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Frequency of comorbidities (HIV, HBV, and diabetes) in patients with pulmonary tuberculosis and resistance profile of strains isolated to first-line anti-tuberculosis drugs at Jamot Hospital of Yaoundé-Cameroon

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Abstract

Tuberculosis (TB) is a significant cause of mortality worldwide with the main risk factor being immunosuppression, which may be due to HIV, HBV or diabetes. Despite current therapeutic progress, effective control of this disease is becoming increasingly difficult in a large number of countries due to co-infection with other pathologies (HIV in particular) and the emergence of strains that have become resistant to anti-tuberculosis treatment. A cross-sectional study was conducted at Jamot Hospital of Yaoundé (JHY) from August 2022 to January 2023, in other to determine the frequency of comorbidities (HIV, HBV, diabetes) in patients with pulmonary tuberculosis and evaluate the resistance profile of strains isolated to first-line anti-tuberculosis drugs. The study population consisted of 82 patients with an average age of 35.84 ± 11.78 years, including 76% men and 24% women. The frequencies of TB-HIV, TB-HBV and TB-diabetes comorbidities were 19.50%, 7.30% and 4.9% respectively. Among the 76 sputum samples that gave positive cultures, 8 cases (10.53%) presented resistance to at least one of the anti-tuberculosis drugs. Resistance to Ethambutol (6.6%) and Isoniazid (5.3%) was the most common compared to Rifampicin (2.6%) and Streptomycin (2.6%). Monoresistance was detected in 4 cases (5.3%), multiresistance in 2 cases (2.6%) and polyresistance in 2 cases (2.6%). It showed that there is a significant association between TB-diabetes comorbidity and overall resistance (P=0.001), streptomycin resistance (P=0.001). However, no significant association between TB-HIV, TB-HBV co-infections and the occurrence of overall resistance (P = 0.138 and 0.481 respectively). Monitoring comorbidities and possible resistance in newly detected tuberculosis patients is of capital importance in order to ensure better care of these patients.

Keywords: Tuberculosis; Comorbidity; Resistance; Anti-tuberculosis drugs

1. Introduction

Tuberculosis (TB) is an opportunistic disease that takes advantage of an abnormal weakness of the immune system to take hold easily and cause damage, which can lead to death. It is one of the ten leading causes of death in the world and the second deadliest infectious disease [1]. It is very common in immunocompromised people such as HIV positive, HBV positive people and diabetics where it reaches conditions that are even more serious. During co-infection with HIV, the latter attacks T4 lymphocytes; making them inefficient and leading to a drop in the CD4 count, which promotes easy onset of active TB [2]. In the case of the TB-diabetes association, the carbohydrate disorder caused by diabetes leads to a deficit in both innate and adaptive immunity [3]; we note a defective Th1-cytokine response, a functional deficit of

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polynuclear neutrophils and monocytes thus favoring the development of *Mycobacterium tuberculosis* (MTB) [4]. During co-infection with HBV, treatment may be more difficult due to the coexistence of a Hepatotropic Virus (HBV) with drugs with hepatic metabolism (first-line drugs against TB) which could be at risk origin of relapses in these patients [5]. Globally, an estimated 10.6 million people contracted TB in 2021 and an estimated 1.6 million people died from TB of whom 187,000 were HIV-positive people [1]. That same year, it was estimated that, more than 250 million people were positive for Hepatitis B [6] and 537 million were diabetics [7]. In Africa, the frequency of TB among people with Diabetes remains high: 4 to 15% [3]. A study carried out in Benin showed that the frequency of the TB-HBV association is 9.35% [5]; the frequency of the TB-HIV association is even higher and varies depending on the country: we note 61% in South Africa, 69% in Zimbabwe, 27% in Nigeria, and 45% in Ivory Coast [8]. In Cameroon, TB-HIV co-infection is around 21% [9]; a study conducted at JHY showed that diabetes was associated with TB at a frequency of 6.2% [10]. However, figures on TB/HBV coinfection are rare. The management of TB is the same for all patients whether they are diabetic, HIV positive, HBV positive or not. However, the emergence of resistance to anti-tuberculosis drugs in a large number of countries has become a public health problem and constitutes an obstacle to the effective control of TB [11]. Furthermore, controlling TB in patients with co-infection is a challenge. In these patients, there is generally resistance to treatment, therapeutic failures and the risks of active TB and relapse after treatment are higher compared to patients with TB only [8-12]. Several studies have been carried out on TB; most turned to the TB-HIV association. On the other hand, there is very little information available regarding the TB-diabetes and TB-HBV associations. In addition, few studies have been carried out on MTB resistance in tuberculosis patients suffering from HIV, diabetes and/or HBV and most studies carried out on resistance to anti-tuberculosis drugs focus much more on resistance to Rifampicin and Isoniazid but very little information is available for resistance to Ethambutol and Streptomycin. This study was therefore carried out with the aim of determining the frequency of these associations at JHY and evaluating the resistance of Mycobacterium tuberculosis Complex (MTBC) strains isolated from patients to first-line anti-tuberculosis drugs.

2. Material and methods

2.1. Study type and population

We carried out a cross-sectional study over a period of 6 months from August 2022 to January 2023. The samples were collected at JHY. The study population consisted of adult patients regardless of gender, with newly detected pulmonary tuberculosis and naive to anti-tuberculosis treatment. Sociodemographic and clinical data were collected by interview with patients. Diabetic status or not was confirmed using the patient's medical file.

2.2. Collection and processing of blood samples

Blood samples were taken from the crook of the elbow by venipuncture, in dry 5 ml tubes. HIV serology was determined by rapid diagnostic tests using Determine™ HIV1/2 strips marketed by Abbott laboratories and HBV serology using strips marketed by Qingdao Hightop Biotech laboratories. To carry out these tests, 50 µL of serum was placed on the different strips and the results were read and interpreted fifteen minutes later [13-14].

2.3. Sputum collection and treatment

Sputum samples were taken in the morning on an empty stomach and their positivity was confirmed by auramine staining. Decontamination was then carried out using the Kubica method [15] then the decontaminated sputum was cultured on MGIT liquid medium and incubated at 37°C in the BACTEC MGIT 960 automated system for a maximum period of 42 days. Growth was detected by the emission of bright orange fluorescence at the bottom of the tube or by the presence of turbidity, small grains or flakes in the culture medium. The BACTEC MGIT 960 SIRE system (Streptomycin, Isoniazid, Rifampicin and Ethambutol) made it possible to perform the Antibiogram on positive cultures as described by Becton Dickinson [16].

2.4. Statistical analysis

The database was created using Excel software and then the data were analyzed using SPSS version 25 software. The chi-square test made it possible to compare the resistance rate between tuberculosis patients with comorbidity and those with none of the comorbidities studied.

2.5. Ethical considerations

The study was carried out in accordance with the ethical principles which help to ensure the well-being and interests of patients. For this, she received ethical clearance No. 1028/CNERSHC/2022 dated 09/19/2022 from the Regional Committee for Ethics in Human Health Research of the Center, then authorization to collect data provided by the

director from JHY. Participants were informed of the objectives of the study and the nature of their participation using the information leaflet and then their consent was obtained using an informed consent form.

3. Results

3.1. Sociodemographic profile of the study population

Among the 82 patients recruited in the study, 62 were male (76%) and 20 were female (24%), therefore a sex ratio of 3.1 in favor of men. The average age was 35.84± 11.78. The age groups most affected were those 20 to 29 years old and 30 to 39 years old (36.60%) and the patient with 60 years old and over were least affected. The patients were predominantly workers with 63.4% workers in the private sector, 19.5% in the informal sector and 6.1% in the public sector. Most of the patients were educated with respectively 20.7%, 57.3%, and 14.6% having primary, secondary and higher levels compared to 7.3% of unschooled patients. 51 patients were single (62.2%) compared to 31.7%, 3.7%, 2.4% of married, widowed and common-law patients respectively. Only 4.9% of participants lived outside the city of Yaoundé (Table I).

Table 1 Sociodemographic profile of the study population

Variables		Numbers	Frequencies (%)
Sexe	Male	62	76
	Female	20	24
Age	20-29	30	36,60
	30-39	30	36.60
	40-49	7	8.50
	50-59	11	13.40
	60 and over	4	4.90
Profession	Student	5	6.1
	Jobless	4	4.9
	Informal sector	16	19.5
	Private sector	52	63.4
	Public sector	5	6.1
Level of study	Not in school	6	7.3
	Primary level	17	20.7
	Secondary level	47	57.3
	Upper level	12	14.6
Marital status	Single	51	62.2
	Married	26	31.7
	Common law	2	2.4
	Widowed or widow	3	3.7
Residency	Out Yaoundé	4	4.9
	In Yaoundé	78	95.1

3.2. Clinical profile of the population

In total, 29.3% of patients were tobacco consumers, 58.5% alcohol consumers and 8.6% drug consumers. The most common clinical signs and symptoms were weight loss (96.30%), productive cough (85.40%), fatigue (80.50%), fever

(74.40%), and shortness of breath (70.70%). 76 (93%) patients were new cases while 6 (7%) were relapse cases (Table II).

Table 2 Clinical profile of the study population

Variables	Numbers	Frequencies (%)
Risk factors		
Tobacco	24	29,27
Alcohol	48	58,54
Drug	7	8,54
Clinical signs and symptoms		
Weight loss	79	96,30
Productive cough	70	85,40
Tiredness	66	80,50
Fever	61	74,40
Shortness of breath	58	70,70
Night sweats	54	65,90
Loss of appetite	51	62,20
Previous TB treatment		
New cases	76	93
Relapses	6	7

3.3. Frequency of comorbidities

We recorded 68% of patients with only tuberculosis compared to 32% with comorbidity. The frequency of TB-HIV, TB-HBV and TB-diabetes associations were 19.50%, 7.30%, and 4.9% respectively (Table III).

Table 3 Frequency of comorbidities

Variables	Numbers	Frequencies (%)
Total number of comorbidities	26	32
TB-HIV co-infection	16	19.50
TB-HBV co-infection	6	7.30
TB-diabetes comorbidity	4	4.9

TB-HIV and TB-HBV co-infections affected men more (56.2% and 100% respectively) in the age group of 30 to 39 years (50%), single (62.5% and 66.7%), and having a secondary education level (57.3% and 33.3%). The TB-diabetes comorbidity was equally distributed between men and women (50% each), but affected patients aged 30 to 39 much more (50%), then elderly people aged 50 and over, married (100%), and having primary (50%) and secondary (50%) levels (Table IV).

Table 4 Frequency of comorbidities according to sociodemographic profile

Variables		TB without co-infection	TB/VIH	TB/VHB	TB/diabetes
Gender	Female	19.6%	43.8%	0%	50%
	Male	80.4%	56.2%	100%	50%
Age	20-29 years	15.5 %	12.2%	33.3%	0%
	30-39 years	50%	50%	50%	50%
	40-49 years	7.7%	12.5%	0%	0%
	50-59 years	19.2%	18.8%	16.7%	25%
	60 years and over	7,7%	6,3%	0%	25%
Marital status	Single	66.1%	62.5%	66.7%	0%
	Married	28.6%	25%	33.3%	100%
	Common law	3.6%	0%	0%	0%
	Widowed or widow	1.8%	12.5%	0%	0%
Level of study	Unschooled	5.4%	12.5%	16.7%	0%
	Primary level	17.9%	18.8%	33.3%	50%
	Secondary level	60.7%	57.3%	33.3%	50%
	Higher level	16%	14.6%	16.7%	0%

3.4. Resistance profile of MTBC strains isolated from patients

After culture on MGIT medium of all 82 positive sputum, growth was only observed in 76 cases. A negative culture was noted for the other 6 cases (Table V).

Table 5 Distribution of participants according to culture result

Culture	Number	Frequencies
Positive	76	93 %
Négative	6	7 %

Among the 76 cases that gave a positive culture, 8 cases, or 10.53%, presented resistance to at least one of the anti-tuberculosis drugs (overall resistance). Resistance to Ethambutol, Isoniazid, Rifampicin and Streptomycin was recorded in 6.6%, 5.3%, 2.6% and 2.6% of cases respectively. Monoresistance was observed in 4 cases (5.3%), multiresistance in 2 cases (2.6%) and polyresistance in 2 cases (2.6%) (Table VI).

Table 6 Resistance profile of isolated strains

Variables	Numbers	Frequencies (%)
Overall resistance	8	10.53
Resistance depending on the type of anti-tuberculosis drug		
Rifampin	2	2.6
Isoniazid	4	5.3
Ethambutol	5	6.6
Streptomycin	2	2.6

Resistance depending on resistance type		
Monoresistance	4	5.3
Multi-resistance	2	2.6
Polyresistance	2	2.6

3.5. Comparison of the resistance profile of the strains between tuberculosis patients presenting the comorbidities and those without any of the comorbidities studied

Overall, the rate of resistance to anti-tuberculosis drugs was more frequent in patients with comorbidity (13.04%) compared to patients without comorbidity (9.43%), but the difference observed was not statistically significant (P = 0.638). Furthermore, more specific analysis of the resistance rate within each comorbidity showed a significant association (P = 0.001) between TB-diabetes comorbidity and the occurrence of resistance, but no significant association between TB-diabetes comorbidities. HIV and HBV-TB and resistance (P = 0.138 and 0.481 respectively) (Table VII).

Table 7 Comparison of the resistance rate between co-infected and non-co-infected patients

Resistance to anti-tuberculosis			
	N=76	n=8	%	*P-value*
TB – Comorbidity				
No	53	5	9.43	0.638
Yes	23	3	13.04	
TB – VIH				
No	61	8	13.11	0.138
Yes	15	0	0	
TB – VHB				
No	72	8	11.11	0.481
Yes	4	0	0	
TB – diabetes				
No	72	5	6.94	0.001
Yes	4	3	75	
N = Number of samples positive for culture n = Number of samples showing resistance to at least one of the anti-tuberculosis drugs % = Frequency of resistance				

Taking into account the rate of resistance to each anti-tuberculosis drug, we noted a significant association (P = 0.001) between TB-diabetes comorbidity and the occurrence of resistance to streptomycin (Table VIII).

Table 8 Association between comorbidity and resistance to each anti-tuberculosis drug

	Rifampicin				**Isoniazid**			**Ethambutol**			**Streptomycin**		
	N=76	n_1=2	%	*P-value*	n_2=4	%	*P-value*	n_3=5	%	*P-value*	n_4=2	%	*P-value*
TB – VIH													
No	61	2	3.28	0.477	4	6.56	0.308	5	8.20	0.251	2	3.28	0.477
Yes	15	0	0		0	0		0	0		0	0	

TB – VHB															
No		72	2	2.78	0.736		4	5.56	0.628		5	6.94	0.586		2	2.78	0.736
Yes		4	0	0			0	0			0	0			0	0	
TB – diabetes																	
No		72	2	2.78	0.736		3	4.17	0.069		4	5.56	0.127		0	0	0.001
Yes		4	0	0			1	25			1	25			2	50	

N : Number of samples positive for culture
n1 : Number of samples resistant to rifampicin
n2 : Number of samples resistant to isoniazid
n3 : Number of samples resistant to ethambutol
n4 : Number of samples resistant to streptomycin
% : frequency of resistance

4. Discussion

Analysis of the results shows a predominance of tuberculosis in the male population (76%) and a sex ratio of 3.1 in favor of men. This can be explained by the mode of contamination of the disease which exposes men as compared to women, through lifestyle habits such as the consumption of drugs, alcohol, tobacco which constitute the major risk factors for this disease [5]. The average age was 35.84± 11.78. Furthermore, we noted a high proportion of TB cases (73.2%) in the young population in the age group of 20 to 39 years. These results are similar to previous studies carried out at the JHY where a predominance of TB cases was recorded in the age group of 25-35 years [17]; this can be explained by the mobility of young people in society due to their socio-economic activities, which increases the risks of being in contact with bacilliferous patients. The patients were mainly workers in the private sector (63.4%) with a very high literacy rate: 20.7%, 57.3%, and 14.6% having primary, secondary and higher levels respectively compared to 7. 3% of patients not in school. Most of the participants (62.2%) were single and almost all (95.1%) of the participants resided in the city of Yaoundé. The latter being a large urban area of Cameroon and full of several higher, secondary and primary institutions, this could partly explain the high proportion of educated patients. Data from the literature show that tuberculosis infections are strongly linked to the habits and lifestyles of the population [18] which was confirmed in this study through a high proportion of participants consuming alcohol (58.5%), tobacco (29.3%) and drugs (8.6%). The clinical signs and symptoms were those usually described in the literature: Weight loss in 96.30% of cases followed by productive cough (85.40%), fatigue (80.50%), fever (74.40%). %), shortness of breath (70.70%). Along the same lines, Bitchong *et al*. found weight loss (71.6%), asthenia (68.5%), fever (61.1%), anorexia (58.9%), night sweats (40%), cough (56.7%) [19].

After evaluating the frequency of TB-HIV, TB-HBV and TB-diabetes comorbidities, the analysis of the results showed that TB-HIV association was the most represented (19.50%), followed by the TB-HBV association (7.30%) and finally the TB-diabetes association (4.9%). The high representativeness of TB-HIV co-infection is explained by the fact that HIV is the main cause of immunosuppression which is one of the major factors favoring the transition from latent tuberculosis infection to TB disease [2]. This frequency of TB-HIV co-infection is comparable to the prevalence of TB-HIV co-infection in Cameroon which is 21% [9] but is very low compared to previous studies carried out at the JHY: 61 .1% for Bitchong *et al*. and 29.9% for Kamgue [17-19]. This decline may be associated with the significant decrease in the prevalence of HIV infections in Cameroon (which fell from 5.6% to 2.7%) and the improvement in patient awareness of the occurrence of opportunistic infections such as than TB. The frequency of the TB-HBV association found in this study is slightly lower than that of a study carried out by Deguenon in Benin in 2018 where the frequency of HBV in TB patients was 9.35% and much lower than that of a study carried out in Conakry where it was 15.5% [5]. However, more in-depth studies on this co-infection in Cameroon would allow these differences to be better appreciated. As for the frequency of TB-diabetes comorbidity, it is similar to that found by Traoré *et al*. (5.2%) in a study carried out in Bamako in 2020 [20] but is slightly lower than that of a previous study carried out at the JHY which was 6.2% [10] which could be explained by our small sample size.

In the literature, it is generally accepted that TB-HIV co-infection is more common in women, because the latter are more at risk and more vulnerable [21]. However, our data show the opposite with the male sex who was more affected for both TB-HIV co-infection and TB-HBV co-infection (56.7% and 100% respectively). We noted a strong predominance of these two co-infections among single people in the age group of 30 to 39 years, and with secondary education, which is consistent with the work of Kamgue [17]; this is explained by the fact that this age group is more sexually active in society and also subject to migration, all of which makes them at risk. A disappearance of cases of HBV-TB was noted above the age of 55, which is consistent with the results of Deguenon who observed a disappearance of cases of TB suffering from hepatitis above the age of 54 [5]. As for TB-diabetes comorbidity, this study showed an identical distribution between the two sexes and it affected much more patients aged 30 to 39, married (100%), and having

primary (50%) and secondary levels (50%).This is contradictory to the data from Tolofoudie who found a high proportion of cases of TB-diabetes in the elderly age group (55-64 years) [3]. This may be linked to hereditary predispositions to diabetes. In addition to this, we can also add the fact that most of our participants were young people. After culture on MGIT, 93% of cases were positive compared to 7% of cases which showed no growth after 42 days of incubation in the machine. This could be explained by the effect of decontaminants (during the decontamination phase) which would have reduced the viability of tuberculosis bacilli, thus affecting their growth [22]. Effective control of TB remains difficult in a large number of countries, due in part to the emergence of strains that have become resistant to anti-tuberculosis treatment. In this study, the overall resistance rate among TB patients was 10.53%. A previous study found a general resistance rate of 8.1% in the Central region and 13.3% in the Western region [23]. Along the same lines, Kamgue found an overall resistance rate of 9.4% [17]. These variations are explained by varying sample sizes. In the literature, it is known that resistance rates to Isoniazid and Streptomycin are highest in sub-Saharan Africa. This observation is consistent with the data reported by Assam *et al*. in 2011 where resistance to Isoniazid and Streptomycin was most encountered and no resistance was observed to Ethambutol [23] and later by Kamgue in 2015 where resistance to Isoniazid (6.81%) and Streptomycin (2.92%) were the most frequent [17]. However, our results show the opposite with resistance to Ethambutol (6.6%) and Isoniazid (5.3%) which were the most common compared to Rifampicin (2.6%) and Streptomycin. (2.6%). This low rate of resistance to Streptomycin could be explained by the fact that this drug is no longer widely used in treatment as in the past [17]. Multi-resistance was recorded in 2 cases, or 2.6%. This frequency is close to that of a study carried out in the Democratic Republic of Congo where multidrug resistance was detected in 2.25% of cases [24]. The chi-square test showed a significant association ($P \leq 0.05$) between TB-diabetes comorbidity and overall resistance ($P = 0.001$), Streptomycin resistance ($P = 0.001$). However, we did not note a significant association between TB-HIV, TB-HBV co-infections and the occurrence of resistance ($P = 0.138$ and 0.481 respectively). These results are contradictory to literature data which generally show that coinfections and comorbidities are the causes of an increase in the resistance rate, therapeutic failures in patients and a low cure rate [8-12]. This can be explained by the fact that coinfections and comorbidities could be associated with resistance acquired during treatment; however, all cases of resistance recorded in this study were cases of primary resistance. In addition to this, we can also add the fact that the patients in this study were recruited upon TB screening; we did not monitor these patients during treatment to be able to note possible cases of acquired resistance.

5. Conclusion

This study shows that 19.50% of tuberculosis patients carry HIV, 7.30% carry HBV and 4.9% suffer from diabetes. It also highlights strains resistant to first-line anti-tuberculosis drugs as well as multi-resistant and even poly-resistant strains to JHY. Finally, it reveals a significant association between TB-diabetes comorbidity and overall resistance, resistance to Streptomycin. All this demonstrates the need for systematic screening of these comorbidities and possible resistance in newly detected tuberculosis patients with a view to having a better diagnosis and better patient care.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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