RESULTS

RESULTS

This study was carried out on 50 pulmonary tuberculous patients, 20 suspected pulmonary tubercu lous patients and 20 apparantly healthy control subjects.

(A) Clinical findings

1. Age & Sex distribution:

Tables (1) a,b,c, show age and sex distribution among the studied groups of proved, suspected and healthy control subjects. The most frequent age group in these study was that of 20-less than 30 years representing 56%, 40% & 30% respectively. The next frequnet age group was that of 30-less than 40 as it represented 32%, 30% & 30% of different investigated groups respectively. Concerning sex distribution among the studied groups as shown in the same tables, it was 34 males (68%) and 16 females (32%) in table (1-a), 14 males (70%) and 6 females (30%) in table (1-b) while in healthy control group table (1-c) the number of males was 13 (65%) & that of females was 7 (35%).

The cases represented a mere sample of pulmonary tuberculous Egyptian patients admitted in the chest department of Tanta TB centre between July 1991, and August 1992.

The cases under study included hospitalized and non hospitalized patients. The controls were apparantly healthy persons. They were comparable in age and number with the patients group.

Among the studied groups. the numnber of smokers was 27 (54%) in proved pulmonary tuberculous patients and in a healthy control group, only 5 smokers (25%) were recorded.

2. Presenting symptoms.

Table (2) Shows the main clinical findings among the proved and suspected pulmonary tuberculous groups from which we can show that cough was the most frequent symptom as it was a complaint of 49(98%) of proved pulmonary tuberculous patients of which 40(80%) was productive cough and 9(18%) was dry cough while in suspected pulmonary tuberculous patients, cough was complaint of 17(85%) of which 13 (65%) was productive cough and 4(20%) was dry cough.

Loss of appetite was a complaint of 48 (96%) of proved tuberculous patients, on the other hand 16(80%) of suspected pulmonary tuberculous group complained from loss of appetite. loss of weight was observed in 46 (92%) of the proved pulmonary tuberculous patients while 17 (85%) of suspected tuberculous group complained of loss of weight. Night fever was present in 44 (88%) and 18 (90%) of proved and suspected pulmonary tuberculous patients respectively. Night sweating was a symptom of 42 (84%) of proved pulmonary tuberculous group and 16(80%) of suspected pulmonary tuberculous group. Dyspnea after exertion was the complaint of 22(44%) and 8(40%) of proved and suspected pulmonary tuberculous groups respectively. Haemoptysis was the least frequent complaint as it

was the complaint of 14(28%) and 7 (35%) of proved and suspected pulmonary tuberculous groups respectively.

Among the proved pulmonary tuberculous group, there were 6 out of 50 patients (12%) suffering from uncontrolled diabetes melitus beside 2 out of 20 patients (10%) suffering from the same disease in suspected pulmonary tuberculous group.

(B) Laboratory investigations: (1) Tuberculin tests:

Table (3) Shows the results of tuberculin test among the studied groups from which, it is to be noticed that among the healthy control group, the number of positive tuberculin reactors was 6 out of 20 subjects (30%) with an average diameter of induration about 12mm. the rest "14" subjects (70%) were negative tuberculin reactors.Among the proved plumonary tuberculous group, the positive tuberculin reactors were "35" out of "50" patients (70%) with average diameter of induration about 15.0 mm. Concerning the suspected pulmonary tuberculous group, this table shows that 13 patients out of 20 (65%) were tuberculin positive reactors with average diameter of induration about 14.0 mm. The same table shows that there is no significant difference between positive tuberculin test in proved and suspected pulmonary tuberculosis.

(2) Elisa test:

The mean optical density (OD) of positive tuberculin reactors in a healthy group was 0.184, while the mean OD of negative tubercutin reactors in the same group was 0.134, so the mean OD of all control subjects (positive & negative

tuberculin reactors) was 0.159. The cut off value was determined by calculating the mean OD of control subjects plus 0.050. So the cut off value was 0.209. so, the mean OD of two wells measurement was considered positive for ELISA, IgG specific to P.P.D. at a concentration of 40 µg per well when tested at a serum dilution 1:1000,if it was equal to or more than the cut off value of control subjects (0.209). The mean O.D. of substrate and conjugate controls were 0.120 and 0.122 respectively. There is no significant difference between the results of optical density (O.D) values in relation to sex among males and females included in the different studed groups in this study.

Table (4) Shows the results of ELISA mean optical density (OD) values of IgG antibody specific to purified protein derivatives (P.P.D) antigen in different studied groups from which, it was found that the PPD specific IgG antibody was positive (optical density greater than the cut off value, 0.209. which has been calculated as mentioned before)in serum of 45 out of 50 proved tuberculous patients (90%). The positive results among 20 healthy control subjects was only 1 (5%). Thus the sensitivity was 90% and the specificty was 95% while the positive results among the suspected pulmonary tuberculous patients was 11 out of 20 (55%) from the same table, it has to be noticed that there is a significant association between results

of ELISA test in suspected and proved pulmonary tuberculous groups.

Analysis of ELISA results.

Table (5) Shows the analysis of optical density values of IgG antibody specific to P.P.D. antigen at a concentration of 40 µg per well when tested at a serum dilution 1:1000 between proved pulmonary tuberculous patients group and healthy control subject group it illustrates that there is increase in O.D. values of IgG specific PPD of the proved pulmonary tuberculous patients group (0.374 ± 0.062) when compared to healthy control subjects group (0.159 ± 0.013). The difference was highly significant.

Also in analysis of optical density values of IgG antibody specific to P.P.D at a concentration of 40 µg/well when tested at a serum dilution 1:1000 between proved and suspected pulmonary tuberculous patient groups.

It illustrates that the mean optical densities of the proved and suspected pulmonary tuberculous groups were 0.374 ± 0.062 and 0.310 ± 0.052 respectively.

Thus, there is no significant difference between proved and suspected pulmonary tuberculous groups was found.

The analysis of the optical density (O.D) values of IgG antibody specific to purified protein derivative (P.P.D) antigen at aconcentration of 40 µg/well suspected pulmonary tuberculous

patients group and healthy control subjects group when tested at a serum dilution 1:1000. It illustrates that the mean O.D of IgG antibody levels were 0.310 ± 0.052 and 0.159 ± 0.013 respectively, thus there is a highly statistically significant difference between suspected pulmonary tuberculous patients group and healthy control subjects group.

Table (6) Shows the analysis of the optical density of IgG antibody levels in relation to the results of tuberculin test in the control group. There were 6 cases with positive tuberculin test, had amean optical density of 0.184 ± 0.012 and 14 cases with negative tuberculin test, had amean O.D. of 0.134 ± 0.012. There is no significant difference between the results of tuberculin test in relation to the "O.D." of IgG antibody in control group.

Table (7) Shows the analysis of the optical density value of IgG antibody levels in relation to the results of tuberculin test in suspected pulmonary tuberculosis. The mean "O.D." of IgG antibody were 0.327 ± 0.052 in 13 patients with positive tuberculin test while the mean "O.D." of IgG antibody were 0.298 ± 0.049 in 7 patients with negative tuberculin test. There is no significant difference between the results of tuberculin test in relation to the optical density in suspected pulmonary tuberculosis.

Table (8) Shows the analysis of the optical density value of IgG antibody levels at aserum dilution 1:1000 in relation to the results of tuberculin test in proved pulmonary tuberculous group. The mean optical densities of IgG antibody levels were 0.417 ± 0.062 in 35 patients with postive tuberculin, while the mean optical densities were 0.246 ± 0.044 in 7 patients, with negative tuberculin test. There is ahighly significant difference between the results of tuberculin test in relation to the "O.D." of IgG antibody levels in proved pulmonary tuberculosis.

Tuberculin Versus ELISA Test.

Table (9) Shows the relation between the results of tuberculin and ELISA tests among the different studied groups from which it is to be noticed that among healthy control subjects group 6 subjects (30%) had a positive tuberculin, from which only one (5%) was also positive ELISA test, and 19 subjects were negative to ELISA test from which only 14 subjects were negative also to tuberculin test. There is no relation between the results of tubercuin & ELISA tests healthy control subjects group. Concerning the suspected pulmonary tuberculous group the same table shows that 13 patients had positive tuberculin test from which 11 patients had postive ELISA test, and 9 patients were negative ELISA tests from which 7 patients were also negative tuberculin test. There is relation between the results of tuberculin & ELISA tests in suspected pulmonary tuberculous patients group.

Lastly concerning the proved pulmonary tuberculous patients group, the same table shows that 45 patients had positive ELISA test from which 35 patients were also positive tuberculin test, and 15 patients were tuberculin negative test from which 5 patients were also negative for ELISA test. There is correlation between the results of tuberculin and ELISA tests in proved pulmonary tuberculous patients group.

Table (1a): Age and sex distribution of the proved pulmonary tuberculous group.

Age in years	No.	%	Males		Females	
			No.	*%*	*No.*	*%*
10-less than 20	5	10	3	6	2	4
20-less than 30	28	56	21	42	7	14
30-less than 40	16	32	9	18	7	14
40-above 40	1	2	1	2	0	0
Total	50	100	34	68	16	32

The most frequent age group in this table is 20 less than 30 years. Then 30 less than 40 years.

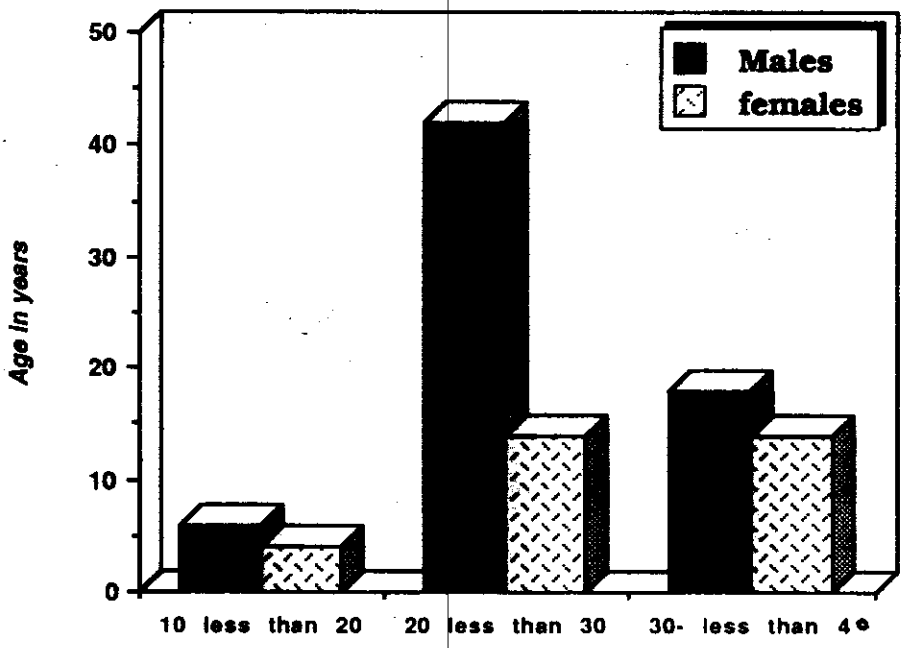

Figure (1 a)

Age and sex distribution among the proved, pulmonary tuberculous

Table (1b): Age and sex distribution of suspected pulmonary tuberculous group.

Age in years	No.	%	Males		Females	
			No.	*%*	*No.*	*%*
10-less than 20	3	15	2	10	1	5
20-less than 30	8	40	6	30	2	10
30-less than 40	6	30	5	25	1	5
40-above 40	3	15	1	5	2	10
Total	20	100	14	70	6	30

The most frequent age group in this table is 20 less than 30 years. Then 30 - less than 40 years.

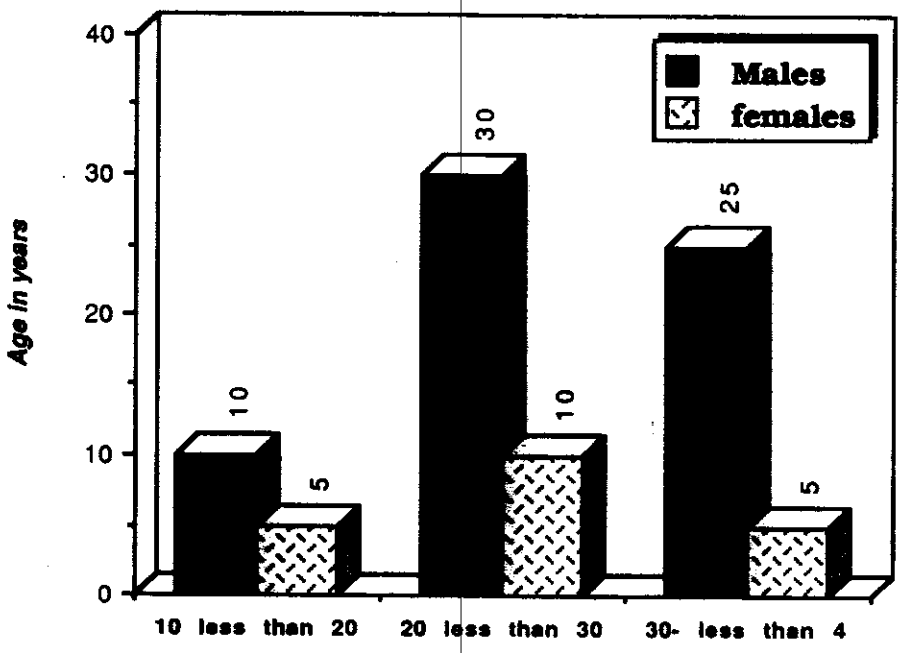

Figure (1 b)

: Age and sex distribution of suspected pulmonary tuberculous group.

Table (1c): Age and sex distribution of healthy control group.

Age in years	No.	%	Males		Females	
			No.	*%*	*No.*	*%*
10-less than 20	6	30	4	20	2	10
20-less than 30	6	30	3	20	2	10
30-less than 40	6	30	4	20	2	10
40-above 40	2	10	1	5	1	5
Total	20	100	13	65	7	35

The most frequent age group in these table was that of 20-less than 30 years.

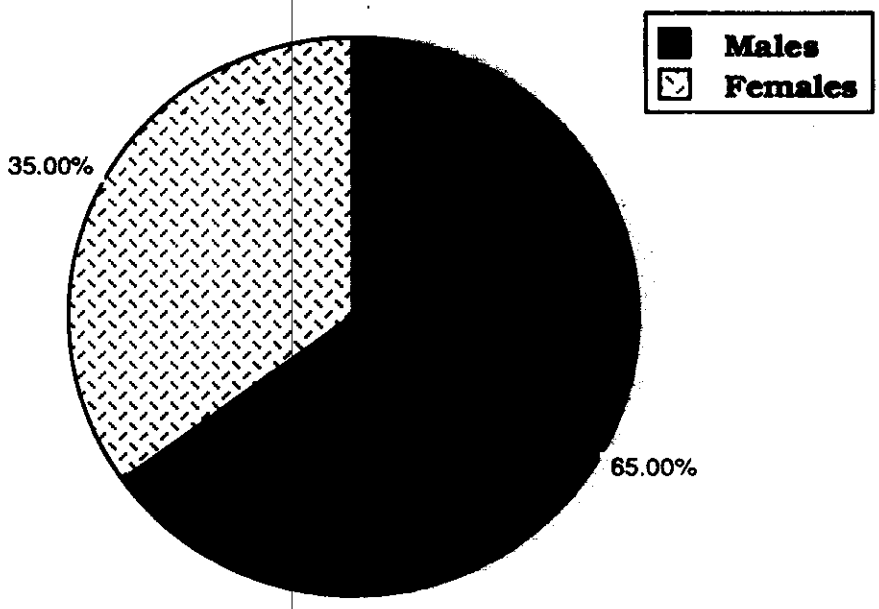

Figure (1 c)

the number of males was 13 (65%) & that of females was 7 (35%).

of healthy control group.

Table (2): The main clinical findings among the studied proved suspected pulmonary tuberculous groups.

Symptoms clinical findings	Proved pulmonary tuberculosis		Suspected pulmoray tuberculosis	
	*No.**	%	*No.***	%
Cough :	49	98	17	85
A) Productive	40	80	13	65
B) Dry	9	18	4	20
Loss of weight	46	92	17	85
Haemoptysis	14	28	7	35
Night fever	44	88	18	90
Sweating	42	84	16	80
Loss of appetite	48	96	16	80
Dyspnea after exertion.	22	44	8	40

* Proved pulmonary tuberculous No. 50 of Patients.

** Suspected pulmonary tuberculous No. 20 of Patients.

Cough is the most frequent symptom.

Then loss of appetite.

The least symptom is Haemoptysis.

Table (3): Results of tuberculin test among the studied groups.

Tuberculin tests	Proved pulmonary tuberculosis *No. 50 of pati.*		Suspected pulmonary tuberculosis *No. 20 of pati.*		Healthy control *No. 20 of Pati.*	
	No.	%	No.	%	No.	%
Positive tuberculin test	35	70	13	65	6	30
Negative tuberculin test.	15	30	7	35	14	70

- No significant difference between positive tuberculin test in proved & suspected pulmonary tuberculous groups. $X^2 = 2.02$ (Chi square test)

- Significant difference between positive tuberculin test in control and proved pulmonary tuberculous groups. $X^2 = 9.44$.

- Significant difference between positive tuberculin test in control and suspected pulmonary tuberculous groups $X^2 = 4.92$

Table (4): Values of IgG antibody specific to purified protein derivatives (P.P.D.) antigen in different studied groups.

Studied group	Total Number	Positive		Negative	
		No.	*%*	*No.*	*%*
1) Healthy control group	20	1	5	19	95
2) Suspected pulmonary tuberculous group	20	11	55	9	45
3) Proved pulmonary tuberculous group.	50	45	90	5	10

There is a significant association between results of ELISA test on sera of both suspected and proved pulmonery tuberculous groups. $X^2 = 10.4$.

Ther is ahighly significant difference between results of ELISA test on sera of both control and proved pulmonary tuberculous groups. $X^2=45.79$.

Table (5): Values of IgG antibody specific to (P.P.D) antigen of proved pulmonary tuberculous patients group, suspected & healtly control subjects group at a serum dilution 1:100 detected by ELISA test.

Patients group	Mean	S.D.
Proved pulmonary tuberculosis	0.374	±0.062
Suspected pulmonary tuberculoss	0.310	± 0.052
Healthy control subject gorup	0.159	± 0.013

t = 22.89

There is high significant difference of the (O.D) values of IgG antibody levels between proved pulmonary tuberculous patients group and healtly control subjects group.

No significant difference of the (O.D) values of IgG antibody levels between proved suspected pulmonary tuberculous groups.

There is high significant difference of the (O.D.) values of IgG antibody levels between suspected pulmonary tuber culous patients group and healthy control subjects group.

Table (6): (Analysis of the optical density (O.D) values of IgG antibody levels in relation to the results of tuberculin test in control group.

tuberculin test	ELISA		
	No.	Mean	S.D.
Positive	6	0.184	± 0.012
Negative	14	0.134	± 0.012

No signifiant difference between the results of tuberculin test in relation to the O.D of IgG antibody levels in control group.

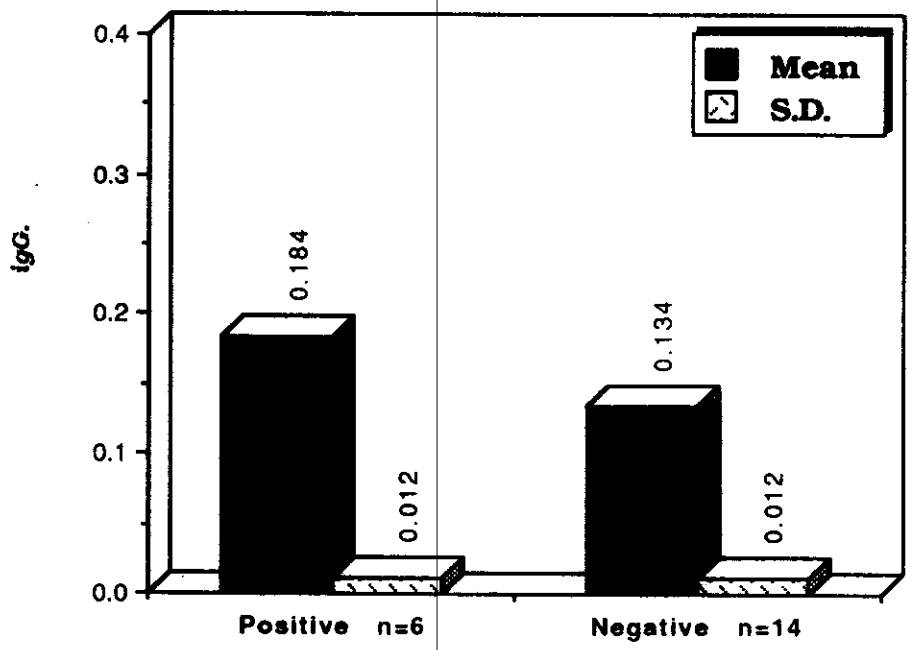

Figure (3)

Table (7): Analysis of the optical density (O.D) values of IgG antibody levels in relation to the results of tuber culin test in suspected pulmonary tuberculous group:

tuberculin test	ELISA		
	No.	Mean	S.D
Positive	13	0.327	± 0.052
Negative	7	0.298	± 0.049

t = 1.254

There is no significant diference between the results of tuberculin test in relation to the O.D. of IgG antibody levels in suspected pulmonary tuberculous group.

Table (8): Analysis of the optical density (O.D) values of IgG antibody levels in relation to the results of tuberculin test in proved pulmonary taberculous group.

Tuberculin test	ELISA		
	No.	Mean	S.D
Positive	35	0.417	± 0.062
Negative	15	0.246	± 0.044

t = 11.03

There is significant difference between the results of tuberculin test in relation to the O.D. of IgG antibody levels in proved pulmonary tuberculous group.

Table (9): Relation between the results of tuberculin versus ELISA tests in the studied groups

Studied group	Total Nomber	Tuberculin test				ELISA test			
		Positive		Negative		Positive		Negative	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
* **Healthy control subjects group**	20	6	30	14	70	1	5	19	95
* **Suspected pulmonary tuberculous group**	20	13	65	7	35	11	55	9	45
* **Proved pulmonary tuberculous group**	50	35	70	15	30	45	90	5	10

*No Relation between the results of tuberculin and ELISA tests among the healthy control subjects group.

* There is a relation between the results tuberculin and ELISA tests among suspected & proved pulmonary tuberculous groups.

DISCUSSION

DISCUSSION

Tuberculosis probably stands as the most important infectious disease in human. Current recommendation for control programs of tuberculosis emphasizes case finding to permit treatment and limit further transmission of infection, *(Daniel & Debanne 1987).*

The diagnosis of mycobacterial diseases depend upon identifying the infecting organism in the secretion or tissues of diseased individual. There are several limitation of this method of diagnosis. Mycobacterium tuberculosis is characteristically present in relatively small numbers, so that it is usually recognized only in these patients with advanced diseases, *(Daniel & Debanne 1987).*

Esmat and El-Maraghy (1978) reported that the importance of sputum analysis in the diagnosis of pulmonary tuberculosis has been emphasized by WHO expert Comitte on tuberculosis in its 8th reports, It was mentioned also that pulmonary tuberculosis proved bacteriologicaly by microscopy is the best available index of the infectious pool in a community. It is evident that the grade of positivity of sputum analysis increases with increasing the extent of the lesion. Although sputum is readily available for examination from most patients with advanced pulmonary tuberculosis. It is difficult to obtain specimens from patients with less extensive disease, from

patients with extrapulmonary tuberculosis, and from children who characteristically do not produce sputum. *(Daniel and Debanne, 1987).*

Cultural identification of mycobacteria is more definitive and more sensitive than direct smear, but this technique is not available in many high prevalence areas of the developing world. Moreover, most mycobacteria are slowly growing organisms and culture from clinical specimens frequently takes 6 to 10 weeks, *(Daniel and Debanne 1987).*

Serodiagnosis is widely used in medicine, and the problems of bacteriologic diagnosis led to the early application of such techniques to tuberculosis. Serological diagnostic tests for tuberculosis has been based on gel precipitation techniques *(Harboe et al., 1977)*, agglutination *(Takahashi, 1962)*, passive hemagglutination, complement fixation and immunofluorescence tests *(Takahashi and Ono, 1961)*. However, none of these techniques has been widely adopted for practical use because of low sensitivity and / or poor specificity *(Bhardwaj et al., 1981).*

The purpose of this study was to use the ELISA technique in measuring specific antibodies of immunoglobulin to PPD antigen in patients with tuberculosis and in control subjects. (Balestrino et al., 1984). demonstrated that ELISA could be used for the serodiagnosis of tuberculosis with a high degree of sensitivity

and specificity. This technique does not require sophisticated instrumentation, and the required reagents are rather inexpensive. It has been widely used for the serodiagnosis of infectious diseases *(Engvall and Perlmann, 1972)*. In the present work, PPD was used as an antigen, because it is readily available, In addition to being extensively studied by many workers in **ELISA** serodiagnosis tests. Antigen 5 is more suitable than PPD for use in ELISA as it yields fewer false positive results (more specific) in healthy persons *(Balestrino et al., 1984)*. Despite the forementioned advantages, antigen 5 was not available at the time of this study. Antigen 6 prepared by immunoabsorent affinity chromatography was not considered in this study, since it was not highly purified and less satisfactory for ELISA test than either antigen 5 or PPD in the comparative study of (Kirant et al., 1985). The specificity of ELISA depends on the antigen used as with crude antigens, a specificity of 97% to 98% has been achieved *(Daniel and Debanne, 1987)*.

The IgG antibody was tested in this study because it has been measured by many investigators and a very low IgM antibody could be demonstrated in tuberculous patients, *Benjamin and Daniel (1982)*. Also in this study PPD was used because of the favorable results, ready availability and it has been used by several investigators in ELISA serodiagnostic test for tuberculosis, *(Balestrino et al. 1984)*.

Several factors were behind the decision of the choice of the topic of this study. The difficulty in construction of a definite diagnosis in many situations of patients with different forms of tuberculous lesions was a main triggering stimulus for searching about new more reliable methods particularly when the tubercle bacilli cannot be detected directly in the samples. Moreover, the diagnosis of tuberculosis with all its forms continues to be difficult in children and the success rate of bacteriological confirmation and histopathological evidence is extremely low especially in extrapulmonary lesions where radiological studies are not helpful and specimens are not easily obtained *(Diena, 1968)*. On the other hand, tuberculin skin test which is a traditional method used to diagnose infection with M. tuberculosis may not induce a reaction for two to eight weeks following infection and does not become positive in all patients with decreasing its reactivity with advanced age, severe or febrile illness or immunosuppressive therapy, and false negative reactions may be recorded in these situations. Meanwhile, radiology appears to be more sensitive but less specific than bacteriology which requires weeks to months for a positive culture and/or identification of species *(Maclean, 1975 and Garcio-Garreno et al., 1986)*.

The successful role of serodiagnosis which has been established in many bacteriological diseases together with the several advantages of serodiagnosis with other factors were behind

the selection of the topic of serology in the diagnosis of tuberculosis. Enzyme linked immunosorbent assay (ELISA) has proved to be a sensitive and reproducible technique widely used with encouraging results in clinical and epidemiological studies of mycobacterial diseases including tuberculosis *(Engvall and Pearlmann, 1971).*

Garcio-Garreno and associates (1986), in their analysis of the results of ELISA test among patients with pulmonary tuberculosis, had confirmed a statisticaly significant difference between the results of immunoenzymatic assay of M. tuberculosis antibodies in sera from patients with clinically active and those with clinically inactive disease. *Mehata and Khuller (1988)* found that antibodies to purified protein derivative were produced in response to the increased microbial load reflecting the progress of the disease and hence, a clear difference was reported in their results between patients with sputum positive bacteriology for acid-fast bacilli and those with negative sputum bacteriology. Similar findings were earlier reported by *Tandon et al. (1980)* who documented that ELISA test using PPD was more sensitive among sputum positive cases than in sputum negative cases.

Diabetes mellitus is an auto-immune disease. There is an increase in the ratio of helper to suppressor T-cells in circulation, this may be a general phenomenon in immune endocrine disease. The increase in this ratio is likely due to a deficiency of suppressor T-cells. The immune-directed

destruction of B-cells probably involves both humoral and cell mediated immunity, *(Foster, 1987).*

Among the proved pulmonary tuberculous group this study included 7 patients (14%) suffering from uncontrolled diabetes melitus beside 2 patients (1[illegible]%) suffering from the same diseaes in suspected pulmonary tuberculous group.

The increased incidence of pulmonary tuberculosis among uncontroled diabetes is well known, and is mainly reactivation of an endogenous lesion rather than superinfection, hence the late development of tuberculosis in these patients *(Turner and Warwick, 1957).*

The disordered metabolism in diabetes apparently creates favorable environment for reactivation of tuberculous lesions. In pre-insulin era the incidence of pulmonary tuberculosis in diabetes was reported to be 5 to 10 times higher than in non-diabetics remains a little higher *(Boucot et al., 1952).*

In the study of *Hassan (1984)*, 9 out of 55 pulmonary tuberculous patients (16.4%) were diabetics.

Hemoptysis is a very important diagnostic evidence in pulmonary tuberculosis recognized since ages. Although there are other common causes of hemoptysis, pulmonary tuberculosis is by far the commonest cause. *Pamra et al. (1970)*, proved that more than half of their patients with a history of hemoptysis

were tuberculous and concluded that tuberculosis is the most frequent cause of hemoptysis in all age-groups.

In this study, 14 out of 50 patients 28% of (proved pulmonary tuberculous patients)& 7 out of 20 patients (35%) in suspected pulmonary tuberculous patients. presented with hemoptysis. *Jaswant-Signgh (1975),* reported that, 30% out of 5000 tuberculous patients presented with hemoptysis. The difference in results may be due to the larger cases number of the *(Jaswant-Singh 1975). (Ebeid (1985),* recorded 37 cases with hemoptysis out of 191 tuberculous patients. (19.4%).

As a rule, every case of active tuberculosis exhibits some degree of pyrexia which is one of the important clinical criteria of activity. The character of the temperature chart varies with the severity of the disease. Early in the disease the fever is low and of short duration, usually occuring in the later afternoon or evening. Night rise above normal if consistently observed, is significant *(Dongerey and Bhatia, 1981).*

In the present work night fever was present in 44 out of 50 patients (88%)of proved pulmonary tuberculous patients & 18 out of 20 (90%) of suspected pulmonary tuberculous patients. This result was in agreement with the result of *Ebeid (1985),* as he recorded fever in 161 out of 191 pulmonary tuberculous patients (84.3%).

Loss of appetite was a complaint of 96% of the proved pulmonary tubculous patients & 80% of suspected pulmonary tuberculous patients in this study. *(Ebeid 1985)*, showed figures identical to the results obtained in the present study (91.9%) These figures were higher than those reported by *(Jaswant-Singh 1975)*, who reported 65% out of 3425 tuberculous patients. The difference may be due to difference in the socioeconomic standard and number of patients included in both studies . No available previous work was concerned with the effect of sex on the yield of the different serodiagnostic tests. However, it came strongly evident in this study that sex has no influence of the results of serodiagnostic techniques as there was no detectable significant relation between the positivity yield of the mean optic density of ELISA among males and females of the control group suspected pulmonary tuberculous group & proved pulmonary tuberculous group.

The mean optical density of two wells measurement was considered positive for ELISA IgG specific to P.P.D. at a concentration of 40 μg/well, if it was equal or more than the mean optical density value of control subject (0.209).

In this study, PPD-specific IgG antibody was positive in serum of 45 out of 50 tuberculous patients (90%) and 1 out of 20 healthy controls (5%), the sensitivity was 90% and specificity 95%. A highly significant differences was detected between

pulmonary tuberculous patients (mean O.D. 0.374 ± 0.062) and the healthy controls (mean O.D. 0.159 ± 0.013).

These results agreed with the results of *Balestrino et al., (1984).* Who reported a positive result in patients with active pulmonary tuberculosis at a sensitivity of 81.4%. Also these results agreed with the results of (Daniel et al., 1986), who reported that a positive result in patients with active pulmonary tuberculosis with a similar technique at sensitivity of 89%.

Also these results agreed with the results of *Kalish et al., (1983)* who reported a positive result with a similar technique and concentration at sensitivity of 80%. and with Daniel et al., (1986), who reported that a positive result at specificity 88%. this results are not compatible with the results of *Zeises (1984)* who reported a positive result at specificity 76%.

The results presented before are in agreement with the study of (Tandon et al., 1980), who used PPD antigen for detection of tuberculosis using ELISA. PPD showed satisfactory significant value ($P < 0.01$), for both sputum positive and negative tuberculous patients. the test detected the disease in 80% of patients with sputum positive tuberculosis.

A comparable result was reported by *(Grange et al. 1980),* who used ELISA to study the level of antibody binding of BCG in

the IgG, IgM and IgA classes in sera from patients with tuberculosis. Seventy five percent of tuberculous patients had significantly elevated levels of anti-M. tuberculosis antibodies in the IgG class. The levels of antibodies in the IgM and IgA classes were much less discriminative. The authors concluded that, for serodiagnosis of tuberculosis, an estimation of anti-mycobacteraial antibodies in the IgG class gives the most discriminative results.

Antibody activity against M. tuberculosis of sera from an area with a high prevalence of tuberculosis was measured by ELISA with a plasma membrane extract from M. tuberculosis . All sera from relapsed tuberculous patients and 82.5% of sera from recent untreated cases gave positive results. Specificity of the test with healthy subjects was 85% *(Karmbovitis 1986).*

Radin et al. (1983) reported that, patients with active tuberculosis clearly had a higher levels of IgG antibody activity to PPD antigen, using the ELISA, than did healthy subjects who were skin test positive or negative. There was a clear difference between the diseased and healthy groups. No correlation between the presence of the disease and the antibody levels was found with IgM. *Radin et al. (1983)* reported that, ELISA was a rapidly reproducible assay using commercially available materials, and might offer a clinically useful test for diagnosis of tuberculosis.

The results obtained in this study were also in agreement with the study of *(Thongkrajai et al. 1989)*, who used ELISA to examine the sera from 86 normal subjects and 54 active pulmonary tuberculous patients. Fourty nine of 54 sera from active cases (91%), and 8 sera from normal subjects gave positive results; a sensitivity and specificity were 91% for each. The results presented here were also in agreement with that of (Deraz 1988), who found that 80% of active tuberculous patients had a positive ELISA for IgG specific to PPD.

In the present study 5 out of 50 active pulmonary tuberculous patients (10%) showed a negative ELISA test for PPD specific IgG. These results were in agreement with the study of *(Deraz 1988)* who reported that 20% of active pulmonary tuberculous patients showed negative ELISA for IgG specific PPD. *(Kalish et al. 1983)*, *(Karmbovitis 1986)* and *Thongkrajai et al. (1989)* reported a nearly similar negative ELISA test for PPD specific IgG. There are several factors which may account for the failure of ELISA to detect antibodies in pulmonary tuberculous patients. *Karmbovitis (1986)* and *Karmbovitis et al. (1986)* reported that, heavy mycobacterium infection may be associated with a low titers of antibodies to mycobacterial antigens, and these antigens may released into the circulation and cause temporary suppression of antibody formation. *Katz et al.(1979)* reported that, increase in the percentage of suppressor T-lymphocytes, demonstrated in

patients with active tuberculosis decrease the activity of B-cells, and partially account for the low level of antibodies observed in some patients with active tuberculosis. Another explanation is that antibodies may bound in circulating immuen complexes which have been demonstrated in many patients with tuberculosis *(Brostoff et al., 1981).*

In this study, positive ELISA results for PPD specific IgG was observed in 1 out of 20 healthy controls (5%). A early similar results were recorded by (Draz 1988) and (Thongkrajai et al. 1989). These positive results may be due to exposure of controls to many types of mycobacterial infection which were endemic in Egypt and BCG vaccination was also widespread. Stanford and Grange(1974) showed that, many antigens were shared by all mycobacteria and to some extent, by other genera such as Corynebacterium and Nocardia. Another possibility is that some controls might have acquired subclinical infections of tubercle bacilli. These small doses of M. tuberculosis were sufficient to boost the antibody concentration but insufficient to cause disease. Unlike the sera from normal subjects, sera from other patients. i.e. VDRL- and RF-positive sera, malaria and inactive tuberculosis sera, gave a high proportion of positive results in ELISA *(Thongkrajai et al., 1989).*

As regard the tuberculin test in patients with proved pulmonary tuberculosis, there was a significant difference between the mean optical densities of positive and negative

tuberculin test. This finding could be accounted for in view of previous discussion.

The results of ELISA method showed no significant difference of the O.D. of the IgG antibody levels between sera of patients with active (proved) and suspected groups. On the other hand comparison of the results of ELISA method of both patients groups and control subject showed a very high significant differences, respectively

These results agreed with the results of *Kalish et al.,(1983)* who reported that patients with active pulmonary tuberculosis had significantlly higher IgG antibody level than control subjects, also these results agreed with the results of (Zeiss and his *Colleagues 1982)* who measured IgG against PPD by ELISA in patients with active tuberculosis and in healthy control subjects there was a sharp delineation between patients and controls subject.

Out of 20 patients with suspected pulmonary tuberculosis 11 (55%) had a positive ELISA for IgG specific to PPD at a sensitivity 55% and 9 (45%) had negative ELISA results. Thus the detection of IgG specific to PPD using the described ELISA method may be considered as an easy and practical diagnostic test in these patients. The negative ELISA results among patient with suspected pulmonary tuberculosis could be attributed either to non mycobacterial infection or infection with atypical

mycobacterial species or tuberculous patients with a very low level of IgG specific to PPD as discussed before.

The results of tuberculin test and the O.D. of IgG antibody level showed no significant difference between results of tuberculin test and the O.D. among patients with suspected pulmonary tuberculosis

Anergic cases in positive ELISA in suspected group had been attributed to malnutrition and to severity of the disease. Also this anergy may be from sequestration of specifically reactive monocytes in tuberculous tissues, or may be mediated by suppressor monocytes *(Daniel et al 1986)* or these suspected cases are non tuberculosis. While negative ELISA and tuberculin test may be as discussed before due to failure of ELISA to detect antibody or other infections rather than mycobacterial diseases or tuberculous patients with very low level of IgG specific to PPD.

Two cases among patients with suspected pulmonary tuberculosis had right sided pleural effusion. ELISA was positive in these cases and these results agreed with the results of *(Daniel et al., 1986).*

Among control subjects there was no significant difference between the O.D. values of IgG antibody levels and positive or negative tuberculin tests

In conclusion the results of ELISA method for the detection of IgG specific to PPD at high serum dilution showed a marked delineation between patients with active pulmonary tuberculosis, suspected pulmonary tuberculosis and control groups regardless of the results of tuberculin skin tests. the same finding were also reported by *(Benjamin & Daniel 1982), (Kalish et al., 1983)* and *(Zeiss et al., 1984).* This technique may have potential use as a rapid diagnostic aid in evaluating patients with suspected pulmonary and extrapulmonary tuberculosis.