

Hematological profiling of tuberculosis-infected and co-morbid patients: A study carried out in central Punjab, Pakistan

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Abstract

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) is a chronic bacterial infection affecting many organs of the body particularly the lungs. Another organ seriously affected by TB is the hematopoietic system. TB patients co-infected with other disorders are more prone to death than TB alone. This study was aimed to investigate the variations in hematological profile of pulmonary tuberculosis (PTB) patients co-infected with chronic infectious and metabolic disorders. The study population (n=366) include PTB patients (positive control) and PTB patients co-infected with hepatitis C virus (HCV) infection, human immunodeficiency virus (HIV) infection, diabetes, and myocardial infarction (MI). Healthy individuals (n=95) were also included as normal control. All the study subjects were screened for PTB, diabetes, HCV infection, MI, and HIV infection. Polymerase chain reaction (PCR) was performed to confirm MTB infection at molecular level. Among 366 Ziehl–Neelsen positive cases, 258 (70.5%) were confirmed PTB positive by PCR. Among them, 36.8% were infected with PTB only, while 24% were co-morbid with diabetes, 17.8% co-infected with HCV, 11.2% with diabetes and HCV, 4.2% with HIV, 2.3% with both HCV and HIV, and 3.4% co-morbid with MI. Significant ($P < 0.01$) variations were observed in hematological profile of different study groups. The study concluded that significant increase in PTB patients co-infected with HCV, HIV, diabetes, and MI suggests the investigation of co-morbidities to rule out PTB co-infection with infectious and metabolic disorders before the start of anti-TB drug therapy.

Keywords

co-morbidity, diabetes, hepatitis C virus, human immunodeficiency virus, myocardial infarction, tuberculosis

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Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) is a chronic infectious disease affecting various organs of the body particularly the lungs and hematopoietic system. Pulmonary TB (PTB) is transmitted mainly through aerosol droplets during sneezing and coughing of infected person. TB is considered the disease of poor people and one among the main causes of morbidity and mortality in both the lower and middle-income countries of the world where the number of TB patients co-infected with diabetes and hepatitis C virus (HCV) infection are increasing rapidly.¹ The

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mortality cases due to TB depend on many factors including the epidemiological status, risk factors, and other associated abnormalities. The most significant conditions which may contribute to the death of TB patients are diabetes, cancer, cardiac diseases, and bacterial infections. Diabetic patients are more prone to chronic infections like TB as the immune system is compromised due to high blood glucose level.² Various studies have reported the involvement of cardiovascular abnormalities in 1%–2% of TB patients favoring atherosclerosis.³ Hepatitis C is an inflammatory liver disease caused by HCV infection which is transmitted mainly through blood transfusion. HCV co-infection with human immunodeficiency virus (HIV) in TB patients is an additive risk factor causing drug-induced hepatitis during TB drug therapy.⁴ Variation in hematological parameters in TB patients represents the dysfunctioning of hematopoietic system which might lead to compromised immunity. It is suggested that co-infection of TB patients with other infectious and metabolic abnormalities like HCV, HIV, diabetes, and myocardial infarction (MI) should be ruled out prior to the start of anti-TB drug therapy. This study was planned to investigate the TB patients co-infected with associated abnormalities and to evaluate the changes in hematological parameters of study subjects.

Materials and methods

This study was carried out on 366 patients having clinical symptoms of PTB with positive Ziehl–Neelsen (ZN) staining for acid-fast bacilli (AFB). The study subjects were grouped as PTB (positive control), healthy individual (normal control), TB patients co-infected with diabetes, HCV co-infected TB patients, HIV co-infected TB patients, TB co-infected with MI, HCV + HIV co-infected TB patients, and diabetes + HCV co-infected TB patients. Informed signed consent has been taken from all the study individuals before inclusion in this research work. The volunteers having clinical sign and symptoms of TB but negative for AFB smear microscopy through ZN Staining were excluded from the study.

The venous blood and sputum samples of each patient under study were collected from different TB hospitals of Faisalabad, Lahore, and Gojra. ZN staining and polymerase chain reaction (PCR)-based analysis was carried out in Molecular Pathology Laboratory, Department of Pathology,

University of Agriculture Faisalabad, Pakistan. The blood samples of study subjects were processed and analyzed for hematological investigations in Biomedical Research Laboratory, Biochemistry Department of University of Agriculture, Faisalabad. The study plan was approved by the research scrutiny/ethical committee of the University of Agriculture, Faisalabad. All the study subjects were screened for pulmonary TB, diabetes, HCV, myocardial infarction (MI), and HIV through sputum microscopy of ZN-stained smear, determining glucose level, HCV infection by immunochromatographic (ICT) method, Troponin-T, and HIV screening, respectively. All the standard protocols were followed during the screening procedure using the reliable, sensitive, and specific methods which are widely accepted for analytical significance.

ZN staining and direct PCR

ZN Staining for AFB sputum microscopy was performed following the method described by Ellis CR and Zabrowarny LA.⁵ Direct PCR analysis was performed on AFB smear positive sputum samples to confirm MTB infection. Sputum decontamination was performed using NaOH by the modified Petroff method,⁶ and the bacterial DNA was extracted by phenol chloroform method. The 16S ribosomal RNA (rRNA) oligonucleotide primer was used to identify *Mycobacterium* genus and MPB70 primer for MTB complex identification. Amplified DNA was run on 1% agarose gel electrophoresis, and bands were visualized under ultraviolet light transilluminator (BioRad; Gel Doc EZ Imager).

Hematological analysis

The blood samples of all the study subjects were analyzed for hematological parameters including hemoglobin (Hb) concentration, red blood cell (RBC) count, packed cell volume (PCV)/hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets count and white blood cells' (WBC) count, and the differential WBC counts were performed following the method described by Patel et al. using ADVIA-360 Automated Hematology Analyzer. Erythrocyte sedimentation rate (ESR) was determined by Westergren method based on the principle that the

rate at which red cells are settled down due to their differential densities and medium which is measured in millimeter/hour.⁷

Statistical analysis

The frequency distribution of diseases among TB comorbid cases and 95% confidence interval (95% CI) were calculated by using WinPIPE software. The obtained data of hematological parameters were analyzed by analysis of variance (ANOVA) technique for statistical significance and means were compared with Duncan New Multiple Range Test (DMRT) by using SAS statistical software. P value <0.01 was considered statistically significant.

Results

All the sputum samples ($n=366$) collected in this study were found positive by ZN staining. Among them, 108 (29.5%) were found PTB negative on PCR and 258 (70.5%) were diagnosed PTB positive on PCR-based study (Figure 1). TB alone was present in 36.8% cases, while 63.2% cases had co-morbidities. Among them, 24% were PTB co-infected with diabetes, 17.8% were co-morbid with HCV, 4.2% with HIV, 11.2% with diabetes + HCV, 2.3% with HCV + HIV, and 3.4% also had MI. Frequency percentage and 95% CI of disease distribution among PCR-positive TB patients with co-morbidities is given Table 1. The results of hematological parameters showed significant variation ($P<0.01$) among healthy control, PTB, and other co-morbid groups (Table 2). The PCV and eosinophils were found reduced, while WBC, neutrophils, and ESR showed a significant increase ($P<0.01$) in TB patients than control group. The

RBC count was decreased significantly ($P<0.01$) in diabetic + HCV co-morbid group. A significant ($P<0.01$) reduction in PCV was observed in diabetes, HCV-infected, and diabetes + hepatitis C and hepatitis C + HIV co-morbid groups. Similarly, significantly ($P<0.01$) lower concentration of Hb was found in diabetics, HCV-infected, diabetic + HCV, and HCV + HIV co-morbid groups. The platelet count was also decreased significantly ($P<0.01$) in MI and HCV + HIV co-morbid groups. The WBC count showed a significant increase ($P<0.01$) in diabetics, MI, and diabetic + HCV-infected co-morbid groups. Significant ($P<0.01$) variation in differential white blood cell count was observed in PTB and co-morbid groups. The ESR was significantly ($P<0.01$) increased in diabetics, HCV-infected, and MI- and diabetic + HCV-infected co-morbid groups.

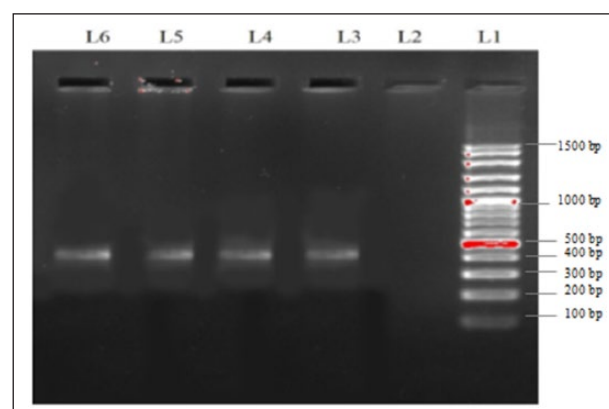


Figure 1. PCR product of MTB complex. From right to left lane 1: DNA ladder with bands at 100 bp intervals starting at 100 bp; lane 2: negative control; and lanes 3–6: positive PCR product of MTB complex.

Table 1. The chi-square test showing percentage and 95% confidence interval of disease distribution among PCR-based positive TB patients with co-morbidities.

Parameters	No. of cases (N)	Frequency percentage (%)	95% CI	
			Lower limit	Upper limit
TB alone	95	36.8	31.1	42.8
TB + diabetic	62	24.0	19.1	29.5
TB + hepatitis C	46	17.8	13.5	22.9
TB + diabetes + hepatitis C	29	11.2	7.8	15.5
TB + HIV	11	4.2	2.3	7.3
TB + hepatitis C + HIV	6	2.3	1	4.8
TB + MI	9	3.4	1.7	6.3

CI: confidence interval; PCR: polymerase chain reaction; TB: tuberculosis; MI: myocardial infarction.

Table 2. Mean and standard deviation values of hematological parameters of different study groups.

Parameters	Healthy control	TB	Diabetic	HCV +ve	HIV +ve	Myocardial infarction control	TB + diabetic	TB + HCV Co-morbid	TB + HIV Co-morbid	TB + myocardial infarction co-morbid	TB + diabetic + HCV	TB + HCV + HIV P value
RBCs ($10^6/\mu\text{L}$)	4.5 ^a ±0.606	4.4 ^a ±1.34	4.2 ^{ab} ±0.634	4.47 ^a ±0.65	3.83 ^{ab} ±0.48	4.22 ^{ab} ±0.91	3.82 ^{ab} ±0.70	3.89 ^{ab} ±1.05	4.54 ^a ±0.28	4.13 ^a ±0.36	3.43 ^b ±0.58	4.5 ^a ±0.069
PCV (%)	41.55 ^a ±3.43	36.6 ^b ±6.5	31.03 ^c ±9.23	32.77 ^{bc} ±5.67	32.81 ^{bc} ±4.55	41.8 ^a ±2.91	34.49 ^{bc} ±5.35	34.36 ^{bc} ±6.38	43.85 ^a ±3.35	43.73 ^a ±5.29	31.04 ^c ±4.65	36.9 ^b ±3.42
HB (g/dL)	13.19 ^{abc} ±1.68	12.3 ^{bcd} ±2.46	9.62 ^f ±3.11	9.99 ^{ef} ±1.84	12.7 ^{abcd} ±1.78	13.14 ^{abc} ±1.33	11.32 ^{def} ±1.86	10.58 ^{ef} ±2.21	13.42 ^{ab} ±1.39	14.2 ^a ±1.51	10.49 ^{ef} ±1.79	11.6 ^{cde} ±0.677
PLT ($10^3/\mu\text{L}$)	314.5 ^a ±61.88	303.79 ^{ab} ±85.14	242.5 ^{abcd} ±105.3	284.1 ^{abc} ±162.5	129 ^e ±1.41	220.3 ^{bcd} ±47.89	307.89 ^{ab} ±93.98	278.73 ^{abc} ±93.88	256.6 ^{abcd} ±98.46	167.33 ^{de} ±29.41	315.8 ^a ±62.80	201 ^{cde} ±119.8
WBCs ($10^3/\mu\text{L}$)	6.65 ^{ef} ±1.22	13.7 ^{ab} ±5.6	10.75 ^{bcd} ±7.9	8.08 ^{def} ±5.12	7.8 ^{def} ±2.29	11.26 ^{bcd} ±2.74	11.59 ^{bc} ±2.68	9.89 ^{cde} ±2.72	9.14 ^{cdef} ±2.38	15.91 ^a ±4.14	11.47 ^{bc} ±4.26	5.86 ^f ±3.60
Neutrophils (%)	66.75 ^{cd} ±10.84	68.92 ^c ±10.95	79.0 ^{ab} ±10.72	66.5 ^{cd} ±8.18	66.13 ^{cd} ±14.26	69 ^c ±11.12	71.87 ^{bc} ±11.19	58.69 ^{de} ±10.58	63.0 ^{cd} ±4.31	82.89 ^a ±3.37	65.60 ^{cd} ±9.73	52 ^e ±19.34
Lymphocyte (%)	27.35 ^{bc} ±9.47	26.75 ^{bc} ±10.21	15.85 ^{de} ±10.8	28.1 ^{bc} ±8.68	28.8 ^{bc} ±13.59	25.08 ^c ±10.22	25.08 ^c ±11.39	34.69 ^b ±8.23	34.45 ^b ±2.91	14.22 ^e ±5.562	22.90 ^{cd} ±7.93	43.7 ^a ±18.42
Monocyte (%)	3.3 ^{bc} ±1.52	2.63 ^{bcd} ±1.18	3.08 ^{bc} ±0.75	3.6 ^b ±1.07	3.33 ^{bc} ±2.38	3.0 ^{bc} ±1.04	2.14 ^{cd} ±1.0	4.7 ^a ±2.37	1.54 ^d ±1.64	2.88 ^{bc} ±1.26	2.18 ^d ±0.58	4.7 ^a ±2.6
Eosinophil (%)	2.6 ^{ab} ±0.82	1.44 ^{def} ±0.66	2.08 ^{bcd} ±0.76	1.8 ^{cde} ±0.79	1.73 ^{cde} ±1.22	2.58 ^{ab} ±0.9	1.54 ^{cdef} ±0.84	1.78 ^{cde} ±1.18	1.0 ^f ±1.09	2.22 ^{bc} ±1.09	1.30 ^{ef} ±0.46	3 ^a ±2.4
ESR (mm/h)	15.7 ^b ±6.32	73.9 ^a ±28.0	77.31 ^a ±37.4	63.50 ^a ±28.39	20.40 ^b ±17.5	34.0 ^b ±31.9	72.04 ^a ±32.96	63.68 ^a ±39.49	20.27 ^b ±22.41	64.44 ^a ±25.42	79.69 ^a ±36.06	34 ^b ±25.2

TB: tuberculosis; RBCs: red blood cells; PCV: packed cell volume; WBCs: white blood cells; ESR: erythrocyte sedimentation rate; HCV: hepatitis C virus. In a row, dissimilar letters in superscript show significant differences among studied group means according to Duncan New Multiple Range Test (DMRT). Statistically significant level was considered at $P < 0.01$.

Discussion

Hematological parameters play important role in treatment strategies and can influence patient's outcome. Evaluation and management of these infectious diseases with reference to hematological markers are of vital importance to improve treatment outcome, patients' survival, and quality of life. This study showed significant variations in blood parameters of studied test groups when compared with healthy controls. Singh et al.⁸ reported anemia as the most common abnormality in all types of TB. Akpan et al.⁹ reported lower HB and PCV in patients suffering from pulmonary TB. WBCs function to fight against infections, and increased count are indicator of active infection in the body. This study also showed significant variations in WBC count in different co-morbid groups. The WBC showed significant increase in diabetic + HCV-infected co-morbid group. Previous studies reported that RBCs, HB, WBCs, ESR thrombocytosis, and body weight loss are useful factors to evaluate the severity of disease in TB patients, and the return of these parameters to normal level is a good indication of disease progression and effectiveness of anti-TB treatment.¹⁰ Differential WBC count also showed significant variations in all co-morbid groups. The neutrophils showed significant decrease in HCV + HIV co-morbid group. The lymphocytes showed significant increase in HCV + HIV-infected group, while a significant decrease in MI co-morbid group. The monocytes showed significant increase in hepatitis C and hepatitis C + HIV co-morbid groups. The eosinophils showed significant decrease in diabetic + hepatitis C co-morbid groups. Shah et al.¹¹ reported neutropenia, lymphocytosis, monocytosis, and eosinophilia in HIV co-infected patients. ESR is considered as a diagnostic tool in many bacterial infections and also as an indicator of disease severity in PTB which is also evident from this study. The ESR showed significant increase in diabetic + HCV co-morbid groups. These results are in agreement with previous studies. Akram et al.¹² also reported significantly higher level of ESR in TB and TB + HCV co-morbid group compared to healthy control group.

This study concluded that significant increase in PTB patients co-infected with HCV, HIV, diabetes, and MI suggests the investigation of co-morbidities to rule out PTB co-infection with infectious and metabolic disorders before the start of anti-TB drug therapy. Significant variations in

blood parameters of PTB and TB-associated co-infected patients suggest the investigation of associated abnormalities in TB patients to rule out the co-infection prior to the start of TB treatment therapy.

Declaration of conflicting interests

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