RESULTS

This study have been conducted on 96 patients with presenting symptoms suggestive of pulmonary tuberculosis such as persistent cough with purulent or mucoid sputum, hemoptysis, chest pain, low grade fever, sweating and loss of weight. There is no history of previous receiving specific treatment for tuberculosis.

Sputum specimens were collected from patients for detection of tubercle bacilli using Ziehl-Neelsen stain and cultivation on L.J medium. Out of 96 sputum samples 36 were positive on culture on L.J. medium, 20 of them were positive by direct Z.N stain and 26 were positive by Z.N staining after processing.

Thirty two isolates out of 36 positive mycobacterial cases were biochemically typed as *M. tuberculosis* whereas the other 4 isolates were MOTT.

I. Results of demographic and clinical data:

- The age of patients under study ranged from 14 to 63 years old. There were 11 cases in age group 14-20 years, 55 cases in age group 21-40 years and 30 cases in age group 41-63 years. In tuberculous group, 2 (6.3%) out of 32 patients were within the 14-20 years group, 21 (65.6%) within the 21-40 group and 9 (28.1%) within the 41-63 years group (Table 1).

As regards sex distribution in this study it was found that out of 96 studied cases, 67 patients were males and 29 were females. 24 (75%) out of 32 tuberculous patients were males and 8 (25%) were females, whereas

43 (67.2%) out of 64 non tuberculous patients were males and 21 (32.8%) were females (Table 1).

- As regards the effect of occupation, residence and smoking on distribution of tuberculous infection table (2) showed that: out of 96 studied cases 53 (55.2%), 16 (16.7%) and 27 (28.1%) were workers, employers and housewives respectively.

Out of 32 tuberculous patients, 19 (59.3%) were workers and 5 (15.6%) were employers while housewives were 8 (25%) cases. Out of 64 non tuberculous patients, 34 (53.1%) were workers and 11 (17.2%) were employers while housewives were 19 (29.7%) cases.

Out of 96 studied cases, 62 (64.6%) were from rural areas while 34 (35.4%) were from urban areas. Out of 32 tuberculous patients, 24 (75%) were from rural areas and 8 (25%) from urban areas. Out of 64 non tuberculous patients, 38 (59.1%) were from rural areas and 26 (40.6%) from urban areas.

41 (42.7%) out of 96 cases under this study were smokers and 55 (57.3%) were non smokers. 19 (59.4%) out of 32 tuberculous patients and 22 (34.4%) out of 64 non tuberculous patients were smokers.

Table (1): Age and sex distribution among 96 suspected tuberculous cases.

	Suspected tuberculous cases (96)					Significance test
	No of cases (96)	**Positive T.B** (N = 32)		**Negative T.B** (N = 64)		
		No	%	No	%	
Age in years						$\chi^2 = 1.87$ P = 0.39 NS
14 – 20	11	2	6.25%	9	14.06%	
21 – 40	55	21	65.62%	34	53.12%	
41 – 63	30	9	28.12%	21	32.81%	
Sex						$\chi^2 = 0.62$ P = 0.43 NS
Male	67	24	75%	43	67.18%	
Female	29	8	25%	21	32.81%	

NS = Non significant

- There is insignificant statistical difference as regards the age and sex distribution between positive and negative tuberculous cases.

Table (2): Effect of occupation, residence and smoking in distribution of tuberculous infection.

	Suspected tuberculous cases (96)					Significance test
	No of cases (96)	Positive T.B (N = 32)		Negative T.B (N = 64)		
		No	%	No	%	
Occupation:						$\chi^2 = 0.35$ P = 0.84 NS
Workers	53(55.2%)	19	59.3%	34	53.12%	
Employers	16(16.7%)	5	15.63%	11	17.19%	
Housewives	27(28.1%)	8	25%	19	29.69%	
Residence:						$\chi^2 = 2.28$ P = 0.13 NS
Rural	62(64.6%)	24	75%	38	59.12%	
Urban	34(35.4%)	8	25%	26	40.62%	
Smoking:						$\chi^2 = 5.45$ P = 0.02 S
+ve	41(42.7%)	19	59.4%	22	34.4%	
-ve	55(57.3%)	13	40.6%	42	65.6%	

S = significant

- There is insignificant statistical difference regarding the role of occupation and residence in distribution of tuberculous infection.
- There is a significant statistical role of smoking in distribution of tuberculous infection.

II. Results of identification of organisms:

- In this study 20 tuberculous cases out of 36 mycobacterial cases were positive for direct Z.N stain and culture on L.J medium. The sensitivity and specificity of direct Z.N were 55.6% and 100% respectively (Table 3).

- Stained smears after concentration revealed positive results in 26 patients who were also positive by L.J culture. The sensitivity and specificity of indirect Z.N stain were 72.2% and 100% respectively (Table 4).

- Direct smear showed 20 positive and 76 negative smear results while stained smears after concentration revealed 26 positive and 70 negative results (Table 5).

- Out of 36 mycobacterial isolates, 32 (88.8%) isolates were biochemically typed as being *M. tuberculosis* whereas the other 4 (11.2%) were revealed to be MOTT (Table 6).

Table (3): Results of direct Z.N stain versus L.J culture for detecting acid fast bacilli in specimens.

Direct Z.N	L.J culture		No of cases
	+ ve	- ve	
+ ve	20	-	20
- ve	16	60	76
Total	36	60	96

For direct Z.N stain:
Sensitivity = 55.6%
Specificity = 100%
Positive Predictive value = 100%
Negative predictive value = 78.9%
Accuracy = 83.3%

Table (4): Results of Z.N stain after concentration (indirect Z.N) versus L.J culture for detecting acid fast bacilli in specimens.

Indirect Z.N	L.J culture		Total
	+ ve	- ve	
+ ve	26	-	26
- ve	10	60	70
Total	36	60	96

For indirect Z.N stain:
Sensitivity = 72.2%
Specificity = 100%
Positive Predictive value = 100%
Negative predictive value = 85.7%
Accuracy = 89.58%

Table (5): Results of direct Z.N stain versus indirect Z.N stain.

Direct Z.N	**Indirect Z.N**		**Total**
	+ ve	- ve	
+ ve	17	3	20
- ve	9	67	76
Total	26	70	96

Sensitivity = 65.4%
Specificity = 95.7%
Positive Predictive value = 85%
Negative predictive value = 88.2%
Accuracy = 89.58%

Table (6): Results of biochemical reactions of mycobacterial isolates.

Culture on L.J	***M.TB***	***MOTT***
N = 36 cases	32	4
100%	88.8%	11.2%

- *M.TB* = Mycobacterium tuberculosis.
- *MOTT* = Mycobacterium other than tuberculosis.

III. Results of antituberculous susceptibility testing:

- The antituberculous susceptibility testing of 32 *M. tuberculosis* isolates revealed that 17 (53.2%) isolates were sensitive to INH and RIF whereas 15 (46.8%) isolates were resistant to one or both of them (Table 7).

- Resistance pattern of 15 *M.tuberculosis* isolates to INH & RIF showed that 13 isolates were single drug resistant distributed as follows 7 (21.8%) isolates were resistant to INH and 6 isolates (18.7%) were resistant to RIF. Multidrug resistant isolates (resistant to both INH and RIF) were 2 (6.2%) (Table 8).

- According to the severity of tuberculosis, the primary drug resistance was the highest in cases with far advanced lesions compared to cases with moderate and minimal lesions. Out of 15 resistant MTB isolates, 9 (60%) had advanced lesions, 4 (26.7%) moderate and 2 (13.3%) with minimal lesions.

Higher incidence of resistance to INH 4 cases (57.1%), RIF 3 cases (50%) and MDR 2 cases (100%) was observed in advanced cases (Table 9).

- Clinical, demographic data and sensitivity test results of 32 tuberculous cases are represented in Table (10). Out of 24 male patients, 13 (54.2%) were harboring sensitive strains while 11 (45.8%) were harboring resistant strains. Out of 8 female patients, 4 (50%) were harboring sensitive strains and the other 4 (50%) were harboring resistant strains.

Twenty six patients were below 45 years old. 14 cases (53.8%) were harboring sensitive strains while 12 (46.2%) were harboring resistant strains. Among 6 tuberculous patients in the age group ≥ 45 years, 3 (50%) cases were sensitive to antituberculous drugs (Table 10).

Table (10) showed that, 8 (80%) out of 10 cases had cavitary lesions were harboring resistant strains while 2 cases (20%) were harboring sensitive strains.

- Table (11) represents a comparison among risk factors of tuberculous patients and their antituberculous drug sensitivity results.

Out of 14 tuberculous patients with history of contact with tuberculous case, 10 patients (71.4%) had resistant strains while the other 4 (28.6%) had sensitive strains.

Three (50%) out of 6 tuberculous cases with history of underlying disease had sensitive strains.

Eleven out of 19 tuberculous smokers (57.9%) were harboring sensitive strains while 8 (42.1%) had resistant ones.

One (50%) out of 2 tuberculous cases had a history of chronic intake of corticosteroid was harboring sensitive strains.

Table (7): Results of antituberculous susceptibility testing of *M.TB* isolates to INH & RIF.

No of *M.TB* isolates	**Sensitive**	**Resistant**
32	17	15
100%	53.2%	46.8%

Table (8): Pattern of resistant strains among 32 *M.TB* isolates.

Drug	**Resistant cases (15)**	**%**
INH	7	21.8%
RIF	6	18.7%
MDR (INH & RIF)	2	6.2%

INH = Isoniazid.
RIF = Rifampicin.
MDR = Multi drug resistant.

Table (9): Relation between the drug resistance pattern of *M.TB* isolates and severity of disease.

Drug	**Clinical Condition**						**Total No of cases**
	Minimal N = 2(13.3%)		**Moderate** N = 4(26.7%)		**Advanced** N = 9(60%)		
	Resistant		Resistant		Resistant		
	No	%	No	%	No	%	
INH	1	14.3%	2	28.6%	4	57.14%	7 (100%)
RIF	1	16.7%	2	33.33%	3	50%	6 (100%)
MDR	-	-	-	-	2	100%	2 (100%)

Table (10): Relation between the clinical & demographic data of tuberculous patients and antitberculous drug sensitivity tests of isolated organisms.

	Susceptibility results				**Total No of cases**		**P value**
	Sensitive (17)		Resistant (15)				
	No	%	No	%	No	%	
Sex:							
Male	13	54.2%	11	45.8%	24	100%	1.00 NS
Female	4	50%	4	50%	8	100%	
Age in years:							
< 45	14	53.8%	12	46.2%	26	100%	1.00 NS
≥ 45	3	50%	3	50%	6	100%	
Cavitary lesions:							
-ve	15	68.2%	7	31.8%	22	100%	0.021 S
+ve	2	20%	8	80%	10	100%	

- There are insignificant statistical differences in the relation between sex and age with the sensitivity test results.
- There is a significant statistical difference as regards the presence of cavitary lesions and sensitivity test results.

Table (11): Relation of risk factors of 32 tuberculous patients with the antituberculous sensitivity tests of isolated organisms.

	***M.TB* isolates (N = 32)**				**Total No of cases**		**Significance test**
	Sensitive (17)		Resistant (15)				
	No	%	No	%	No	%	
History of contact with tuberculous cases:							$\chi^2 = 6.026$
+ve	4	28.6%	10	71.4%	14	100%	P = 0.014
-ve	13	72.2%	5	27.8%	18	100%	S
History of underlying disease:							FET, P = 1.0
+ve	3	50%	3	50%	6	100%	NS
-ve	14	53.8%	12	46.2%	26	100%	
Smoking:							FET,
+ve	11	57.9%	8	42.1%	19	100%	P = 0.513
-ve	6	46.2%	7	53.8%	13	100%	NS
Chronic intake of corticosteroids:							FET, P = 1.0
+ve	1	50%	1	50%	2	100%	NS
-ve	16	53.3%	14	45.6%	30	100%	

FET = Fisher's Exact Test.

- There is a significant statistical value between the history of contact with tuberculous cases and resistance of organisms to antituberculous drugs.
- There are insignificant statistical differences regarding history of underlying disease, smoking and chronic intake of corticosteroids and resistance of organisms to antituberculous drugs.

IV. Results of apoptosis:

- Tables (12a, 12b and 12c) & Figures (1 & 2) showed the comparison between 10 control subjects and 14 sensitive cases (3 susceptible cases were excluded) regarding the assessment of *M.TB* induced apoptosis in lymphocytes and IL-2 level in culture supernatant. Before treatment of susceptible cases, the mean percentage of apoptotic lymphocytes by Giemsa and acridine orange stains were 58.2 ± 7.1 and 51.4 ± 16.7 respectively while the mean percentage of apoptotic lymphocytes for control subjects was 25.9 ± 4.04. However, the mean level of IL-2 in susceptible cases was 60.4 ± 18.02 while its mean level for control group was 175.7 ± 27.9.

There is a highly significant statistical difference regarding lymphocyte apoptosis (detected by Giemsa and acridine orange stains) and the level of IL-2 between control group and 14 tuberculous cases sensitive to antituberuclous drugs before treatment.

There are insignificant statistical differences regarding lymphocyte apoptosis (detected by Giemsa and acridine orange stains) and the level of IL-2 between control group and 14 tuberculous cases sensitive to antituberuclous drugs after treatment.

Table (12a): Mean percentage of apoptotic lymphocytes (Giemsa stain) for control group and 14 tuberculous cases sensitive to antituberculous drugs.

Giemsa	Control (N = 10)	Cases (N = 14)	Significance test	
			t	P
Before treatment				
Mean ± SD	25.9 ± 4.04	58.2 ± 17.1	6.81	< 0.001 (HS)
After treatment				
Mean ± SD	---	27.28 ± 6.06	0.69	0.53 (NS)

HS = high significant.

- There is a highly significant statistical difference regarding lymphocyte apoptosis (detected by Giemsa) between control group and 14 tuberculous cases sensitive to antituberuclous drugs before treatment.
- There is insignificant statistical difference regarding lymphocyte apoptosis (detected by Giemsa) between control group and 14 tuberculous cases sensitive to antituberuclous drugs after treatment.

Table (12b): Mean percentage of apoptotic lymphocytes (Acridine orange) for control group and 14 tuberculous cases sensitive to antituberculous drugs.

Acridine orange	Control (N = 10)	Cases (N = 14)	Significance test	
			t	P
Before treatment				
Mean ± SD	20.6 ± 5.19	51.42 ± 16.71	5.65	< 0.001 (HS)
After treatment				
Mean ± SD	---	25.0 ± 7.71	1.43	0.167 (NS)

- There is a highly significant statistical difference regarding lymphocyte apoptosis (detected by acridine orange stains) between control group and 14 tuberculous cases sensitive to antituberuclous drugs before treatment.
- There is insignificant statistical difference regarding lymphocyte apoptosis (detected by acridine orange stains) between control group and 14 tuberculous cases sensitive to antituberuclous drugs after treatment.

Table (12c): Mean level of Interleukin-2 in control group and 14 tuberculous cases sensitive to antituberculous drugs.

IL-2	Control (N = 10)	Cases (N = 14)	Significance test	
			t	P
Before treatment				
Mean ± SD	175.7 ± 27.9	60.35 ± 18.02	12.33	< 0.001 (HS)
After treatment				
Mean ± SD	---	163.71 ± 20.55	1.21	0.23 (NS)

- There is a highly significant statistical difference regarding the level of IL-2 between control group and 14 tuberculous cases sensitive to antituberuclous drugs before treatment.
- There is insignificant statistical difference regarding the level of IL-2 between control group and 14 tuberculous cases sensitive to antituberuclous drugs after treatment.

The 14 tuberculous cases sensitive to antituberculous drugs were classified according to the severity of disease, into mild group (4 patients had minimal lesions) and advanced group (3 had moderate advanced and 7 had far advanced lesions).

- Table (13) & Figures (3 and 4) represent the comparison between the control group and mild tuberculous group as regards the mean percentage of apoptotic lymphocytes and IL-2 level before and after treatment. The mean percentage of apoptotic lymphocytes detected by Giemsa stain for control group was 25.9 ± 4.04 while in mild tuberculous group it was 33.5 ± 2.4 before treatment and 24.5 ± 6.1 after treatment. By acridine orange stain detection, the mean percentage of apoptotic lymphocytes in control group was 20.6 ± 5.2 while in mild tuberculous group it was 28.3 ± 2.4 before treatment and 17.5 ± 5.97 after treatment. The mean level of IL-2 in control group was 175.7 ± 27.9 while it was 80.9 ± 20.1 in mild tuberculous group before treatment and 173.8 ± 13.8 after treatment.

- In advanced tuberculous group, the mean percentage of apoptotic lymphocytes detected by Giemsa stain was 68.1 ± 6.4 before treatment and 28.6 ± 5.8 after treatment compared to that of control group 25.9 ± 4.04, by acridine orange stain it was 60.7 ± 7.8 before treatment and 32.0 ± 2.7 after treatment compared to that of control group 20.6 ± 5.2. The IL-2 level in advanced tuberculous group was 52.5 ± 9.8 before treatment

and 159.7 ± 22.0 after treatment while it was 175.7 ± 27.9 in control group (Table 14 & Figures 5 and 6).

The percentage of apoptotic lymphocytes from patients with minimal TB (mild group) was significant lower than in lymphocytes of patients with advanced TB (advanced group).

- Morphological assessment of apoptosis by light microscope (Giemsa stain) and flourescnece microscope (acridine orange stain) was confirmed in 14 tuberculous cases by agarose gel electrophoresis DNA fragmentation with characteristic pattern of internucleosomal ladder. There was increase of DNA fragmentation (an indicative of apoptosis) in tuberculous patients lymphocytes compared to that of control before treatment. DNA fragmentation in the internucleosomal pattern reduced when restudied after treatment of tuberculous patients (Fig 13).

- In mild group, the mean percentage of apoptotic lymphocytes by Giemsa and acridine orange stains were 33.5 ± 2.4 and 28.3 ± 2.4 respectively before treatment while their mean percentage after treatment were 24.0 ± 6.1 and 17.5 ± 5.9 respectively. The mean level of IL-2 before treatment was 80.9 ± 20.1 but after treatment it was 173.8 ± 13.8 (Table 15).

- In advanced group, the mean percentage of apoptotic lymphocytes by Giemsa stain before treatment was 68.1 ± 6.4 but after treatment it was 28.6 ± 5.8. The mean percentage of apoptotic lymphocytes by acridine orange before treatment was

60.7 ± 7.8 but after treatment it was 32 ± 2.8. The mean level of IL-2 was 52.5 ± 9.8 before treatment while it was 159.7 ± 22.0 after treatment (Table 16).

Table (15): Mean percentage of apoptotic lymphocytes and IL-2 level in mild group (N = 4) before and after successful chemotherapy for *M.tuberculosis.*

	Before treatment Mean ± SD	**After treatment** Mean ± SD	**Significance test**	
			t	**P**
Lymphocyte apoptosis:				
Giemsa	33.5 ± 2.35	24.0 ± 6.055	3.61	0.016 (S)
Acridine orange	28.25 ± 2.36	17.5 ± 5.97	3.2	0.017 (S)
IL-2 level	80.9 ± 20.1	173.75 ± 13.76	6.06	< 0.001 (HS)

- There is a significant statistical value in lymphocyte apoptosis in mild group before and after treatment.
- There is a highly significant statistical value in IL-2 level before and after treatment.

Table (16): Mean percentage of apoptotic lymphocytes and IL-2 level in advanced group (N = 10) before and after successful chemotherapy for *M.tuberculosis.*

	Before treatment Mean ± SD	**After treatment** Mean ± SD	**Significance test**	
			t	**P**
Lymphocyte apoptosis:				
Giemsa	68.1 ± 6.38	28.6 ± 5.83	17.1	< 0.001 (HS)
Acridine orange	60.73 ± 7.84	32 ± 2.78	10.19	< 0.001 (HS)
IL-2 level	52.5 ± 9.78	159.7 ± 22.005	16.89	< 0.001 (HS)

- There is a highly significant statistical value in lymphocyte apoptosis and IL-2 level of advanced group before and after treatment.

Figure (7): Normal lymphocytes stained by Giemsa stain and examined by light microscope from control subject.

Figure (8): Apoptotic lymphocytes stained by Giemsa stain from advanced tuberculous case before treatment.

Morphological changes of apoptosis in lymphocytes:

a- Cell shrinkage and decrease cell volume.
b- Eccentric nucleus.
c- Vaculated cytoplasm.
d- Budding cells.
e- Apoptotic bodies.

Figure (9): Apoptotic lymphocytes stained by Giemsa stain from advanced tuberculous case after treatment.

Little number of apoptotic bodies show that apoptosis decreased after treatment (arrows refer to apoptotic bodies).

Figure (10): Normal lymphocytes stained by acridine orange stain and examined by fluorescent microscope from control subject.

Figure (11): Apoptotic lymphocytes stained by acridine orange stain from advanced tuberculous case before treatment.

Apoptotic cells have bright fluorescent, condensed nuclei and many apoptotic bodies are seen.

Figure (12): Apoptotic lymphocytes stained by acridine orange stain from advanced tuberculous case after treatment.

Little number of apoptotic bodies show that apoptosis decreased after treatment.

Figure (13): Assessment of apoptosis by using DNA electrophoresis.

Lanes 1 and 2 show DNA electrophoresis of apoptotic lymphocytes from advanced tuberculous cases before treatment demonstrating multiple DNA fragmentation with more prominent banding pattern.
Lanes 3 and 4 show DNA electrophoresis of apoptotic lymphocytes from advanced tuberculous cases after treatment demonstrating a very faint apoptotic ladder.
Lanes 5 and 6 show DNA electrophoresis of lymphocytes from control subject showing no bands.