never smoked were considered non-smokers.

Also, data was recorded regarding the age at which the subject started smoking (<10 years; 10-20 years; 20-30 years; >30 years), the period during which the subject smoked (<5 years; 5 -10 years; 10-20 years; >20 years), the number of cigarettes smoked per day (<10; 10-19; 20-39; 40-59; ≥60), the period that has elapsed since the subject gave up smoking (<5 years; 5-10 years; 10-20 years; >20 years).

Regarding alcohol consumption, data was recorded regarding the frequency of alcohol use (daily; 1-4 days/week; 5-6 days/week; 1-3 days/month; less often than once a month).

- Data on corticosteroid administration at enrollment in the study, route of administration (oral, inhaler, topical, intravenous, intramuscular), the duration of corticotherapy (<2 weeks, ≥2 weeks), or corticotherapy within the last 30 days.

- Laboratory tests:

- HbA1c: the results were divided into several categories: <7%; 7-7.9%; 8-8.9%; 9-9.9%; 10-10.9%; 11-11.9%; 12-12.9%; 13-13.9%; >14%;
- total cholesterol;
- triglycerides;
- serum creatinine;
- calculation of eGFR – categories: ≥90 ml/min/1.73m^2; 60-89.9 ml/min/1.73m^2; 45-59.9 ml/min/1.73m^2; 30-45.9 ml/min/1.73m^2; 15-29.9 ml/min/1.73m^2; <15 ml/min/1.73m^2.
- ACR – categories: <30 mg/g; 30-299 mg/g; ≥300 mg/g;
- UTI presence, UTI etiology.

Blood and urine samples were also collected for the study of genetic material (DNA or RNA). The samples will be stored for a period of 25 years in order to carry out further research on the association between DM and TB.

Methods:

a. Determination of height, using a taliometer.
b. Determination of weight, using an electronic scale.
c. Calculation of BMI, according to the formula: BMI = weight (kg) / height^2 (m).

The calculated values are classified as follows:

- BMI <18.5 kg/m^2 – underweight;
- BMI 18.5-24.9 kg/m^2 – normal weight;
- BMI 25-29.9 kg/m^2 – overweight IMC;
- BMI 30-34.9 kg/m^2 – grade 1obesity;
- BMI 35-39.9 kg/m^2 – grade 2 obesity;
- BMI ≥40 kg / m2 – grade 3obesity.

d. Determination of WC, hip circumference, using a circumferential taliometer.

WC was determined midway between the costal rim and the iliac crest. Waist circumference values ≥ 80 cm for women and ≥ 94 cm for men, respectively, are an indicator of abdominal obesity.

The hip circumference was determined at the level of the large trochanter.

e. WHR calculation, according to the formula: WHR = waist circumference (cm) / hip circumference (cm), a value ≥ 0.85 in women, respectively ≥ 0.9 in men, being an indicator of abdominal obesity.

f. WHtR calculation according to the formula: WHtR = waist circumference (cm) / height (cm), a value ≥ 0.5 also being an indicator of abdominal obesity.

g. BP measurement was performed with the subject in sitting position, after five minutes of rest, with the cuff at heart level, on both arms. We considered subjects with hypertension the study participants who had values of SBP ≥140 mmHg and / or DBP ≥90 mmHg and / or those with antihypertensive treatment at home.

h. The temperature measurement was performed with the subject in sitting position. The subject's arm was raised, the thermometer was placed with the tip in the center of the axilla, parallel to the chest. The arm was then approached to the trunk, the subject having the forearm flexed on the anterior region of the thorax. The thermometer stays in this position until the beep is alerted. The results thus obtained were divided into the following categories:

- normal temperature: < 37°C;
- subfebrility: 37-38°C;
- fever: > 38°C.

i. The tuberculin skin test (TST), also known as the Mantoux test, was performed by intradermal injection of 0.1 ml of PPD, containing 5 units of tuberculin, on the inner face of the forearm. The injection was performed with a tuberculin syringe, with the needle cut facing up. The test result was read 48-72 hours after PPD administration and took into account the size of the induration, not the erythema. The diameter of the induration was measured transversely on the long axis of the forearm, the result being noted in millimeters. The positive result of the tuberculin skin test was considered to be ≥ 10 mm, all patients having DM, compared to a healthy person, where the test is considered positive at a dimension of the induration ≥ 15 mm.

j. The chest x-ray was performed at a distance of 2 meters, in postero-anterior incidence, the subject being positioned in orthostatism, in apnea after deep inspiration or after forced expiration.

k. The BCG vaccine is given to newborns, up to 2 months of age or to children aged 5 to 10 months, who do not have BCG scars or who have a scar below 3 mm. 0.1 ml of BCG vaccine suspension is injected intradermally on the postero-external side of the left arm, in the middle third. The injection is performed with a 0.5 ml or 1 ml syringe, with the needle cut facing up. After administration an ulceration may appear, after healing, thus leaving a specific scar.

l. HbA1c was performed using a Nationally Glycohemoglobin Standardization Program (NGSP) certified and standardized to the Diabetes Control and Complications Trial (DCCT) assay, with no need for fasting.

Subjects with DM were considered those with a personal history of DM and newly diagnosed cases, according to the American Diabetes Association criteria [26]:

- Fasting blood glucose ≥ 126 mg/dl; fasting blood glucose is the glucose harvested after a minimum of 8 hours of fasting; or
- Blood glucose level ≥ 200 mg/dl at two hours after the administration of 75g of anhydrous glucose, within OGTT; or
- HbA1c ≥ 6.5%; the laboratory must use a NGSP certified and standardized to the DCCT assay; or
- A random plasma glucose ≥ 200 mg/dl in a patient with classic symptoms of hyperglycaemia;
- In the absence of unequivocal hyperglycemia, DM diagnosis requires two modified test results from the same sample or two different samples.

m. Total cholesterol was harvested after a 12-14 hours of fasting.

Hypercholesterolemia was defined in subjects with total cholesterol ≥ 200 mg/dl and/or statin treatment at home.

n. Triglycerides: the sample was harvested after a 12-14 hours of fasting.

Hypertriglyceridemia was defined in subjects with triglyceride levels ≥ 150 mg/dl or in treatment with fibrates or other hypolipemic agents.

o. Serum creatinine was harvested fasting.

p. eGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation: eGFR = 141 x min (SCr / κ, 1) α x max (SCr / κ, 1) -1,209 x 0.993 Mean x 1,018 [if woman] x 1,159 [if black].

q. ACR: A sample of spontaneous urine (first morning urine) was collected.

CKD was defined as eGFR value <60 ml/min/1.73 m^2 and/or ACR value ≥ 30 mg/g. The obtained values were classified as follows:

- Stage 1 CKD: eGFR ≥ 90 ml/min/1.73 m^2 + ACR ≥ 30 mg/g;
- Stage 2 CKD: eGFR 60-89,9 ml/min/1.73 m^2 + ACR ≥ 30 mg/g;
- Stage 3a CKD: eGFR 45-59,9 ml/min/1.73 m^2 +/- ACR ≥ 30 mg/g;
- Stage 3b CKD: eGFR 30-44,9 ml/min/1.73 m^2 +/- ACR ≥ 30 mg/g;
- Stage 4: eGFR 15-29,9 ml/min/1.73 m^2 +/- ACR ≥ 30 mg/g;

- Stage 5 CKD: eGFR <15 ml/min/1.73 m^2 +/- ACR ≥ 30 mg/g.

2.4. STATISTICAL DATA ANALYSIS

The data was recorded on the computer, in an electronic database (REDCap), then transferred to Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM, Armonk, New York, USA), coded and analyzed using this program. The data was analyzed according to the presence or absence of TB infection in the subjects included in the study. The value $p \leq 0.05$ was considered statistically significant.

We used the chi-square test to compare the percentages between the two groups. The distributions of the continuous variables were tested for normality using the Kolmogorov-Smirnov test; data with Gaussian distribution were presented as mean ± standard deviation (SD); data that were not normally distributed were presented as median and interquartile range (IQR). In order to determine the significance of the differences between the groups, the Student's t-test and ANOVA were used to compare the means, respectively the Mann-Whitney U and Kruskal-Wallis tests for comparing the medians.

We also used: Pearson correlations, Spearman correlations, logistic regression, Levene test, Principal Component Analysis, 95% confidence intervals (95% CI).

2.5. RESULTS AND DISCUSSIONS

1. Primary objective: Analysis of the prevalence of MTB infection in a group of patients with DM, not selected, from Dolj County

603 subjects with DM who met the inclusion and exclusion criteria were enrolled in the study. Of these, 53.2% were women and 46.8% were men. Most of the subjects were in the age group 60-79 years (45.9%), followed closely by the age group 40-59 years (45.1%).

Most subjects presented type 2 DM (88.9%). The duration of DM (median [IQR]) was 8.00 [12.00] years, most of the subjects (41.4%) being into the 6-15 year group. Regarding the antidiabetic treatment at the time of the interview, a large percentage of subjects (66%) had metforminum treatment. Also, a large percentage of subjects had insulin treatment (67.8%), a possible explanation for this percentage being the reason for hospitalization, most of the subjects (92.9%) being hospitalized for metabolic imbalance.

A significant percentage of subjects presented hypertension (70.6%). Also, hypercholesterolemia was present in 69% of the subjects. Moreover, overweightness and obesity are present in over three quarters (79%) of the study participants. CKD was present in over one third (38%) of the subjects, most with stage 2 (11.3%) and stage 3a (10.1%) CKD.

Regarding smoking, the majority of the subjects (53.7%) stated that they were non-smokers at the time of inclusion in the study, only 14.9% being smokers, respectively 31.4% former smokers. In addition, a significant percentage of subjects (56.3%) were alcohol consumers at the time of enrollment, 10.4% of them consuming alcohol daily.

Almost all the subjects included in the study (98.7%) presented BCG scar, being vaccinated at birth.

Personal pathological history of TB was present in 3.5% of the enrolled subjects, the majority of them (90.4%) being diagnosed with TB only once. In most cases (85.7%) the anti-TB treatment was administered for more than 5 months.

A percentage of 5% of the subjects had a family history of TB, thus: 3.2% had parents diagnosed with TB, while only 1.5% had children with TB, and 1.2% had siblings with TB. Moreover, 11.8% of the subjects included in the study had extrafamilial TB contact.

Regarding the weight change, almost half (44%) of the subjects presented unintentional weight loss in the last 3 months. This can also be caused by the metabolic imbalance, the majority of the subjects (92.9%) being hospitalized for this reason.

At the time of enrollment, 15.4% of the subjects presented cough, lasting more than three weeks in the case of over one third of them (35.2%). In addition, 39.6% of the participants presented productive cough, and 5.6% of those with productive cough stated the presence of blood in the sputum. A possible explanation for this number of subjects who had cough at the time of the inclusion in the study may be due to the high percentage of patients with hypertension treated with angiotensin-converting enzyme (ACE) inhibitors (55% of those with cough had treatment at home with ACE).

Three quarters (75.6%) of the subjects had no radiographic changes at the enrollment. A significant percentage of subjects (18%) had lesions suggesting inactive TB, and 0.8% of the study participants had suggestive changes of active TB.

Other changes, which do not suggest TB, were present in 5.6% of the subjects. (Figure 1)

The prevalence of MTB infection was 48.4%, almost half of the subjects enrolled in the study presenting a positive tuberculin skin test. (Figure 2)

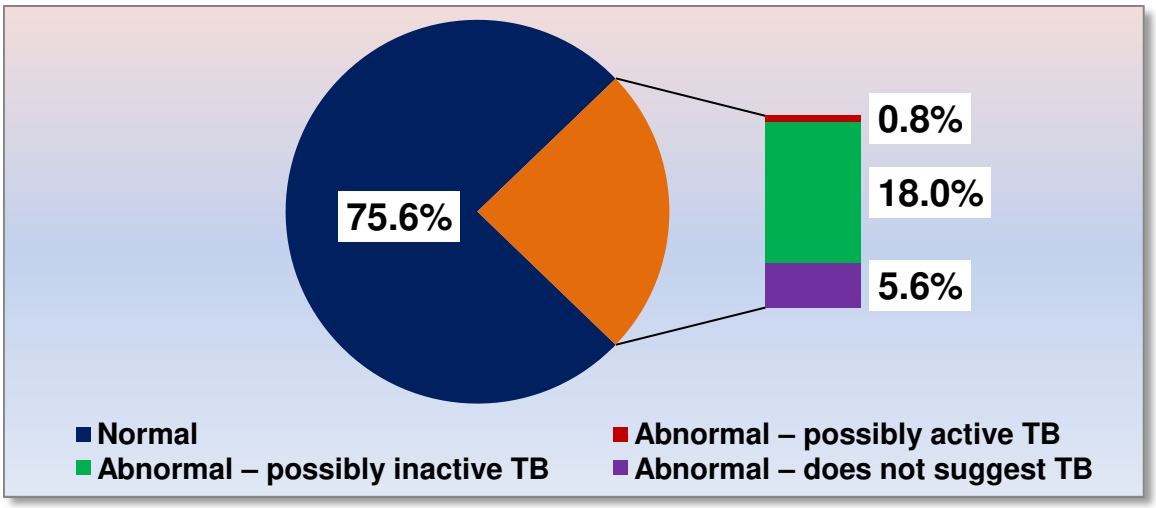

Figure 1. Group distribution according to thoracic x-ray interpretation

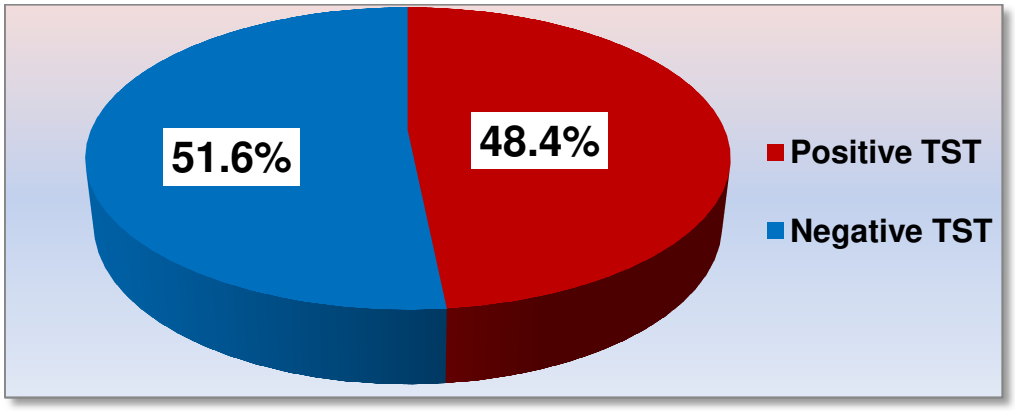

Figure 2. Group distribution accordind to TST

2. Primary objective: Analysis of personal history of TB and other risk factors for TB in patients with DM and MTB infection (GROUP 1), compared to the control group (GROUP 2)

The demographic data of the subjects are presented in Table 1.

Table 1. Demographic data

	TST		p (TST+ vs TST-)	Total
	TST +	TST -		
Gender				
Women	120 (37.6%)	199 (62.4%)	**<0.001**	319 (100%)
Men	170 (60.7%)	110 (39.3%)		280 (100%)

Age (categories)				
< 40 years	22 (47.8%)	24 (52.2%)	**0.002**	46 (100%)
40-59 years	152 (56.3%)	118 (43.7%)		270 (100%)
60-79 years	115 (41.7%)	161 (58.3%)		276 (100%)
> 80 years	1 (14.3%)	6 (85.7%)		7 (100%)
Age (years) (median [IQR])	58.00 [15.00]	61.00 [15.00]	**0.002**	59.00 [15.00]
Etnicity				
Romanian	287 (48.5%)	305 (51.5%)	0.767	592 (100%)
Roma	3 (42.9%)	4 (57.1%)		7 (100%)
Environment				
Urban	158 (46.7%)	180 (53.3%)	0.264	338 (100%)
Rural	131 (51.4%)	124 (48.6%)		255 (100%)
Marital status				
Married	236 (50.4%)	232 (49.6%)	**0.029**	468 (100%)
Unmarried	48 (39.3%)	74 (60.7%)		122 (100%)
Educational level				
No formal education	2 (20%)	8 (80%)	**0.016**	10 (100%)
Less than primary school	1 (12.5%)	7 (87.5%)		8 (100%)
Primary school	32 (48.5%)	34 (51.5%)		66 (100%)
Secondary school	97 (48.7%)	102 (51.3%)		199 (100%)
High school	132 (53.7%)	114 (46.3%)		246 (100%)
College/University	19 (33.3%)	38 (66.7%)		57 (100%)
Post graduate studies	1 (33.3%)	2 (66.7%)		3 (100%)

MTB infection was found to be significantly higher in subjects living with children (53.5%), while 46.5% of those without MTB infection were living with children.

Most patients (52.7%) with BP ≥ 140/90 mmHg (with and without treatment) had MTB infection.

MTB infection is found to be statistically significantly higher in smokers and former smokers, compared to those without MTB infection. (Figure 3)

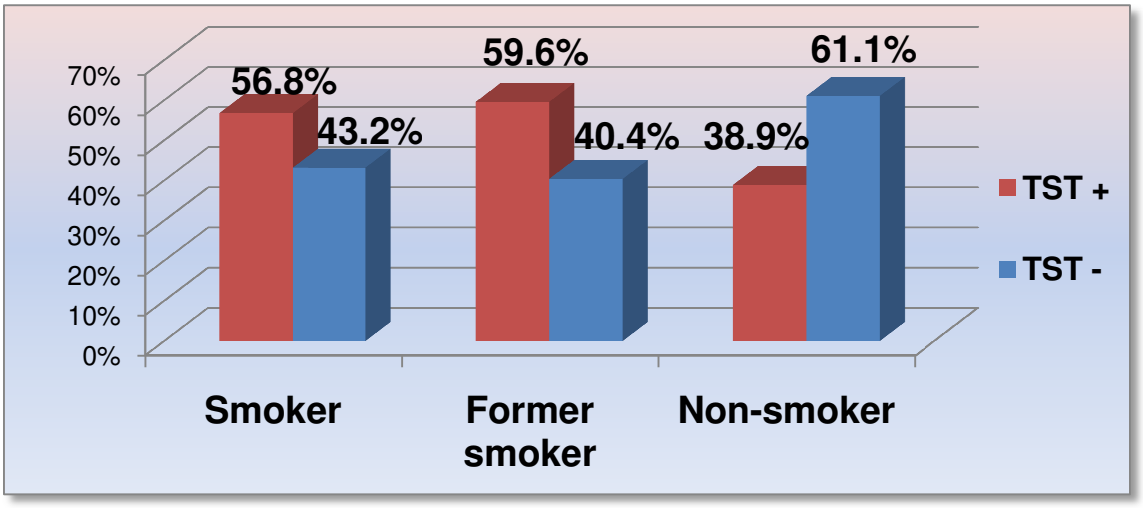

Figure 3. Group distribution according to TST and smoking status

The percentage of subjects with MTB infection is statistically significantly higher among alcohol consumers (52.8%), compared to those with negative TST (47.2%).

The percentage of BCG vaccinated subjects was similar in the two studied groups.

Although the percentage of subjects with personal pathological history who had a positive TST is double compared to those with a negative TST (66.7% vs. 33.3%), the difference between the two groups did not reach statistical significance. The personal pathological history of TB was present in 21 subjects (3.6%), 14 (4.9%) with positive TST and 7 (2.3%) with negative TST.

MTB infection is almost twice as high in subjects with family history of TB and extrafamilial TB contact, compared to the group without MTB infection (65.6% vs. 34.4%). MTB infection is found to be twice as high in subjects with family history of TB, compared to the group without MTB infection (68.2% vs. 31.8%). MTB infection is found to be twice as high among subjects with extrafamilial TB contact, compared with negative TST (65.2% vs. 34.8%).

3. Secondary objective: Analysis of pulmonary changes revealed by chest x-ray in patients with DM and MTB infection (GROUP 1), compared to the control group (GROUP 2)

Radiographic changes suggestive of active and inactive TB were more frequent in subjects with latent TB infection, compared to the control TST group. Regarding gender and age, no statistically significant differences were found in either of the two studied groups, depending on the result of the lung x-ray.

In the group of subjects with DM and MTB infection, radiographic changes suggestive of active and inactive TB were more frequent in those from rural environment, compared to urban environment. Also, the highest percentage of changes suggestive of inactive TB is found in households and retired subjects, and active TB changes are more frequent in unpaid and retired subjects.

Regarding the personal history of TB, statistically significant differences were found in the positive TST group ($p=0.002$), depending on the lung radiographic aspect, both lesions of active TB and inactive TB being more frequent in subjects with personal history of TB. Regarding the family and extrafamilial history of TB, statistically significant differences ($p=0.003$) were found in the positive TST group,

depending on the lung x-ray aspect, inactive TB lesions being more frequent in subjects with a family and extrafamilial history, compared to those without history.

4. Secondary objective: Analysis of the clinical symptoms suggestive of active TB in patients with DM and MTB, compared to the control group

Symptoms suggestive of active TB were more frequent in subjects with HbA1c value above 10%, in smokers and former smokers and in alcohol consumers.

FINAL CONCLUSIONS

The study includes the evaluation of the prevalence of MTB infection, the analysis of risk factors for TB, as well as of the radiographic lung changes and of the suggestive symptoms of active TB in a group of adult subjects with DM from Dolj County, who were enrolled in the international TANDEM project.

Our study led to the elaboration of the following conclusions, which may have applicability in clinical practice regarding prevention, but also delaying the occurrence of MTB infection and risk factors for active TB:

- The prevalence of MTB infection was 48.4% among the subjects with DM enrolled in the study. In contrast, the prevalence in the general population of Romania was lower (44.7%) [18].
- A significant percentage of subjects (18%) presented lesions suggesting inactive TB, and 0.8% of the study participants had suggestive changes of active TB. Compared, in 2015, the frequency of new cases of active TB in the general population of Romania was 0.1% [17], being a much lower percentage compared to the result of the screening of the subjects with DM enrolled in our study.
- 3.5% of the subjects included in the study stated, anamnestic, personal history of TB, the majority (90.4%) being diagnosed with TB only once. This percentage is much lower than the percentage of subjects with radiographic lesions of inactive TB, which is explained by the lack of recognition of TB symptoms by patients, which are non-specific, and may be confused with symptoms of metabolic imbalance; also, if the symptomatology was not severe, it did not made the patient to go to the doctor. Another explanation for the lower percentage of subjects with personal history of TB, compared to those with inactive TB lesions may be the limited access to medical services in some disadvantaged areas. Thus, some of the

subjects could not be diagnosed with TB, and they healed spontaneously without anti TB treatment. As a result, economic conditions and education are extremely important for effective prevention, diagnosis and management.

- The anti TB treatment performed at TB diagnosis in the past was administered for a period of more than 5 months in most subjects (85.7%).
- The MTB infection was more commonly found in male subjects, younger, married, from rural environment, who completed secondary school and high school education, those with BP ≥ 140/90 mmHg, those who lived with children, smokers and former smokers and alcohol consumers.
- Also, MTB infection (detected by TST +) was more frequent in subjects with personal history of TB and in those with family history and/or with extrafamilial TB contact, especially in those who worked in hospitals or other medical units.
- The percentage of BCG vaccinated subjects was similar in the two groups of subjects, with positive TST and negative TST, the explanation being given by the duration of the immunity after vaccination, the existing data indicates a decrease of immunity after a period of 10 years, the vaccination being performed on newborns.
- Radiographic changes suggestive of active and inactive TB were more frequent in the group of subjects with MTB infection, compared to the control group, as expected.
- In the group of subjects with MTB infection, radiographic lesions suggestive of active and inactive TB were more frequent in those from rural environment. Suggestive lesions of inactive TB were more frequent in households and retired subjects, and lesions of active TB in unpaid and retired subjects.
- Also, in the group of subjects with MTB infection, both the radiographic lesions suggestive of active and inactive TB were found more frequently in subjects with personal history of TB and in those with family history and/or with extrafamilial TB contact.
- Symptoms suggestive of active TB were more frequent in subjects with HbA1c value above 10%, in smokers and former smokers and in alcohol consumers.

Although the number of TB cases in subjects with DM is low, the prevalence is higher than in the general population, DM being considered a risk factor for TB. Routine TB screening is difficult for economic reasons, but chest x-ray screening is

recommended in patients with DM who have symptoms and signs suggestive of active TB, followed by Xpert MTB/RIF, if chest x-ray lesions are present [27] .

The increasing prevalence of DM will also influence the prevalence of MTB infection, eradicating TB being practically impossible in the absence of reducing DM incidence.

As a result, the vital need to stop the global epidemic of DM, as part of the TB control strategy, through the orientation of national programs and through effective integrated management from clinicians in both diseases, is imperative.

SPECIAL CONTRIBUTION

The present paper represent the first study in Romania that aimed to analyze the prevalence of pulmonary tuberculosis in patients with diabetes mellitus (from our research on the population studies published so far).

Also, this paper is the first study in our country that analyzes the presence of risk factors for pulmonary tuberculosis, but also the lung radiographic aspect and the suggestive symptomatology of active pulmonary tuberculosis in patients with diabetes mellitus.

Moreover, this is the first study that analyzed the frequency of personal pathological history of pulmonary tuberculosis in subjects with diabetes mellitus who have tuberculosis sequelae highlighted by chest x-ray.

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