*Research Article*

# Profile of Interferon-gamma levels in patients infected with *mycobacterium tuberculosis* and coinfected with human immunodeficiency virus in Yaoundé.

**[1]Tata Gloria Berinyuy, [2]Diderot Fopa, [1]Fabo Yatchou Sosthene, [3]Matcha Fotso Laetitia, [4]Mme. Kamdjeu Rita, [5]René Essomba,**

[1]MSc. Health Sciences option Microbiology; Catholic University of Central Africa.

[2]Ph.D. Immunology and Infectious Diseases; M.Sc. Immunology and Regenerative Medicine; D.U. Sécurité Transfusionnelle Infectieuse; Hematology and Transfusion Service Yaoundé University Teaching Hospital

[3]MSc. Health Sciences option Immuno-hematology; Catholic University of Central Africa.

[4]Laboratory Technician, Yaoundé University Teaching Hospital.

[4]National Public Health Laboratory, Yaoundé, Cameroon; Department of Microbiology, Parasitology, Hematology, Immunology, and Infectious Diseases. Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1; Head of Immunology Department, Catholic University of Central Africa, Yaoundé Cameroon.

Corresponding Author: Tata Gloria Berinyuy (Beritata7@gmail.com)

## ABSTRACT

Tuberculosis remains a major public health problem globally with HIV/AIDS being the main predisposing factor to *Mycobacterium tuberculosis* (MTB) infection. Interferon Gamma (IFN- γ) is one of the most important cytokines in the host immune response against this mycobacterium. This study aimed to determine the profile of IFN-γ levels in patients infected with Mycobacterium tuberculosis and co-infected with HIV in Yaoundé. A descriptive cross-sectional study was carried out in the Yaoundé Jamot Hospital and the Yaoundé University Teaching Hospital from August - November 2021. Ninety (90) participants were enrolled (45 pulmonary tuberculosis (PTB) cases and 45 healthy controls). **A**LERE DETERMINE HIV-1/2 rapid diagnostic test plus HIV AB/AG ELISA HUMAN COMBO was used for HIV screening, and IFN-γ levels were measured by sandwich ELISA method. Among the 45 cases, 35 were PTB infected only, while 10 (22.2%) were HIV/TB co-infected. IFN-γ levels were higher in the TB-HIV co-infected group (7.24pg/ml ± 6,9) and the TB mono-infected group (4.11pg/ml ± 3.02) as compared to healthy controls (3.31 ± 2.14). In TB patients, a negative correlation was observed between the IFN-γlevels and the duration of anti-tuberculosis therapy, indicating the importance of IFN-γ in accelerating recovery from PTB following treatment. Considering the limited number of cases studied, further studies are needed to demonstrate the value of this cytokine in the management of MTB in Cameroon.

Keywords: IFN-γ levels, Mycobacterium tuberculosis, HIV, PTB, co-infection, Cameroon.

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**RÉSUMÉ**

La tuberculose reste un problème majeur de santé publique au niveau mondial, le VIH/sida étant le principal facteur prédisposant à l'infection par *Mycobacterium tuberculosis* (MTB). L'interféron gamma (IFN-γ) est l'une des cytokines les plus importantes dans la réponse immunitaire de l'hôte contre cette mycobactérie. Cette étude visait à déterminer le profil des niveaux d' IFN-γ chez les patients infectés par *Mycobacterium tuberculosis* et co-infectés par le VIH à Yaoundé. Une étude transversale descriptive a été menée à l'hôpital Jamot de Yaoundé et au CHU de Yaoundé d'août à novembre 2021. Quatre-vingt-dix (90) participants ont été enrôlés (45 cas de tuberculose pulmonaire (PTB) et 45 témoins sains). Le test de diagnostic rapide ALERE DETERMINE HIV-1/2 plus HIV AB/AG ELISA HUMAN COMBO a été utilisé pour le dépistage du VIH, et les niveaux d' IFN-γ ont été mesurés par la méthode ELISA sandwich. Parmi les 45 cas, 35 étaient uniquement infectés par la tuberculose, tandis que 10 (22,2 %) étaient co-infectés par le VIH et la tuberculose. Les taux d' IFN-γ étaient plus élevés dans le groupe co-infecté VIH-TB (7,24pg/ml ± 6,9) et dans le groupe mono-infecté par la TB (4,11pg/ml ± 3,02) par rapport aux témoins sains (3,31 ± 2,14). Chez les patients tuberculeux, une corrélation négative a été observée entre les niveaux d' IFN-γ et la durée de la thérapie antituberculeuse, indiquant l'importance de l' IFN-γ dans l'accélération de la guérison de la PTB après le traitement. Compte tenu du nombre limité de cas étudiés, des études complémentaires sont nécessaires pour démontrer l'intérêt de cette cytokine dans la prise en charge de la MTB au Cameroun.

Mots-clés : Taux d' IFN-γ , *Mycobacterium tuberculosis*, VIH, PTB, co-infection, Cameroun.

## INTRODUCTION

Tuberculosis is a communicable disease caused predominately by *Mycobacterium tuberculosis* or other members of the Mycobacterium complex, such as *Mycobacterium Africanum* and *Mycobacterium bovis* (Liu et al., 2018). Globally, tuberculosis is among the top 10 diseases that cause death, and it is the leading cause of mortality from a single infectious agent (WHO, 2019). In 2019, an estimated 10 million people fell ill with tuberculosis, resulting in a total of 1.4 million deaths, including 208,000 people living with HIV/AIDS (PLWHA). TB is present in all countries and age groups, and about a quarter of the world's population is infected with *Mycobacterium tuberculosis,* so this fraction is at risk of developing tuberculosis disease (WHO, 2019; Assam et al., 2013). HIV/AIDS predisposes to *Mycobacterium tuberculosis* (MTB) infection and increases the rate of recurrent TB as well as rapid progress to active TB among individuals with recently acquired infection. The risk of progressing from latent to active TB is estimated to be about 20 times greater in PLWHA than among those without HIV infection, with a higher risk of transmitting the infection to others (Tanue et al., 2019).

IFN-γ production in MTB-infected individuals has been exploited in research and clinics to evaluate and develop new tools for the prevention, diagnosis, prognosis, and treatment of the infection. (Lalvani & Millington, 2008). Interferon-gamma (IFN-γ) is a soluble protein secreted by a number of immune cells and is a dimeric cytokine that belongs to the interferon family. At the site of MTB infection, it is the major cytokine of Th-1 cells, therefore, as a pro-inflammatory immune response cytokine, it protects against MTB principally by activating macrophages to kill intracellular mycobacteria (Hussain et al., 2010).

The purpose of this study was to determine the serum IFN-γ levels in PTB patients regardless of their status of the disease (that is, whether

active, inactive, acute, or chronic), pulmonary TB patients co-infected with HIV, and then to compare the levels of IFN-γ between TB patients and healthy controls.

## METHODOLOGY

**Ethics:** For the commencement of this project, ethical clearance was obtained from the Institutional Ethics Committee on Research for Human Health of ESS-UCAC. A letter of authorization for data collection and sample analysis from the Director of the Jamot Hospital and the Director General of YUTH and, informed consent signed by all those who accepted to participate in the study.

**Selection and description of participants:** A total of 90 participants were recruited in this study, including 45 pulmonary tuberculosis (PTB) cases and 45 healthy controls. The PTB cases were included based on the following criteria; (i) patients diagnosed positive for TB (TB -LAMP (+), microscopy (+)), (ii) >18 years of age, (iii) nonpregnant/lactating females, and selected blood donors served as controls. Participants were excluded if they did not meet these criteria.

**Blood collection:** Four milliliters of whole blood samples were collected from each participant into tubes with ethylenediaminetetraacetic acid (EDTA). All blood samples were centrifuged at 4000 rpm for 5 minutes to extract plasma for the determination of IFN-γ levels and HIV screening. The plasma samples were stored at "20°C until analysis.

**HIV screening:** The first line HIV test used was ALERER determine™ HIV1/2, which is an immunochromatographic test for the qualitative detection of antibodies to HIV 1 and HIV 2. The test was performed according to the manufacturer's instructions. As a confirmatory test, the HIV 1, 2 Ag/Ab ELISA human combo, a two-step incubation sandwich enzyme immunoassay test, was used. The test was also performed according to the manufacturer's instructions.

**Determination of Interferon-gamma levels:** Circulating levels of IFN-γ, was determined in plasma samples using a sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (ELABSCIENCE Human IFN-γ (Interferon Gamma) ELISA_Kit).

**Statistical analysis:** All the data were presented as the mean ± standard error of the mean. Data analysis was conducted using R software. Comparisons in age and IFN-γ levels between cases and controls werre performed using the Mann Whitney *U* test. The Kruskal Wallis test was used for associations between IFN-γ levels and clinical state. A linear regression test was carried out between IFN-γ levels and the number of weeks of treatment using the Spearman correlation test.

## RESULTS

### Baseline characteristics of the study population

A total of 90 participants were recruited in this study, 35 of whom were PTB infected only, while 10 (22.2%) [95% CI 9.9%-34.1%] were HIV/TB co-infected and 45 healthy controls were recruited as well. The mean ages for the PTB group and the control group were 32.79 (range 17-49) and 28.42 (20-48) respectively. There was no significant difference in the ages of both the cases and the controls ($p = 0.171$). All 45 (100%) cases had a cough, among which 31 (68.9%) had chest pain, 23 (51.1%) had fever, 41 (91%) experienced weight loss, 25 (55.6%) had a loss of appetite, 38 (86.7%) had fatigue, and 32 (71.1%) had night sweat. Regarding the treatment phase, 2 (4.4%) of MTB participants were untreated, while 36

(80%) were in the intensive phase (2 months) of their treatment. In addition, 15 (33.3%) of the patients were hospitalized, while 30 (66.7%) were treated at home.

Table 1: Baseline demographics of the study population

| | **TB only** N (%) | **TB-HIV** N (%) | **Control N (%)** |
|---|---|---|---|
| **Gender** | | | |
| Female | 11(31.43) | 3(30) | 24(53.33) |
| Male | 24(68.57) | 7(70) | 21(46.67) |
| **Age**(years) | | | |
| < 21 | 3(8.57) | 0(0) | 5(11.11) |
| [21-30] | 17(48.57) | 5(50) | 28(62.22) |
| [31-40] | 8(22.86) | 4(40) | 7(15.56) |
| 40 + | 7(20.00) | 1(10) | 5(11.11) |

Table 2: Clinical characteristics of the case group

| | |
|---|---|
| **Clinical information** | |
| HIV | 10 (18.1%) |
| Coughing | 45 (100%) |
| Chest pain | 31 (68.9%) |
| Fever | 23 (51.1%) |
| Weight loss | 41 (91%) |
| Loss of appetite | 25 (55.6%) |
| Weakness or fatigue | 38 (86.7%) |
| Night sweets | 32 (71.1%) |
| **Therapeutics information** | |
| Place of treatment | Hospitalization: 15 (33.3%)<br>Home: 30(66.7%) |
| Treatment's phase | Intensive phase (<2months) :36 (80%)<br>Continuation phase(>2months): 7(15.6%)<br>Untreated: 2 (4.4%) |
| Days of treatment | Mean = 42,7 days<br>Min = 0, max = 140 days |

## Circulating interferon-gamma levels

Our results showed that IFN-γ levels were higher in the TB-HIV co-infected group (7.24 pg/ml ± 6,9) and in the TB mono-infected group (4.11 pg/ml ± 3.02) as compared to healthy controls (3.31 ± 2.14). However, the difference between the TB-HIV coinfected group and the TB mono-infected group was not statistically significant (p = 0.09), and likewise, there was no significant difference in IFN-γ levels between the TB-HIV coinfected group as compared to healthy controls (p-value = 0.09). Correlations between IFN-γ levels in the different groups (TB-HIV, TB only, and controls) are represented in Figure 1.

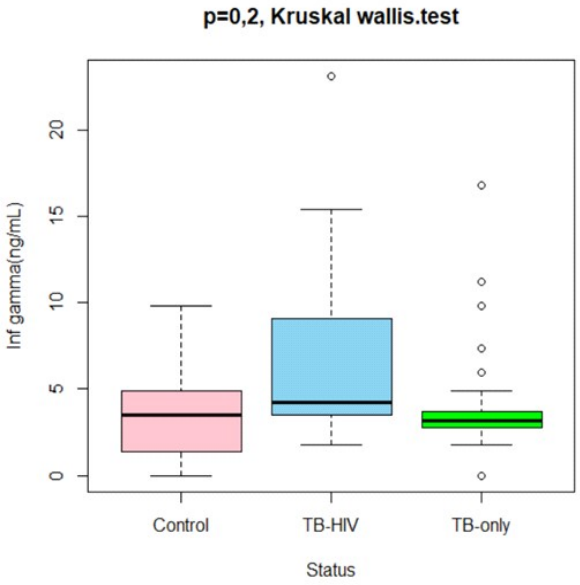


Figure 1: Comparison of serum IFN-γ levels between TB-HIV, TB only, and the Control Group. Krukal-Wallis (non-parametric test) was performed to see if the distribution of IFN-γ is different according to status (Tb-HIV, Control and TB only. We obtained a non-significant value of P=0.2. Box plots are shown with the horizontal line indicating the median levels; the lower and upper edges of each box indicate the 25th and 75th percentiles, respectively, while the lower and upper whiskers show the 10th and 90th percentiles. Dots represent outliers.

In TB patients, a negative correlation was observed between the IFN-γ levels and the duration of tuberculosis therapy. These results were, however, not statistically significant but highlighted the fact that with an increase in the duration of antituberculosis therapy, serum IFN-γ levels decrease. This is represented in Figure 2.

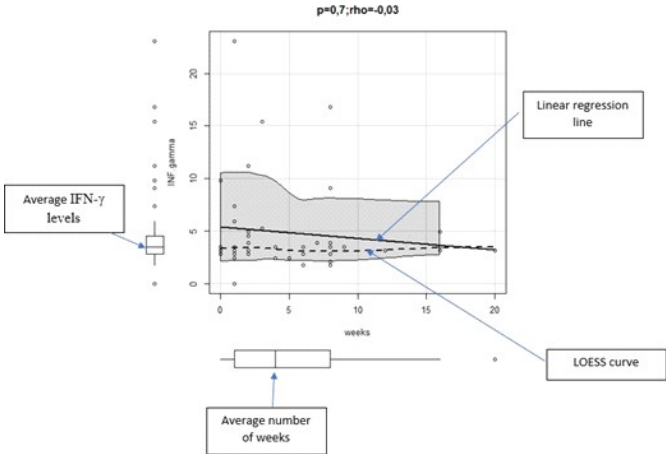


Figure 2: IFN-γ levels and the duration of tuberculosis therapy. A linear regression test was carried out using the Spearman method to evaluate the relationship between IFN-γ levels and the weeks of treatment. We obtained a negative coefficient of correlation (rho = -0.03). Despite the fact that rho is different from 0, the linearity was not statistically significant (p = 0.7).

## DISCUSSION

The development of TB or reactivation of the latent TB infection depends on the host's immune status. HIV, which remains the main risk factor in TB infection and co-infection, has been suggested to alter blood cell populations and change the Th1/Th2 balance, which affects the course of TB progression (Tanue et al., 2019). The protective immunity against this pathogen is mediated by certain cytokines, which are important immunomodulating agents of the immune system. Cytokines are thought to be transiently produced following antigenic stimulation in vivo. Thus, elevated cytokine levels are generated following a prolonged stimulus and this could be expected in patients with tuberculosis. In the host defense mechanism against MTB, IFN-γ is the most important cytokine (Cavalcanti et al., 2012).

This cross-sectional study determined the circulating levels of IFN-γ in patients infected with MTB and co-infected with HIV compared to healthy controls. The main findings of this study were: a) there was a higher but insignificant difference in serum IFN-γ levels between the TB mono-infected group and healthy controls; b) equally, IFN-γ levels were higher but the difference was statistically insignificant in the TB-HIV co-infected group as compared to the TB mono-infected group and the healthy control group; c) serum IFN-γ levels decreased with increasing weeks of treatment. Serum IFN-γ levels in tuberculosis patients can be explained by the hypothesis that there is leakage of cytokines from the tissue into the circulation due to increased local vascular permeability that favors the diffusion of IFN-γ into the bloodstream (Köksal et al., 2006).

The elevated circulating IFN-γ levels in TB cases suggest upregulation in the pro-inflammatory response, indicative of an innate protective response during MTB infection (Shaviya et al., 2016). This may be because, in mycobacterial infections, IFN-γ is required to activate the macrophage, stimulating the antimicrobial activities that destroy the intracellular mycobacteria and, therefore, providing protection to the host. As a protective cytokine against MTB, elevated IFN-γ in TB-infected patients may also indicate host adaptive mechanisms used to clear the bacteria or as a marker of disease severity (Feng et al., 2018). Indeed, this can be further explained by the fact that individuals deficient in IFN-γ receptors are more susceptible to infection by this mycobacterium with increased disease severity (Warren Levinson, 2014).

Our study showed that the high levels of IFN-γ in the TB-mono-infected group were insignificant when compared to the healthy controls. These results tie in with those of Al-Jubouri et al., 2018 in Iraq. However, they are at variance with the results obtained by Bolajoko, 2020 and Ei et al., 2019 in a similar setting in Nigeria. There was a

higher but insignificant difference in the serum levels of IFN-γ levels between the TB-HIV co-infected group as compared to the TB mono-infected group and the healthy control group. These results are similar to those of Mihret and collaborators in Ethiopia. This could be due to both tuberculosis and HIV infection inducing immune activation in a similar pattern (Mihret et al., 2014). Conflicting data are available in the literature review based on the levels of IFN-γ in TB patients. Studies of the systemic cytokine response in TB patients have either focused on serum cytokine levels (in-vivo) or cytokine production by peripheral blood mononuclear cells (ex-vivo) (Köksal et al., 2006). Most of the studies determining serum IFN-γ levels show that the levels are higher in TB patients as compared to healthy controls (Yamauchi et al., 2020; Bolajoko, 2020; Ei et al., 2019). In studies determining the ex-vivo cytokine production capacity of PBMCs after stimulation with MTB antigens, results report decreased cytokine production in TB patients (Feng et al., 2018); however, findings from this study conducted by Feng and collaborators did not demonstrate that ex-vivo production of cytokines by PBMCs in IFN-γ Release Assays (IGRA) can evaluate the host immunity and treatment outcome.

Few studies have been carried out to correlate clinical signs and levels of IFN-γ in TB patients. One study that clearly stands out is that of Feng and collaborators in Taiwan. They demonstrated in their study that non-TB specific IFN-γ response was a significant factor associated with more severe clinical signs and on-treatment mortality (Feng et al., 2018). This, however, contradicted our findings. We summed the score of clinical signs and associated it with IFN-γ levels. Our findings showed no association between these two variables. Further studies are needed to evaluate IFN-γ levels with respect to clinical signs. Equally, our study demonstrated no significant association between IFN-γ levels and the age of the participants. This ties in with studies conducted by Feng and collaborators in 2018.

Several studies have revealed that anti-tuberculosis treatment reduces serum IFN-γ levels. Hussain and collaborators demonstrated that the mean IFN-γ levels of confirmed TB patients were higher than those of clinically diagnosed TB patients. This may be due to the fact that most of the clinically diagnosed TB patients were on anti-tuberculosis therapy, and therefore the healing effect on granulomas could reduce the number of local and circulating IFN-γ producing activated T cells. This explanation was supported by the observation of a negative correlation between the levels of interferon-gamma and the duration of ATT in TB patients, which demonstrated that drug therapy caused a decrease in the level of IFN-γ (Hussain et al., 2010). In our study, a negative correlation was also observed between the levels of IFN-γ and the increase in weeks of treatment. However, these results were not statistically significant compared to those of Hussain and collaborators.

Our study had several limitations. Firstly, IFN-γ levels were only measured once, depending on the clinical stage of the patient at the time of the study. Therefore, dynamic changes in IFN-γ levels for each patient during the course of anti-TB treatment were not evaluated. Furthermore, the levels of some relevant inflammatory cytokines in host defense against MTB, such as tumor necrosis factor-alpha (TNF-α) were not measured in this present study.
There is still a need to further investigate the host immune response against MTB in our setting.

## CONCLUSION

This study aimed to determine the plasma levels of IFN-γ in patients infected with MTB and co-

infected with HIV in Yaoundé. The seroprevalence of HIV infection among MTB-infected patients was 22.2%. IFN-γ levels were found to be higher, but the difference was not statistically significant, in the MTB-HIV coinfected group as compared to the MTB mono-infected group and the healthy control group. We also observed a negative correlation between IFN-γ levels and the number of weeks of treatment. There was no significant association between IFN-γ levels and the age of participants as well as the clinical signs. IFN-γ has been found useful in developed countries either as a diagnostic marker, as in the case of IGRAs, as a prognostic marker, or even for therapeutic purposes. The importance of IFN-γ has not been fully explored in our setting; therefore, further studies are needed to demonstrate the value of this cytokine in the management of MTB in Cameroon.

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Conflict of interest: None

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