EVALUATION OF LDH AND GAMMA INTERFERON AS BIOCHEMICAL MARKERS FOR DIAGNOSIS OF PULMONARY AND EXTRA-PULMONARY TUBERCULOSIS

SK Baul[1], SMR Hossain[2], D Parvin[3], M Hadiuzzaman[4], MS Islam[5], K Fatema[3]

[1]Dept of Biochemistry, Satkhira Medical College, Satkhira; [2]Dept of Microbiology, Sheikh Hasina Medical College, Tangail; [3]Dept of Clinical Biochemistry, NICVD, Dhaka; [4]Dept of Biochemistry, Sheikh Shayera Khatun Medical College, Gopalgon; [5]Planning and Research, DGHS, Mohakhali, Dhaka

ABSTRACT

Tuberculosis is a common disease throughout the world, especially in developing countries. The most common form of the disease is pulmonary tuberculosis (PTB). Extra pulmonary tuberculosis (EPTB) is also a growing problem worldwide. The diagnosis of PTB and EPTB is even harder when smears and cultures are negative. Due to its nature, the diversity of clinical pictures, the diagnosis of EPTB is often difficult and late. Advances in rapid diagnostic techniques are urgently required both for the early management of the new cases of TB and for the individuals already infected with *Mycobacterium tuberculosis* and are at risk of developing disease. In addition to standard TB diagnostic techniques, use of new biochemical surrogate markers like LDH and gamma interferon are new suggested. This study was undertaken to evaluate the diagnostic value and accuracy of serum LDH and gamma interferon for the diagnosis of PTB and EPTB. This cross-sectional study was carried out in the Department of Pathology and Microbiology, NIDCH, Dhaka and by convenient and purposive sampling, 131 patients (male 98, female 33) having age range of 20-90 years were enrolled for the study. Serum LDH and QuantiFERON-TB gold (QFT-G i.e. gamma interferon) were estimated and the result was compared with the culture and histopathology results. Performance tests were done. Prevalence was measured at 95% CI. Statistical significance was set at $p < 0.05$. Of 131 patients 89 (67.9%) had pulmonary tuberculosis (PTB) and the rest 42 (32.1%) had extra-pulmonary tuberculosis (EPTB). Level of LDH and gamma interferon in serum of all patients was found significantly high. TB pleural effusion was detected in 64% cases. The sensitivity, specificity, positive predictive values, negative predictive values and accuracy of LDH and gamma interferon wre calculated both in PTB and EPTB. For LDH it was (SEN 89.22%, SPE 85.66%, PPV 88.82%, NPV 86.11%) and for gamma interferon (SEN 93.01%, SPE 91.64%, PPV 93.73%, NPV 91.18%) in PTB; and for LDH (SEN 89.27%, SPE 87.18%, PPV 84.35%, NPV 90.36%) and for gamma interferon (SEN 94.88%, SPE 93.65%, PPV 90.04%, NPV 95.82%) in EPTB respectively. When calculated in combination, the sensitivity and specificity was 100% and 50% respectively in both PTB and EPTB, and positive and negative predictive values of LDH and gamma interferon in combination were 94.00% and 91.58% in PTB and 90.43% and 96.71% in EPTB respectively. The study concludes that the diagnostic accuracy of LDH and gamma interferon is low when single test is done but it is significantly high when done in combination.

Key Words: PTB, EPTB, LDH, QFT-G

Introduction

Tuberculosis is a common disease throughout the world, especially in developing countries. Millions of people die each year from tuberculosis (TB), a leading chronic infectious killer of all age groups and the second most common infectious disease worldwide. It is an infection with human strains of *Mycobacterium tuberculosis*[1,2]. The most common form of the disease, which is responsible for the spread of TB, is pulmonary tuberculosis (PTB). Tuberculosis can affect any organ in the body. Extra-pulmonary tuberculosis (EPTB) is referred to as isolated TB at any site in the body outside the lungs. Although TB can be fully cured with the use of appropriate drugs, the major hurdle to treatment for TB lies in the late diagnosis of the disease due to the lack of simple and cost-effective diagnostic procedures. Although the isolation of tuberculous bacilli from clinical specimens is the gold standard for diagnosis, chest X ray and the histopathological detection of granulomatous lesions in addition to clinical findings generally lead to true diagnosis. The diagnosis of PTB and EPTB is even harder when smears and cultures are negative. Extra pulmonary tuberculosis (EPTB) is a growing problem worldwide[1-3] and due to the nature of the disease, diversity of clinical pictures as well as its minor epidemiological importance, the diagnosis is often difficult and late. Definitive diagnosis of tuberculosis includes a demonstration of the presence of M. tuberculosis by microbiological culture and histopathological methods. Classical methods of TB diagnostics have significant limitations for diagnosis of EPTB. Culture is a lengthy process, while these patients often requires rapid diagnosis. Material for PCR diagnosis is often not possible to obtain (except cerebrospinal fluid and urine), and histopathological diagnosis requires surgical procedure, which is an invasive technique. The most reliable diagnostic criteria, is still to confirm the presence of bacilli in the patient's material by culture. In some forms of EPTB, material can be taken for culture from renal, meningeal, pleural, and pericardial fluid. Pleural effusion is a common reason for admission in hospitals and tuberculosis is found most common infectious cause. Pleural tissue histopathology and culture for Mycobacterium tuberculosis is considered the gold standard but the procedure is invasive, requires skilled clinicians and does not provide immediate results[2,4-9]. Advances in rapid diagnostic techniques are urgently required both for the early management of the new cases of TB and for the individuals already infected with Mycobacterium tuberculosis who are at risk of developing disease. In addition to standard TB diagnostic techniques, use of new biochemical surrogate markers (ADA, LDH, gamma interferon) are increased. An immunological test depending on the measurement of levels of gamma-interferon, the QuantiFERON-TB gold (QFT-G), specific to M. tuberculosis antigen produced by T cells have been developed in recent years. It is somewhat faster and more reliable test for diagnosing latent and active tuberculosis. Since there is no single test to diagnose M. tuberculosis infection, invasive procedures are needed in such cases, and diagnosis is usually delayed[8,10-23]. Therefore, to address these limitations, we evaluated lactate dehydrogenase (LDH) and gamma interferon in serum and to find out the combined efficacy of these two biochemical markers for rapid diagnosis of PTB and EPTB.

Materials and Methods

This hospital based cross-sectional study was conducted in National Institute of Diseases of the Chest and Hospital (NIDCH), Mohakhali, Dhaka at Pathology and Microbiology Department during the period of July 2014 to December 2014. After getting the approval of the research proposal from the ethical committee of the Institution and BMRC, consent was taken from each individual prior to inclusion. From selected patients, after aseptic precaution 5 ml of whole venous blood from antecubital vein was collected in clean and dry test tube and was kept standing for clotting. Then it

was centrifuged at 3000 rpm to separate serum. Three samples of adequate amount of sputum were collected in a dry clean plastic cup from all the patients. The subjects were patients from OPD and indoor of Institute of Diseases of the Chest and Hospital (NIDCH), who were clinically suspected TB patients, hospitalized for investigation for fever of unknown origin. In addition to standard examinations, X- ray of the lungs, USG of abdomen, hematological and biochemical tests, abdomen, chest or pelvic CT scan, echocardiography was done depending on the presentation of illness. The histological processing of tissues of clinical interest (biopsy of the liver, lymph nodes, peritoneum, small bowel and adnexa) were also done. Histological processing of tissue samples was performed at the Pathology department of the institute. LDH and Gamma interferon (using QFT-G) in serum of all patients were measured by using standard procedure and method. After collection, all the data was checked and edited. Then data was entered into computer with the help of software SPSS for windows version 12. After frequency run, data was cleaned and frequencies were checked. An analysis plan was developed keeping in view with the objectives of the study (CI 95%, p<0.05). Due to the heterogeneity of data, although the observed parameters were continuous, we used non-parametric tests (χ^2 test). Sensitivity and specificity of test results were calculated.

Results

For this study 208 clinically suspected tuberculosis (pulmonary and extra-pulmonary) patients were enrolled. Among them 131 patients (male 98, female 33) were proved to have tuberculosis by any one or in combination of sputum smear (Z-N or Auramine stain), sputum or tissue culture and histopathological examination. Age of the study subjects was between 20 to 90 years. Mean($\pm$SD) age of study subjects was 44.65$\pm$17.84 years, that in male and female were 44.66$\pm$17.94 and 44.61$\pm$17.77 years.

Distribution of male and female is shown in Figure-1.
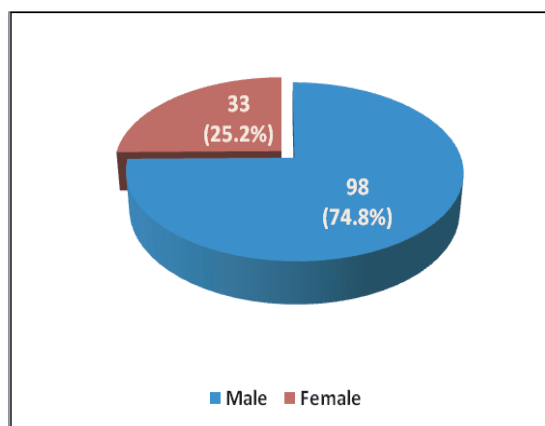
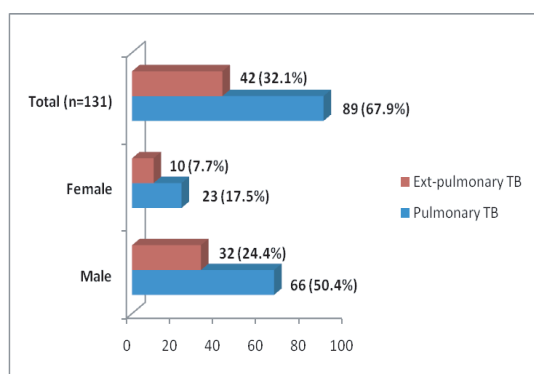**Figure-1:** Sex distribution of the study subjects.

Table-I: Demographic Profile of the study Subjects

		Male		**Female**	
		n=98	(74.8%)	n= 33	(25.2%)
*Income	Low	59	(74.8%)	23	69.7%
	Medium	31	60.2%	10	30.3%
	High	08	31.6%	00	00.0%
Education	Primary	68	08.2%	24	72.7%
	SSC	22	69.4%	07	21.2%
	HSC	05	22.4%	02	06.1%
	Above	03	05.1%	00	00.0%
Smoking Habit	Smoker	87	03.1%	04	12.1%
	Non-smoker	11	88.8%	29	87.9%
Residence	Kancha	57	58.2%	24	72.7%
	Semipacca	38	38.7%	09	27.3%
	Pacca	02	03.1%	00	00.0%

*Income (BD Tk./month): Low<10,000/-; Medium> 10,000/- to <30,000/-; High>30,000/-.

Demographic Profile of the study subjects are shown in Table-I. High frequency was found in low income, low education, smokers and dwellers of kancha residence among both male and female.

Figure-2: Disease distribution of the study subjects.

Eighty-nine (67.9%) of total 131 patients had pulmonary TB and of them 66 (50.4%) was male, 23 (17.5%) was female. Other patients had extra-pulmonary TB. Among the pulmonary TB positive patients 74.2% was male and 25.8% was female whereas that of extra-pulmonary TB it was 76.2% and 23.8% respectively. No significant difference between types of diseases found between male and female (Figure-2 and 3).

**Table-II:** Results of Biochemical markers in Pulmonary TB & Extra-Pulmonary TB

	Pulmonary TB (n=89) number (%)	Extra-Pulmonary TB(n=42) number (%)	Total(n=131) number (%)
S. QFT-G			
Positive	73(82.0)	33(78.6)	106 (80.9)
Negative	16(18.0)	09(21.4)	25(19.1)
S. LDH			
Positive	66 (74.2)	30 (71.4)	96(73.3)
Negative	23 (25.8)	12 (28.6)	35(26.7)
Both S. QFT-G & S. LDH			
Positive	88(98.9)	40(95.2)	128(97.7)
Negative	01(01.1)	02(04.8)	03(02.3)

Table-II shows the results of individual serum Quanti FeronTB-Gold (QFT-G), serum lactate dehydrogenase (LDH) and combined result of all the two biomarkers. When combined, the biomarkers show a high number of positive results, 128(97.7%) out of 131.

Table-III: SEN, SPE, PPV, NPV & Accuracy of the Biochemical markers

Biochemical markers	Pulmonary TB					Extra-Pulmonary TB				
	SEN	SPE	PPV	NPV	Acc	SEN	SPE	PPV	NPV	Acc
S. LDH	89.2	85.7	88.8	86.1	78.7	89.3	87.2	84.4	90.4	73.8
S. γ-Interferon (QFT-G)	93.0	91.6	93.7	91.2	74.2	94.9	93.7	90.0	95.8	71.4
Both S. QFT-G & S. LDH	100.0	50.0	98.9	100.0	98.9	100.0	50.0	95.0	100.0	97.7

All results are in %. SEN (Sensitivity), SPE (Specificity), PPV (Positive predictive value), NPV (Negative predictive value), Acc (Accuracy)
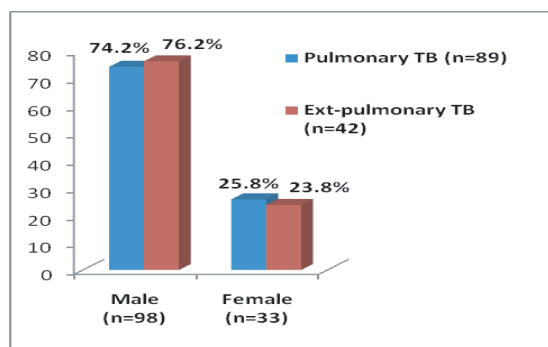
Figure-3: Distribution of Study Subjects according to disease conditions

Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of serum Quanti FeronTB-Gold (QFT-G), serum lactate dehydrogenase (LDH) and those of combined of all the two biomarkers have been shown in Table-III. Each biomarker individually and all in combination showed good sensitivity and specificity.

Discussion

This hospital based cross-sectional study was conducted enrolling 208 clinically suspected tuberculosis (pulmonary and extra-pulmonary)

patients of 20-90 years of age, among them 131 patients were proved to have tuberculosis by any one or in combination of sputum smear (Z-N or Auramine stain), sputum or tissue culture and histopathological examination. In our study, like other several previous studies, demographic profile of the diseased persons and the prevalence of the disease in male and female were consistent[1-9]. Definitive diagnosis of tuberculosis includes a demonstration of the presence of M. tuberculosis by microbiological, cytological or histopathological methods. But, classical methods of TB diagnostics have significant limitations for diagnosis of EPTB. Cultivation is to long, while these patients often requires rapid diagnosis. Material for PCR diagnosis is often not possible to collect and histopathological confirmation requires biopsies, which are invasive. The most reliable diagnostic criteria, is still to confirm the presence of bacilli in the patient material by cultivation[9,10-18]. In some forms of EPTB material can be taken for culture but, unfortunately, successful culture is very variable[19-23]. Because of these problems in exact diagnosis of tuberculosis, numerous additional tests are in use with the intention to facilitate the diagnosis. In the recent years, biochemical markers for tuberculosis infection, such as levels of serum LDH or the level of interferon gamma is found to be much diagnostic importance. Serum LDH still remains as one of the important parameters to assess the cell/tissue specific stress or pathology, caused by Mycobacterium tuberculosis[24].

In our study, we also found higher concentration of LDH in the serum of both PTB and EPTB patients. Our finding is consistent with that of Sharma et al.[24]. Some studies were done to assess the usefulness of gamma interferon estimation for diagnosis and management of immunocompromised individuals, in children and in those with extrapulmonary TB[25]. Our study also demonstrated the strong evidence of

QFT-G to diagnose both PTB and EPTB. Performance test of each biomarker was done to see the SEN, SPE, PPV and NPV to evaluate the efficacy of the biomarkers.

In conclusion, the diagnostic accuracy of LDH and gamma interferon may be low when single test is done. But, when they are used in combination, it is significantly high. Biochemical tests mentioned above may be useful adjunct tests for diagnosing TB and EPTB for early and appropriate management to reduce morbidity and mortality. Larger studies are needed in our country to determine more accurately the diagnostic value of new biomarkers, for PTB and EPTB.

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