SUMMARY AND CONCLUSION

The resurgence of tuberculosis and increases in the resistance of *M. tuberculosis* to antituberculous agents have focused attention on the need for simple and rapid means that help to diagnose tuberculosis. The rapid diagnosis of *M.tuberculosis* and accurate antituberculous susceptibility tests are essential for treatment of infected patients and to control the spread of the disease.

The aim of this work is to detect the prevalence of isoniazid and rifampicin resistant mycobacterium tuberculous clinical isolates among the newly diagnosed pulmonary tuberculous patients and to assess the value of apoptosis as a laboratory technique in the diagnosis of pulmonary tuberculosis in comparison to the conventional identification methods (Z.N stain and culture on L.J medium).

This study was carried out on 96 cases suspected of having pulmonary tuberculosis based on full history, clinical and radiological examination. They had never receive antituberculosis treatment.

- Three successive morning sputum samples were collected from each patient and subjected to bacteriological study which includes:
  1- Microscopical examination of Z.N stained sputum smears before and after concentration and decontamination by NALC-NaOH method.
  2- Culture on L.J media and identification of isolates by biochemical reactions.

3- Antimicrobial sensitivity testing were done for the isolated strains to two antituberculous drugs, INH & RIF, using the conventional proportion method on middle brook 7H10 agar.

- Blood samples from 14 cases harboring sensitive strains to INH and RIF from the newly diagnosed pulmonary tuberculous cases and 10 control subjects were collected for assessment of lymphocyte apoptosis (by Giemsa & acridine orange stains and agarose gel electrophoresis) and detection of T cells activity by measuring IL-2 level by ELISA method.

Out of 96 studied patients 36 (37.5%) showed mycobacterial growth on L.J medium, by biochemical reaction identification 32 (88.8%) cases out of 36 mycobacterial isolates were *M.TB* and 4 (11.2%) were MOTT. Twenty one (65.62%) tuberculous cases out of 32 were in the age group (21-40 years). Most of tuberculous cases were males (75%) and manual workers (59.3%), this may be due to their activity, more exposure to overcrowding, lack of health education and smoking.

Most of tuberculous cases were from rural areas (75%), this may be due to overcrowding, bad habits in addition to illiteracy.

There was a significant increase in the number of smokers among positive tuberculous cases (59.4%) compared to negative cases (34.4%) (P = 0.02). The significant increase in Goza smokers especially between young adult may play a role in transmission of tuberculosis.

Thirty six (37.5%) cases out of 96 showed mycobacterial growth on L.J medium, 20 of them were positive by direct Z.N stain and 26 were positive by Z.N stain after concentration and decontamination. The

sensitivity of direct stained smear versus L.J culture was 55.6% while the sensitivity of stained smear after concentration and decontamination was 72.2%.

Regarding the drug sensitivity tests, 15 (46.8%) *M.TB* isolates out of 32 were resistant to antituberculous drugs; 7 (21.8%) isolates were INH resistant, 6 (18.7%) were RIF resistant and 2 (6.2%) were MDR. Resistant *M.tuberculosis* isolates were more common among the advanced and moderate cases (9 and 4 respectively) than minimal tuberculous patients (2 cases).

Clinical, demographic data and antituberculous sensitivity results in addition to risk factors of 32 tuberculous patients were analyzed to determine whether these factors attribute to emergence of the primary drug resistance. There were insignificant association between the drug resistance and each of the following: sex, age, underlying diseases, smoking and chronic intake of corticosteroids but there were significant association between the incidence of resistance in patients having cavitary lesions ($P = 0.02$) and in those having history of contact with known tuberculous case ($P = 0.01$).

Regarding the cellular immune response of tuberculous and control subjects. Apoptosis was significantly increased in tuberculous patients than control subjects ($P < 0.001$) while IL-2 level was significantly decreased in tuberculous patients than control subjects ($P < 0.001$). Morphological assessment of apoptosis was confirmed by agarose gel electrophoresis. DNA fragmentation was increased in patients' lymphocytes compared to that in control.

The effect of successful antituberculous chemotherapy on apoptosis and IL-2 level was examined. Before treatment of mild tuberculous group, apoptosis showed significant statistical difference than that in control while after treatment there was insignificant difference between them. Also, IL-2 level in mild tuberculous group showed insignificant difference after treatment compared with that of control subjects.

Apoptosis showed significant difference between advanced group restudied after treatment compared with that of control subjects. While IL-2 level in advanced group after treatment showed insignificant difference compared with that of control subjects. The significance of apoptosis in advanced group after treatment may be due to the severity of disease as the test was done after 6 months of treatment. In advanced group, the percentage of apoptotic lymphocytes was significant higher than in mild group. Further follow up may be needed to established the relevance of apoptosis in the pathogenesis of tuberculosis.

Conclusion & Recommendations:

1- It is evident from the results of the present study that the incidence of primary drug resistance in our locality is high and that tuberculosis control is not performed well in Egypt. Further studies are needed to evaluate these programmes.

2- Antismoking program should clarify the significant association between smoking in the form of cigarette or Goza and tuberculosis. Goza smoking should be prohibited.

3- The sensitivity of microscopical examination of Z.N stained smears for detection of AFB in sputum directly was 55.6% and 72.2% after

concentration, thus microscopic examination of stained smears is a good microbiological method for confirmation of pulmonary tuberculosis as it is rapid and cost effective for diagnosis of pulmonary tuberculosis inspite of its low sensitivity in comparison to culture on L.J medium.

4- The high prevalence of RIF and INH resistance stressed the needs for limitation of their use for treatment of tuberculous patients only and not to be prescribed as broad spectrum antibacterial agents.

5- Antituberculous sensitivity should be performed to each *M.TB* isolates. The high incidence of resistance in patients having history of contact with known tuberculous case ensures that the examination and search for tuberculous cases should include their contacts for the benefits of family and community.

6- It should be stressed that tuberculous patients having cavitary lesion must be under attention as they are more expected to have resistant strains. They might become an epidemiological problem with the spread of drug resistant disease in the community.

7- Apoptosis during active *M. tuberculosis* infection may contribute to deletion of reactive T cells and limiting the cellular response in this disease.

8- Apoptosis as a test for T cell function and responsiveness can be used for diagnosis of pulmonary tuberculosis besides Z.N, sputum culture and other investigations. It helps in diagnosis of pulmonary negative stained smears tuberculosis.