



## Histological and Epidemiological study on *Mycobacterium tuberculosis* in Nineveh Governorate

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### ABSTRACT

Tuberculosis (TB) is a common infection especially in developing countries and can involve almost all bodily systems. Iraq has been identified as middle TB burden country in the world. The aims of the study are to determine the main factors that lead to prevalence of TB in people living in Nineveh governorate, to study the histopathological effect of *M. tuberculosis* on the tissues. The objective of epidemiological study is to find the relationship between *M. tuberculosis* and age, sex, education level, occupation, type of TB and sectors of people living in Nineveh governorate. This study included 100 patients infected with *Mycobacterium tuberculosis* ( 51 males and 49 females ) the age of the patients ranged from 1–75 years through the period from september 2010 to september 2011. In order to determine the main factors that lead to TB infection, data collected from all 100 patients infected with TB. Tissue sections were taken from patients with active TB from (lung, lymph node and breast mass) and fixed in (10%) formaldehyde and (4µm) paraffin blocks sections stained with hematoxylin and eosin or Ziehl Neelsen stain for histopathological examination. Data for epidemiological study of patients diagnosed with TB were collected from consultant clinic for chest and respiratory diseases in Nineveh governorate over a 16-year period (1994-2010). The results showed that The main factors that lead to TB infection were diabetes with a percentage of (25%) of the patients, followed by smoking with a percentage of (20%) . Changes in the tissues infected with tuberculosis is the formation of tuberculous granulomas .The result of epidemiological study showed that the number of Tuberculosis cases in Nineveh governorate increased between 1994-2002 and decreased between 2002-2010. The number of TB cases in males is more than in females . The main age groups infected with TB was the productive ages (15-34 years). Pulmonary TB is more than Extrapulmonary TB .The highest percentage of TB in sectors of Nineveh governorate (25%) for each of left sector and right sector, followed by (13.7%) in Sinjar.

### Introduction

*Mycobacterium tuberculosis* is the etiologic agent of tuberculosis in humans [1] .

Tuberculosis most commonly affects the lungs (Pulmonary TB). TB can also occur outside the lungs (Extrapulmonary) most commonly in central nervous , lymphatic or genitourinary systems, or in the bones and joints and even the skin [2] . Tuberculosis which occurs scattered throughout the body is referred to as miliary TB . Miliary TB result from the passage of *M. tuberculosis* into the systemic arterial circulation [3].

Virtually any organ of the body can be infected by *M. tuberculosis*; additional uncommon manifestations include gastrointestinal infection, peritonitis, cutaneous TB, laryngitis, otitis, and involvement of the adrenal glands, eyes, and breast infections[4,5]. The exact symptoms of extrapulmonary TB vary according to the site of infection of the body[4] .

Not everyone infected develops the disease asymptomatic, latent infection is most common however one in ten latent infections will progress to

active TB disease which if left untreated kills more than half of its victims[6] .

diagnosis of tuberculosis depends on history, physical examination of patient, chest X-ray, tuberculin skin test, Microscopic smears and culture, molecular technique, and histopathological examination[7] .

Tuberculosis is a major health problem worldwide. In 2011 World health organization

(WHO) estimated that 2 billion people (about one third of world populatin) are infected with *M. tuberculosis*, with 9 million new TB cases a year resulting in 2 million deaths a year , and there is about 310,000 cases of multidrug resistant-TB (MDR-TB) among TB patients with pulmonary TB in the world in 2011. TB is one of the top ten leading causes of death .

WHO aims to dramatically reduce the global burden of TB by 2015 [8] .

The prevalence continues to increase worldwide particularly in developing countries , this has been attributed to the Human Immunodeficiency Virus (HIV) pandemic and virulence of TB bacilli and emergence of drug resistant strains, also inadequate health care generally attributed to poor performance of NTPS. TB control programs should be able to manage those who are suspected to have the disease and provide assessment and diagnostic services.[9]

In Iraq tuberculosis remains a serious health problem affecting 16,000 people every year . Each year around 3000 people in Iraq die from this respiratory disease and primarily spreads by coughing and sneezing[10] . Iraq has been identified as middle-TB burden country in the world and ranks 108<sup>th</sup> worldwide among countries with a high TB burden and 9<sup>th</sup> among Eastern Mediterranean countries in the region[8,11]. According to WHO and UNICEF 93% of the population were immunized against tuberculosis with the BCG vaccine in 2001[10] .

WHO Global TB report, 2008 the incidence of all TB cases in Iraq is 56/ 100,000 population/ year and incidence of new sputum smear positive cases is 25/ 100,000 population per year[12]. WHO Global TB report, 2011 the incidence of all TB cases in Iraq is 45/ 100,000 population/ year[8] . And there is 14,500 TB cases in Iraq in 2011[13] , and the magnitude of *M. tuberculosis* drug resistance in Iraq found to be relatively high[14,15,16].

The objective of this study has been to determine the main factors that lead to prevalence of TB in people living in Nineveh governorate. And to study the histopathological effect of *M. tuberculosis* on the tissues.

The objective of epidemiological study is to find the relationship between *M. tuberculosis* and age, sex, education level, occupation, type of TB and sectors of people living in Nineveh governorate.

### Patients and Methods

This study included 100 patients with clinical signs or symptoms of pulmonary or extrapulmonary tuberculosis (51 males and 49 females) who attended Alkamalia, Ibn Sena, Alsalam, Ibn Alatheer general hospitals and consultant clinic for chest and respiratory diseases in Nineveh governorate, through the period from september 2010 to september 2011. Their ages were ranging from 1 – 75 years .

In order to determine the main factors that lead to TB infection, data collected from all 100 patients infected with TB, the data collected included : diabetes, smoking, alcoholisim, industrial factors, hepatitis, malnutrition, cancer, steroid drug users, gastrectomy[17] .

50 Out of 100 patient, sputum samples were collected by using sterile container for the isolation and identification of *M. tuberculosis* using smears stained with Ziehl - Neelsen staining and culture on lowenstain - Jensen medium and PCR .

Tissue sections were taken from patients with active TB from (lung, lymph node and breast mass) and fixed in 10% formaldehyde and(4µm) paraffin blocks sections stained with hematoxylin and eosin (H & E) or Ziehl Neelsen stain for histopathological examination [18]

Data for epidemiological study of patients diagnosed with tuberculosis were collected from consultant clinic for chest and respiratory diseases in Nineveh governorate over a 16-year period (1994-2010), and these included age, sex, education level, occupation, date of diagnosis, address at the time of diagnosis , type of TB and sectors .

### Statistical Analysis

t-test was used for statistical analysis of epidemiological data at significant level for the test ( $P < 0.05$ ) .

### Results

Out of 100 tuberculosis patients 51 (51%) were male and 49 (49%) were female, the age of the patients ranged from 1 – 75 years .

This study revealed that the highest percentage of main factors that lead to TB infections in Nineveh governorate in the year 2011 was: (25%) in diabetes patients followed by (20%) in smoking, (12%) in industrial factors, (11%) in alcoholism, (9%) in hepatitis, (8%) in malnutrition, (6%) in cancer, (5%) in steroid drug users, finally (4%) in gastrectomy, as shown in (Figure 1).

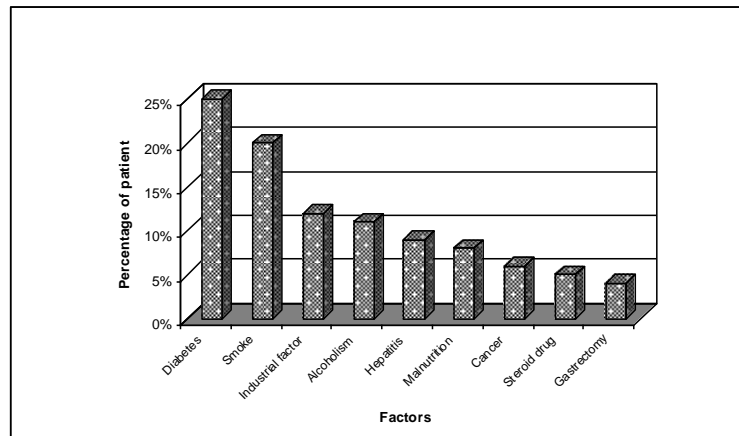


Figure (1): The percentage of factors that lead to tuberculosis cases in Nineveh governorate in the year 2011

The most important histological changes in the (lung, lymph node and breast mass) is the formation of tuberculous granulomas shown in Figures (2, 3, 4, 5).

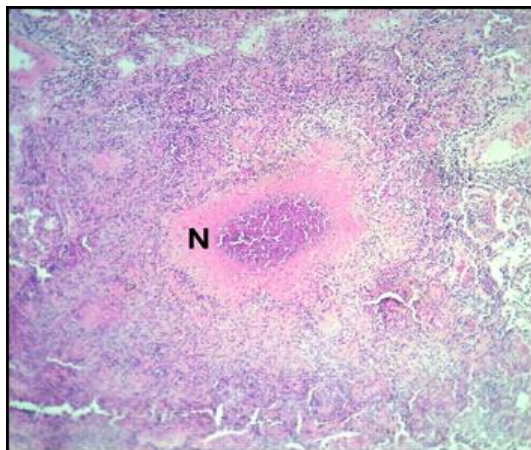


Figure (2) : A Photomicrograph of lung of 34 year old male with tuberculous granuloma showed necrosis surrounded by a rim of epithelioid cells .N : Gaseous Necrosis , E : Epithelioid cells, L: Lymphocyte cells. (H & E 100X)

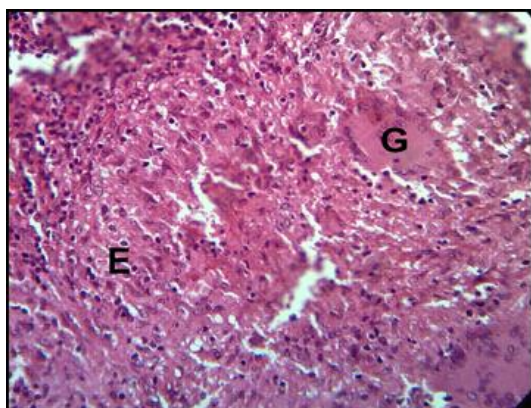


Figure (3) : A Photomicrograph of lymph node of 30 year old male with tuberculous granuloma showed central necrosis with focal collection of epithelioid cells and scattered langhans giant cells . G: Giant cell , E : Epithelioid cells, L : Lymphocytes ( H & E 400X)

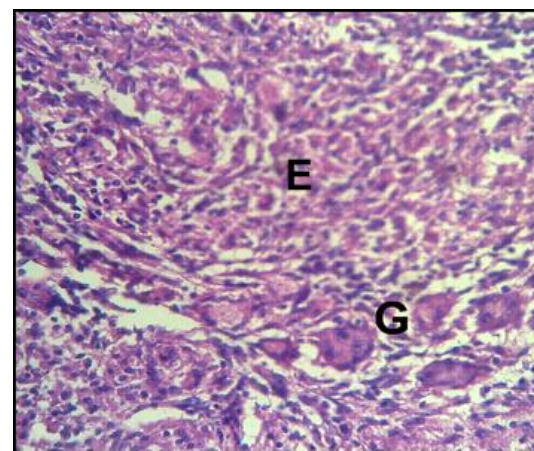


Figure (4) : A Photomicrograph of breast mass of 30 year old female with tuberculous granuloma showed focal collection of epithelioid cells with scattered langhans giant cells. G : Giant cells , E : Epithelioid cells , L: Lymphocyte cells .( H & E 400X )

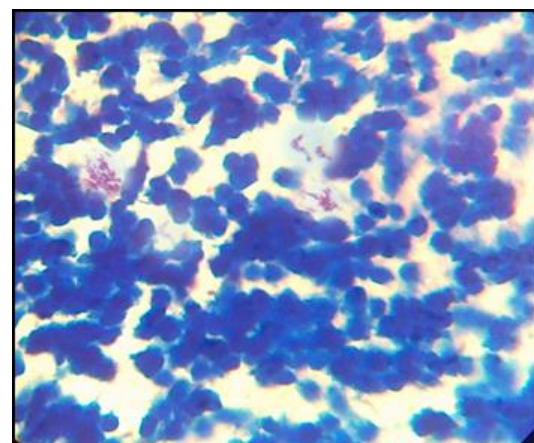


Figure (5) : A Photomicrograph of breast mass of 30 year old female with tuberculous granulomas showed *Mycobacterium tuberculosis* in tissue section which stain as red rods by Ziehl Neelsen stain . (ZN stain 1000X).

#### Epidemiological study

This study revealed that there is increase in the number of TB cases in Nineveh governorate from the year 1994 to 2002, and the highest number of TB

cases were recorded in year 1996 and 1997. From 2002 to 2010 there was a decrease in the number of TB cases. But, In 2010 the number of TB cases began to increase. From 1994 to 2010 the number of TB

cases for male was more than female however, there was no significant difference in TB cases between male and female (P=0.205), as shown in (Figure 6) .

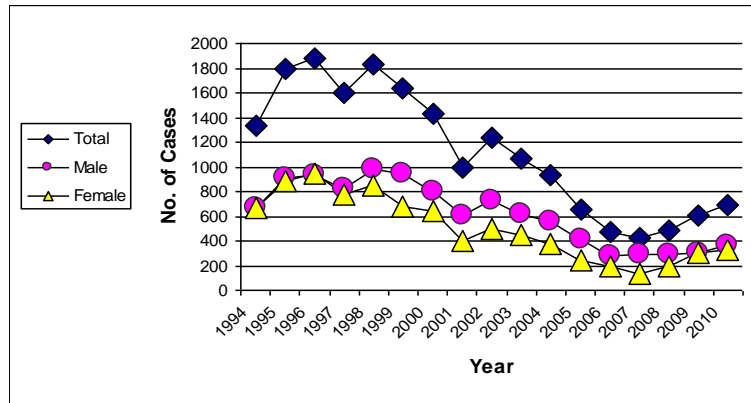


Figure (6) : Reported TB cases according to the total number of TB cases and sex in Nineveh governorate from the year 1994 to 2010

In 2010 the percentage of TB cases for male 52.5% , and 47.5% for females as Shown in (Figure 7).

This study revealed that the main age groups infected with TB in the years 2002, 2004, 2006 and 2010 was the productive age (15 - 34 years). The cases among children <5 years is under registered . This is mostly due to the misdiagnosis of TB with other communicable diseases, as shown in (Figure 8).

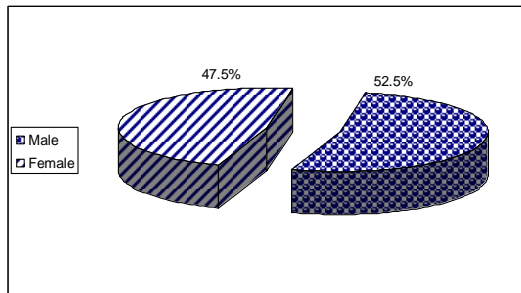


Figure (7) : Male to female percentage of TB cases recorded in year 2010

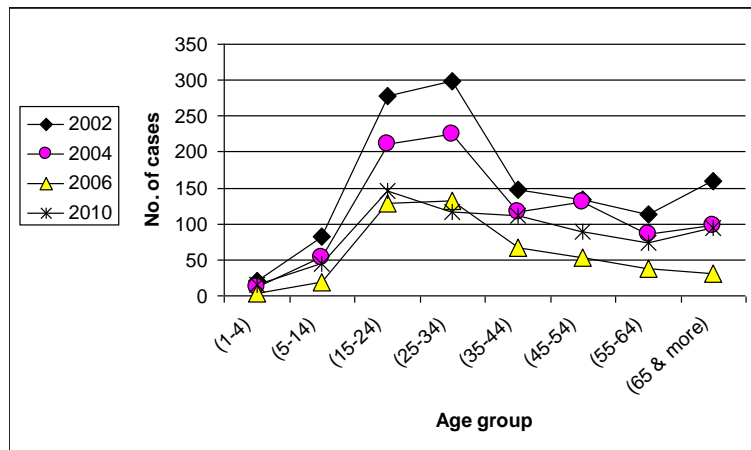


Figure (8) : Distribution of TB cases by age groups in Nineveh governorate in years 2002, 2004, 2006, 2010

In the year 2010 the main age groups infected with TB ( 15 – 25 years ) and the number of TB cases in

male is more than the number for female , as shown in (Figure 9).

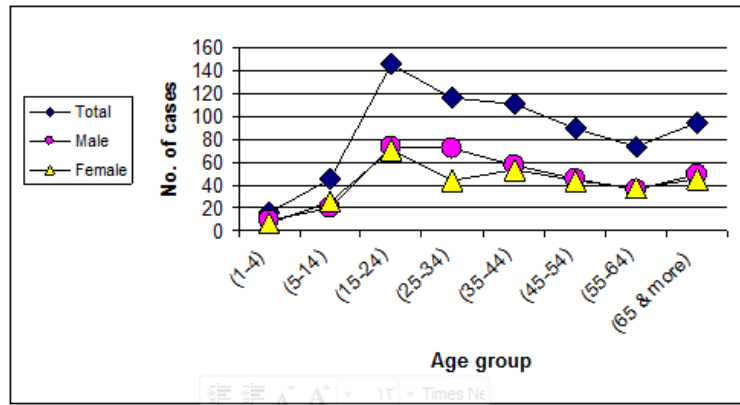


Figure (9) : Distribution of TB cases by age and gender in Nineveh governorate in the year 2010

This study revealed that there was a significant increase in the number of pulmonary TB cases in Nineveh governorate from the year 1994 to 2010 in comparison with extrapulmonary TB (P= 0.001), (Figure 10).

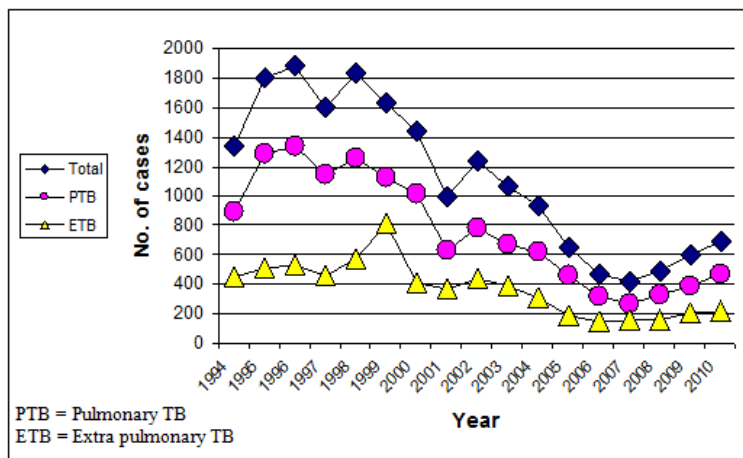


Figure (10): Number of TB cases according to the type of TB in Nineveh governorate from the year 1994 to 2010

And there is increase in the number of new sputum smear positive cases as represented in (Figures 11, 12 and 13).

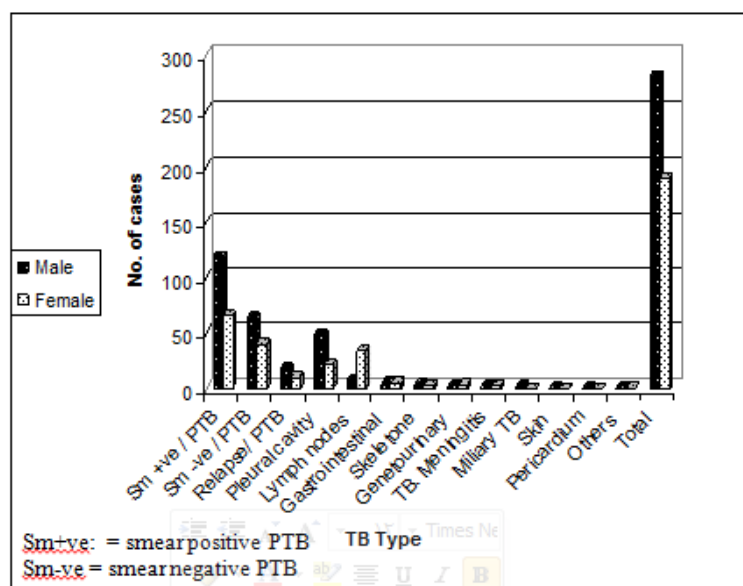


Figure (11): Number of TB cases according to the type of TB and sex in Nineveh governorate in the year 2006

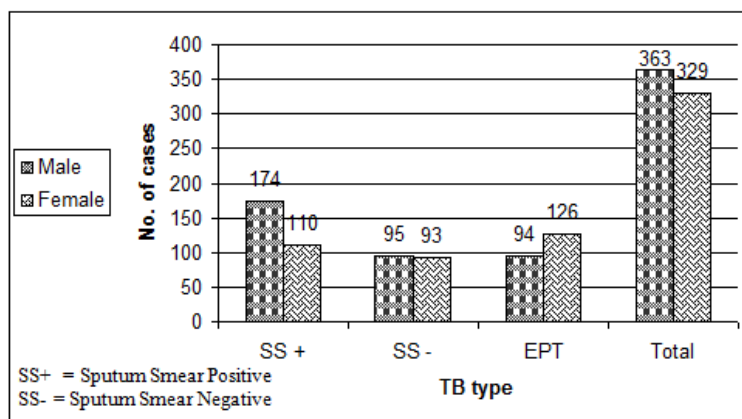


Figure (12) : Number of TB cases recorded in year 2010 according to TB type and sex

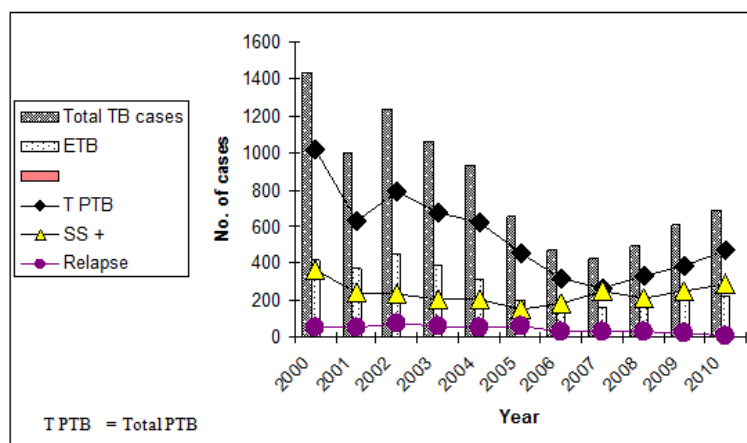


Figure (13) : TB cases notification in Nineveh governorate in years 2000-2010

It was clear that the highest percentage of TB cases in sectors of Nineveh governorate in the year 2010 : (25%) was in the right sector and (25%) in left sector followed by (13.7%) in Sinjar, (8.4%) in Talafar,

(7.6%) in Hamdania , (6.6%) in Qayyarah, (4.9%) in Baaj, (3.5%) in Telkif, (2%) in Makhmor, (1.7%) in Sheikhan, finally (1.6%) in Hatra, as shown in (Figure 14).

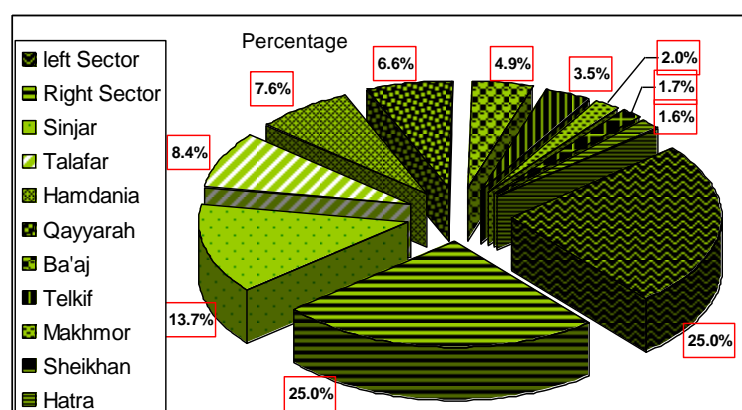


Figure (14) : The percentage of TB cases according to the sectors in Nineveh governorate in the year 2010

**Discussion**

Anyone can get TB, however some groups are at higher risk to get active TB disease. People at high risk include those with medical conditions that make the body less able to protect itself from disease ( for example : Diabetes, or people undergoing treatment with drugs that can suppress the immune system, such as long term use of corticosteroids ) [19].

Person with diabetes mellitus have a risk for developing active TB that is two to four times greater than persons without diabetes mellitus and this risk is likely to be greater in persons with insulin-dependent or poorly controlled diabetes, therefore there is association between diabetes and TB [20]. This study revealed that the main factors that lead to TB infection is diabetes , the result of this study is consistent with other studies done in different

governorate in Iraq[10,13,16,21] and other countries [17,22].

Smoking increases the TB risk may be through a decreased immune response, mechanical disruption of cilia function, defects in macrophage responses, thereby increasing the susceptibility of pulmonary TB[17]. WHO in 2011 reported that more than 20% of TB cases worldwide are attributed to smoking[8] as in this studies.

Alleban[10] asserted that tuberculosis has been linked with Industrial factor, people with silicosis have an approximately 30-fold greater risk for developing TB, silica particles irritate the respiratory system causing immunogenic responses such as phagocytosis, which as a consequence result in high lymphatic vessel deposits. Crystalline silica is found in concrete, masonry, sandstone, rock, paint, and other abrasives. Secondary tuberculosis is due to reactivation of dormant *M.tuberculosis* and is usually a consequence of impaired immune function resulting from some other cause such as malnutrition, infection (eg. Hepatitis), chemotherapy for treatment of malignancy. And several malignancies, especially haematological ones, might also lead to increased rates of TB, and several drugs induce susceptibility to TB [23].

Some drugs including rheumatoid arthritis drugs that work by blocking tumor necrosis factor-alpha (an inflammation causing cytokine) raise the risk of activating latent infection due to the importance of the cytokine in the immune defense against TB[24].

Other clinical conditions that have been associated with TB include gastrectomy with attendant weight loss and malabsorption, jejunioileal bypass[10].

In contrast to our study Human Immunodeficiency Virus (HIV) has come forward as the most important risk factor for development of TB in persons infected with *M.tuberculosis* in the world[25,26,27]. Because of people living with HIV and infected with TB are 21 to 34 times more likely to develop active TB disease than people without HIV, due to the immunosuppression caused by HIV infection, and HIV will enhance the spread of multidrug resistant TB[26,27].

In the present work, The most important histological changes in the (lung, lymph node and breast mass) is the formation of tuberculous granulomas which composed of central necrosis (caseation) containing TB bacilli surrounded by transformed macrophages called epithelioid cells, lymphocytes, scattered plasma cells and fibroblasts. The macrophages that are stimulated by cytokines such as interferon –  $\gamma$  secreted by nearby T- lymphocytes, may fused together to form Langhans giant cell. The process of granulomatous inflammation takes place over months to years[23,28,29,30].

#### **Epidemiological Study**

As shown in this study there is an increase in the total number of TB cases in the Nineveh governorate. From the year 1994 to 2002, a number of factors

contributed to this trend including: Low socioeconomic states, Social factors (poverty, overcrowding, homelessness), Delay in identification and treatment of patients infected with these bacteria particularly poor people in rural area, immunocompromised patients, prisoners, war, immigrant from countries with high rates of TB[31]. Longer antituberculosis treatment regimens[7]. Emergence of multidrug resistant strains of *M. tuberculosis* (virulent strains) due to badly managed TB care, for example incorrect drug prescribing, poor quality drugs and non-compliance[14,15,16].

From 2002 to 2010 there was a decrease in the number of TB cases in Nineveh governorate because of improved public health measures including Directly Observed Treatment Short course (DOTS) strategy of tuberculosis treatment recommended by WHO has been adopted in Nineveh governorate since 1999 and socioeconomic development.

The result of this study is consistent with other studies done in different governorates all over Iraq[10,11,21,32,33] who confirmed that the most TB patients belonged to the productive age group with a male dominance as compared to female, and outside like Qatar[34]. And the cases among children <5 years is under registered. This is mostly due to the misdiagnosis of TB with other communicable diseases.

Mohammed et al. [21] concluded that TB is difficult to diagnose in children because of poor yield on standard diagnostic laboratory tests and lack of characteristic symptoms in majority of the children in comparison to adults.

Age and sex variations in the prevalence of tuberculosis infection and disease have been reported worldwide in both developed and underdeveloped countries[35,36]. Age and sex differences may be reflected greater exposure among adult males because of differentiated social roles and economic activities[37]. It is probable that in addition to genuine age and sex differences in susceptibility related to biologic mechanisms, socioeconomic and cultural factors may play a role in determining age and sex differences in rates of infection, progression to disease and treatment outcome[17].

The result of this study is consistent with results of other studies[11,32,33,38] who confirmed that pulmonary TB is more than extrapulmonary TB, and pleural cavity and lymph nodes are the highest percentage in extrapulmonary TB in all governorates all over Iraq.

Tuberculosis cases are classified according to the site of lesion as either pulmonary or extrapulmonary, pulmonary cases are further classified as either sputum smear positive or sputum smear negative (which includes a patient with two sets (taken at least two weeks apart) of at least two sputum specimens negative for Acid Fast Bacilli). Relapse: a patient who has been declared cured of any form of TB in the past by a physician after one full course of

chemotherapy and now has become smear positive[7].

Extra pulmonary cases are further classified into gastrointestinal disease, genitourinary, lymphadenitis, meningitis, miliary, pericarditis, pleural disease, skeletal (Pott disease), and the ability of *M. tuberculosis* to disseminate and cause extra pulmonary TB is considered a marker of virulence[7,39].

This study shown the increased percentage of TB in rural areas like Sinjar and Talafar may be due to the

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difficulties that prevent most of patients to attend the health institutions asking for investigations and treatment .

## Conclusion

There is relationship between *Mycobacterium tuberculosis* and the age, malnutrition, Immunologic status, coexisting diseases (eg, Diabetes, Silicosis) and other individual host resistance factors. And there is a high prevalence of TB disease in diabetic patients in this setting.

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## دراسة نسيجية ووبائية عن عصابات السل (التدرن) في محافظة نينوى

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### الملخص

التدرن هو اصابة خمجية عامة خاصة في الدول النامية وغالبا ما يشمل جميع اجهزة الجسم, ويعد العراق دولة ذات ترتيب وسط بين دول العالم بالتدرن. تهدف الدراسة الى تحديد العوامل الرئيسية التي تؤدي الى انتشار مرض التدرن في الناس المقيمين في محافظة نينوى و دراسة التأثيرات المرضية النسيجية لجرثومة عصابات السل (التدرن) في الانسجة وتهدف الدراسة الوبائية الى ايجاد العلاقة بين جرثومة عصابات السل والعمر والجنس والمستوى التعليمي والمهنة ونوع التدرن والقطاعات للناس المقيمين في محافظة نينوى.

تضمنت الدراسة 100 مريض مصاب بعصابات السل (التدرن) كان عدد الذكور 51 بينما كان عدد الاناث 49 و تراوحت اعمار المرضى بين 1-75 سنة خلال الفترة من ايلول 2010 الى ايلول 2011. ولغرض تحديد اهم العوامل التي تؤدي الى الاصابة بمرض التدرن تم اخذ المعلومات من جميع المئة مريض المصابون بالتدرن. كما تم اخذ مقاطع نسيجية من المرضى المصابون بالتدرن الفعال من مناطق (الرئة والعقد للمفاوية وعقدة الثدي) وتم تثبيتها بالفورمالين بنسبة تركيز عشرة بالمئة وتم تحضير الشرائح البارافينية وصبغها بصيغة الهيماتوكسلين والايوسين او صبغة زيل نلسن وذلك لغرض الفحص النسيجي الخلوي. ولغرض الدراسة الوبائية تم جمع المعلومات عن المرضى المشخصة حالاتهم بالتدرن من العيادة الاستشارية للأمراض الصدرية والتنفسية في محافظة نينوى ولفترة 16 عاما (1994-2010).

اظهرت النتائج ان اهم العوامل التي تؤدي الى الاصابة بالتدرن هي السكري وبنسبة (25%) من المرضى يليه التدخين وبنسبة (20%) وان اهم التغيرات النسيجية في الانسجة المصابة بالتدرن هو تكون الورم الحبيبي التدرني. واظهرت نتائج الدراسة الوبائية ارتفاع عدد حالات التدرن في محافظة نينوى بين الاعوام 1994-2002 وانخفاضها بين الاعوام 2002-2010. وان عدد حالات التدرن في الرجال اكثر من النساء, وان اهم فئة عمرية تصاب بالتدرن هي عمر التكاثر (15-34 سنة), وان التدرن الرئوي اكثر من التدرن غير الرئوي, وان اعلى نسبة تدرن في قطاعات محافظة نينوى كانت (25%) في كل من الجانب الايسر والجانب الايمن يليه (13.7%) في سنجار.