Antineutrophil cytoplasmic antibodies in patients with tuberculosis

Sahib A. Hussein[1*], May K. Ismael[1], Nahlah G. Abdulmajeed[2]
[1]Department of Biology, College of Science,University of Baghdad, Baghdad, Iraq.
[2]The Medical City/Teaching Laboratories/Baghdad.

Abstract

Tuberculosis is caused by *Mycobacterium tuberculosis;* it is considered as one of the most common, infectious diseases and major causes of morbidity and mortality worldwide. A prospective study was conducted to obtain more clarification about the impact of causative agent and its treatment to enhance autoantibodies production such as ANCA and BPI which used as diagnostic markers for several diseases, and to provide further insight into the classical risk factors (age and sex).Seventy patients with tuberculosis involved in this study, 35 of them were untreated and 35 with treatment administration these patients were attending to directorate of general health national reference laboratory in Baghdad during the period between November/ 2012 and March/ 2013 as well as 20 apparently healthy volunteers as control group. Their ages ranged between 11-70 years.The present study revealed that most patients at the third decades of age; male were affected more than female (1.8:1).Estimation of serum ANCA were done by indirect immunofluorescent (IIF) whereas BPI measured by enzyme linked immune sorbent assay (ELISA) and comparing with healthy control (H.C) group.The current study revealed that high significant increasing of ANCA and BPI in tuberculosis patients 71.43% and 15.71% respectively as compared with H.C 15% and 0% respectively. Also the data of the research showed significant differences of ANCA between untreated group 82.85% and treated group 60%. As well as our results showed differences of BPI percentage between, before treatment group 17.14% and after treatment group 14.28% but non-significant (p> 0.05).These results showed that *Mycobacterium tuberculosis* plays pivotal role in stimulation autoantibodies production. In contrast to our study on the treatment influences had yielded controversial results. Clinically, present of positive ANCA in patients with TB confused the diagnosis of Wegener's granulomatosis as many of the clinical features of this disease eg. Haemoptysis and pulmonary infiltrate may also be found in patients with TB.

Keyword: ANCA, BPI, Tuberculosis.

الاجسام المضادة ضد بعض مستضدات كريات الدم البيضاء العدِلة في المرضى المصابين بالسل الرئوي

صاحب عبد الله حسين*[1]، مي خليل اسماعيل[1]، نهلة غانم عبد المجيد.[2]
[1]قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق، [2]مدينة الطب، المختبرات التعليمية، بغداد

الخلاصة

يعتبر مرض السل الرئوي Tuberculosis الذي تسببه بكتريا *Mycobacterium tuberculosis* من الامراض المعدية والشائعة وأحد الاسباب الرئيسية لحالات الوفاة في العالم ، ولاهميته اجريت هذه الدراسة

*Email: Saat828480@yahoo.com

لتسليط الضوء اكثر حول تأثير البكتريا المسببة لهذا المرض والعلاج المستخدم لها على تحفيز انتاج الاجسام المضادة الذاتية مثل الاجسام المضادة ضد مستضدات الخلايا العدِلة ANCA و BPI والتي تستخدم كمعلمات تشخيصية لامراض اخرى بالاضافة الى دراسة تأثير عوامل الخطورة التقليدية مثل العمر والجنس في هذا المرض. شملت هذه الدراسة 70 مريضا مصاباً بالسل الرئوي (35 مشخصون حديثا وغير معالجين و 35 بعد العلاج) ممن كانوا يراجعون المختبر المرجعي للامراض الصدرية والتنفسية في بغداد خلال المدة بين تشرين الثاني/ 2012 واذار/2013؛ فضلا عن 20 متطوعاً من الأشخاص الأصحاء كمجموعة سيطرة ، تراوحت اعمار مجاميع الدراسة بين 11–70 سنة. بينت الدراسة ان اعمار المرضى الذين يعانون من مرض السل الرئوي هو ضمن حدود العقد الثالث من العمر ، كذلك تبين ان عامل الجنس كان له تأثير ايجابي على المرض اذ ان نسبة اصابة الذكور الى الاناث كانت 1:1.8. استخدمت تقنية التألق المناعي غير الم باشر Indirect immunofluorescent لتقدير مستوى ANCA في حين استخدمت تقنية الامتزاز المناعي المرتبط بالانظيم ELISA لقياس مستوى BPI في مصول مجاميع المرضى والسيطرة . تبين من خلال هذه الدراسة ان نسبة ANCA و BPI قد ارتفعت بشكل ملحوظ وعالي المعنوية في مرضى السل الرئوي 71,43% و 15,71% على التوالي مقارنة مع مجموعة السيطرة 15% و 0% على التوالي. كما اظهرت النتائج وجود فروق معنوية في نسبة ANCA بين مجموعتي المرضى غير المعالجين 82,85% والمرضى المعالجين 60%، كذلك فقد اظهرت نتائج قياس مستوى BPI وجود فروق بين المجموعتين لكنها غير معنوية اذ شكلت النسبة 17,14% في مجموعة غير المعالجين و 14,28% في مجموعة المعالجين($p > 0.05$). وعلى ضوء نتائج هذه الدراسة نجد ان بكتريا *Mycobacterium tuberculosis* تؤدي دورا حيويا في انتاج الاجسام المضادة الذاتية في حين اظهرت نتائج جدلية حول تأثير العلاج على انتاج هذه الاجسام المضادة . ان ظهور ANCA في مرضى السل الرئوي يتداخل مع تشخيص مرض Wegener's granulomatosis كما ان العديد من العلامات السريرية لهذا المرض مثل النفث الدموي Haemoptysis والترشيح الرئوي pulmonary infiltrate كذلك ممكن ان تكون موجودة في مرضى السل الرئوي.

Introduction

Tuberculosis (TB) is a contagious disease caused by a bacterium called *Mycobacterium tuberculosis* (MTB). [1].TB is still one of the major causes of morbidity and mortality worldwide.According to WHO, eight millions cases of tuberculosis occur each year resulting in three millions death cases [2]. Iraqi data show a prevalence of 27/100000 case/yr. with a mortality rate of 11/100000 case/yr. for TB in 2012 [3]. The bacterium spreads from person to person through the air. When people have TB in their lungs it is called pulmonary tuberculosis but when isn't in the lungs it is called extrapulmonary TB [4]. The first line of immune defense against MTB represented by phagocytosis of bacilli by alveolar macrophage and dendritic cells (DCs), these cells release inflammatory mediators; cytokines and chemokines that regulated inflammatory reactions [5]. Other immune cells such as monocyte, polymorphoneuclear, CD4+ and CD8+ migrate to the site of infection but they are unable to kill the bacteria efficiently [6].Polymorphonuclear cells are abundant, motile cellsinvolved in the innate immune response and form an early line of defense against mycobacterial infection. [7] In addition to a direct bactericidal or immunomodulatory effect, neutrophils readily undergo apoptosis, and phagocytosed microbe-containing apoptotic neutrophils can have a stimulatory effect on macrophages and DCs. [8] Neutrophils abundantly express a unique set of neutrophil serine proteases (NSPs), namely proteinase 3 (PR3), myeloperoxidase (MPO), bactericidal permeability increasing protein (BPI), neutrophil elastase (NE), cathepsin G (CG) and lactoferrin (LF), which are stored in the cytoplasmic, azurophilic granules. [9] All of these are implicated in antimicrobial defense by degrading engulfed microorganisms inside the phagolysosomes of neutrophils. [10]Anti-neutrophil cytoplasmic antibodies (ANCA) are auto antibodies react with antigens found in the cytoplasmic granules of polymorphonuclear andmonocytes [11] and can be produce as a result of mycobacterial infection. [7] They have been especially useful in the diagnosis of primary systemic vasculitides (PSV); and are reasonably sensitive and highly specific for small-vessel vasculitides, such as Wegener's granulomatosis (WG), microscopic polyangiitis and Churg–Strauss syndrome. [12] Up to now, there has been no study investigatesome serum autoantibodies like ANCA and anti-BPI,

amongtuberculosis patients in Iraq. Thus, the current study is designed to explore the seroprevalence of anti-neutrophil cytoplasmic antibody (ANCA) and anti-BPI among Iraqi patients with tuberculosis and its possible contribution in pathogenesis of TB which may lead to the false diagnosis of Wegener's granulomatosis (WG) or vice versa.

Materials and method

Seventy consecutive patients with pulmonary tuberculosis (PTB) were enrolled in the study. They were admitted to directorate of general health national reference laboratory in Baghdad between November 2012 and March 2013. Their mean ages were 31.34±1.7 ranged between 11 and 70 years. They had clinical symptoms and radiological signs as well as positive acid fast bacilli under direct microscopic observation with ziehl-Neelsen staining of the sputum and became culture positive for *M. tuberculosis*.Twenty apparently healthy volunteers included in this study as a control group, with mean 29±1.66 years ranged between 10-65 years. These patients were divided into two clinical subgroups 35 before treatment administration and 35 after treatment administration. None of them had a history of any underlying autoimmune diseases or chronic infectious ones. This prospective study was carried out after obtaining the requisite ethics committee permission and informed consents from patients. ANCA were detected in patients' sera by using a commercially available indirectImmuno-fluorescent (IIF) technique on a substrate of ethanol-fixed human neutrophil (Immco, Canda). Briefly, the method is as follows. Neutrophil were used to prepare a substrate and the slide had two lines; one of these lines was contained wells fixed with 96% ethanol and another with 0.45% formalin. After reacting with patient's sera at 1:20 was added polyvalent anti-human IgG labeled with fluorescent stain; then examined under fluorescent microscope. While; anti-BPI identified in patients' sera by antigen bindings ELISA (DIA LAB, Algeria). Sera to be tested were diluted 1:100. A value of <10 U/ml considered as negative and >10 U/ml as positive.

Statistical analysis

All the statistical analyses were done byF-test, Chi square and the calculation of the 95% confidence intervals. A p value less than 0.05 were considered statistically significant [13]

Results and Discussions:

A total of 70 cases with pulmonary tuberculosis (PTB) and 20 apparently healthy control (HC) subjects. Their mean ages were31.4±1.7 years with a range of 11-70 and 29±1.66 with a range of 10-65 years for patients and healthy control respectively. Patients were divided into two groups according to treatment administration, 35 cases newly diagnosed before treatment administration(B.T) and 35 cases were collected after treatment administration(A.T). The drug most widely taken before ANCA testing was streptomycin, isoniazid (INH), rifampicin and ethambutol. The mean of disease duration was 3.5±0.27 weeks and 153±17.8 weeks in before treatment and after treatment groups respectively. The male:female ratio was 1.8:1(M:F = 45:25) and 1.5:1 (M:F = 12:8) for patients and H.C respectively.

ANCA were assessed in the three studied groups. The overall incidence of ANCA in TB patients was 71.43% (50/70) patients that had positive ANCA. Of these positive ANCA patients; 47 patients (94%) showed cytoplasmic immuno-fluorescence pattern (c-ANCA) and three patients (6%) showed perinuclear (p-ANCA) the percentage was significantly higher than that in healthy control which revealed 3/20 patients (15%) had positive ANCA with only c-ANCA pattern (100%) ($p< 0.001$). On the other hand; the percentage of positive ANCA in before treatment group was 82.85% (29/35). Ofthese positive ANCA patients; 28 patients (96.55%) were c-ANCA and one only patient (3.45%) was p-ANCA. Similarity, 60% (21/35) of patients in after treatment group had positive ANCA; 90.47% (19/35) were c-ANCA and 9.53% (2/35) were p-ANCA. Both groups revealed significant differences with each other ($p< 0.05$) and with healthy control group ($p< 0.001$ and $p< 0.02$) respectively. The percentage of positive ANCA patients was illustrated in figure 1; while the ANCA patterns which had been detected by IIF were illustrated in figure 2.

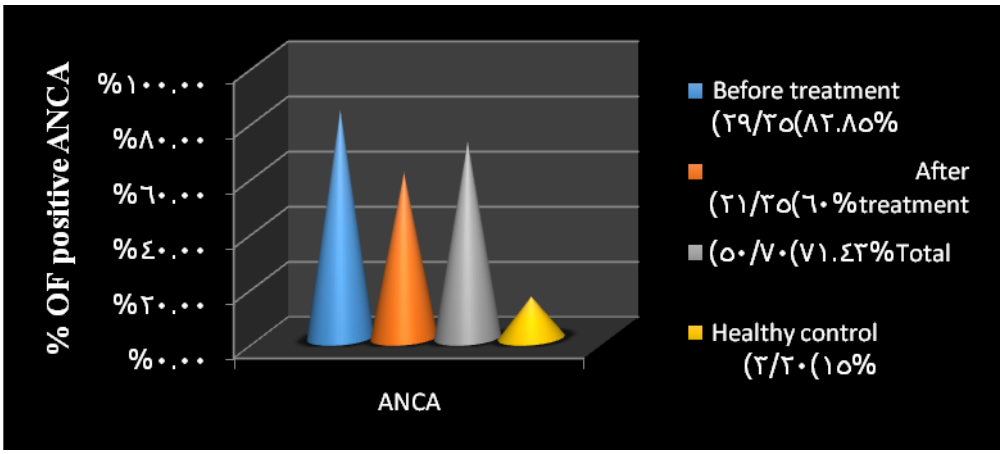

Figure1- Distribution of positive ANCA according to studied groups

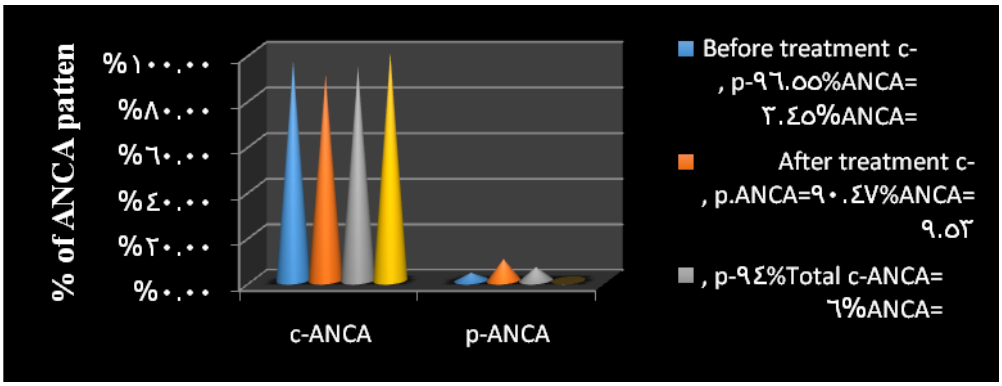

Figure 2- Distribution of positive ANCA Patterns (c-ANCA & p-ANCA) among studied groups

On the other hand; the current study revealed significant differences in the percentage of seropositive anti-BPI between before treatment (B.T) patients group 17.14% (6/35), after treatment (A.T) patients group 14.28% (5/35) and total TB 15.71% (11/70) versus healthy control (H.C) 0.0%.($p< 0.05$, $p< 0.05$, $p<0.01$ respectively). While the seropositive Percentage of anti-BPI was non-significantly higher in B.T patients group than A.T patients group ($p> 0.05$) as illustrated in Figure 3.

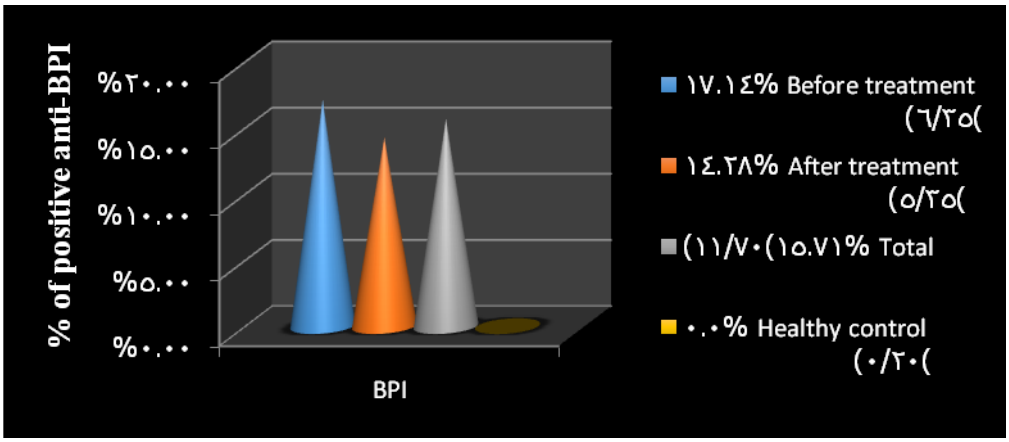

Figure 3- Distribution of positive anti-BPI according to studied groups

These results were compatible with results of local study Al-Kareemi, [2] and another study Pradhan*et al.* [17] who mentioned that the tuberculosis occurs at age mean 32.8 years and the age is considered one of the absolute risk factors to tuberculosis occurrence.

Accordingly, the age is consider as one of the risk factors for tuberculosis in Iraqi population, and third decade of age represented the dangerous age for tuberculosis disease. The explanation for this

may be related to nature of lifestyle, socioeconomic relationships, psychical factors, beside the stress that people exposed. All these agents may act together and enhances the disease development early.

In addition the results showed that most of the patients were male (1.8/1 male to female). This study agrees with previous Iraqi study submitted by Al-Kareemmi [2] as well as Teixeira *et al.*, [19] and Sherkat*et al.*, [15]. It is unclear why more males than females are afflicted with tuberculosis. The conclusion of a recent research was that a combination of biological and social factors responsible for these differences. The immune response to tuberculosis may also be closely related to differences between females and males in type and concentration of non-sex-steroid and sex-steroid hormones secreted. Animal models studies suggest that pregnancy induces Th2 activity, which would be detrimental to the course of tuberculosis [12]. Infection occurs in male more than female may be due to direct contact with bacilli because of carry on life expense in Iraqi society.

ANCA can be divided into four patterns when visualized by IIF; cytoplasmic ANCA (c-ANCA), C-ANCA (atypical), perinuclear ANCA (p-ANCA) and atypical ANCA (a-ANCA), also known as x-ANCA but the main types are c-ANCA and p-ANCA[14].ANCA, especially anti-proteinase 3 (PR3) and Anti-myeloperoxidase (MPO) have been used as diagnostic markers of primary systemic vasculitis [12]. Antineutrophil cytoplasmic autoantibodies (ANCA's) directed to proteinase 3 (PR3-ANCA) or myeloperoxidase (MPO-ANCA) are strongly associated with the ANCA-associated vasculitides: Wegener's granulomatosis, microscopic polyangiitis, and its renal limited form, idiopathic necrotizing crescentic glomerulonephritis, and Churg-Strauss syndrome[15]. In several studies the positivity of ANCA's subtypes in non-vasculitis diseases such as invasive amoebiasis, infective endocarditis, atypical pneumonias, respiratory tract infections, and others have been shown [16]. The results of current study revealed that large number of TB patients exhibited increased percentage of c-ANCA ($p< 0.001$) and significant differences in anti-BPI between groups (B.T, A.T and total T.B) versus apparently healthy control ($p< 0.05$). Interestingly; the percentage of c-ANCA and anti-BPI were reduced after treatment with $p< 0.05$, $p> 0.08$ respectively. The presence of ANCA in TB has been a matter of controversy. Flores-sua'rez and his colleagues using IIF showed that ANCA's were present in 44.4% of the tuberculosis patients, while only 40% of patients showed anti-PR3 and anti-MPO [12]. Another study showed an increased proportion of p-ANCA in up to 25% of Iranian patients suffering from TB[15]. Moreover, Pradhan and coworkers, [17] has reported the presence of ANCA in 3% of TB patients (52.4% P-ANCA, 38.1% c-ANCA and 9.5% atypical Pattern) [17]. In agreement with our results; Elkayam and his colleagues, showed substantial proportion of patients had ANCA before treatment; while the percentage reduced in treated patients [18].On the other hand, Teixeira and his colleagues detected a low prevalence of ANCA in 10% of the patients with TB who had been received treatment including 4% c-ANCA and 6% atypical ANCA [19].The development of autoantibodies in TB patients could have a multifactorial etiology; the presence of T lymphocytes reactive to heat shock proteins (Hsp), and the important aim of immune response against certain intracellular auto antigens such as MPO from PMN, also the mechanism of molecular mimicry, could explain the association of ANCA and TB in patients with severe alterations of their immune response. It has been shown that *M. tuberculosis* can stimulate the release of oxygen metabolites from neutrophils that are activated through interaction with the phenol glycolipids of the cell wall of *M. tuberculosis* [20]. This activation most likely leads to the release of lysosomal enzymes from the neutrophils in the initial stages of the mycobacterial infection, and autoantibodies against the granular components of those cells can develop. Another plausible explanation for the production of ANCA in these patients is the therapy received. It is well known that some drugs, especially isoniazid in the tuberculosis, can be transformed by MPO into active metabolites with the development of cytotoxic products [21]. Presumably, cytotoxicity could damage the neutrophil, in theory, subsequent synthesis of PR-3 and MPO-ANCA, which would explain the observed presence of anti-MPO autoantibodies in some subjects taking drugs like isoniazid. Though one of the studied groups was treated patients, we did not find a correlation between drug and the presence of MPO-ANCA, because the untreated patients group had not received treatment when ANCA testing was performed. Additionally; most patients reacted against PR3 (94% c-ANCA) as well as the percentage of ANCA in after treatment group revealed significant failing in ANCA level compare with before treatment group which indicated that treatment had no influential role to stimulate ANCA synthesis. No relationship was found between any of the drugs patients were taking and the ANCA test results. Tuberculosis may mimic many diseases including Wegener's granulomatosis [17]. ANCA is an important biological

biomarkers used in diagnosis of later disease in association with clinical symptomes. Thus, presence of ANCA positivity in TB patients may fail the diagnosis of Wegener's granulomatosis as many of the clinical features of this disease eg. pulmonary infiltrate and hematuria may also be found in patients with tuberculosis.

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