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Evaluating the Diagnostic Value of Serum Neopterin Levels for Pulmonary Tuberculosis

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Abbreviation used: ADA: adenosine deaminase

DOTS: Directly Observed Treatment, Short Course

ELISA: Enzyme-linked Immunosorbent Assay

NPT: Plasma Neopterin

ROC: Receiver Operating Characteristic Curve

The study was approved by the ethical committee, Deputy of Research and Development, Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1396.544). The ethical committee composition and expertise, including the number of members, and the scope of the review is depicted online on <http://ethics.research.ac.ir/PortalCommittee.php?code=IR.AJUMS.REC>. All participants recruited for the study with informed consent, including permission for publication.

Introduction:

Tuberculosis (TB) is one of the oldest known diseases of human beings, which is still delayed diagnosed and treatment is a major clinical issue worldwide (Ebrahimi Kalan *et al.*, 2018; Gebreegziabher *et al.*, 2016; Martinez *et al.*, 2017). Even higher than AIDS, malaria, and measles, TB is the top cause of death among infectious diseases, claims the 10th rank in the global burden of disease (GBD Tuberculosis Collaborators, 2018). This disease is predicted to keep its current position until 2020 or may stand at the seventh rank (García-Basteiro *et al.*, 2017). Mycobacterium tuberculosis is usually transmitted through the respiratory droplets generated by a patient with infectious tuberculosis (Torrelles & Schlesinger, 2017).

Early diagnosis of tuberculosis is useful for the timely treatment and preventing the development of the bacteria.

Abstract

The aim of the present study was to evaluate the diagnostic value of serum Neopterin levels, an indicator of the cellular immune system activity, in diagnosing pulmonary tuberculosis. A total of 121 individuals including 36 patients with a definite diagnosis of pulmonary TB, 37 healthy individuals (with negative tuberculin skin test), 25 patients with lung cancer and 23 patients with pneumonia were included. The serum Neopterin level measured by the ELISA method using Brahms ELitest Neopterin and coated plate technique. The diagnostic value of serum Neopterin level investigated using ROC curve. We found the male subject had statistically higher serum Neopterin levels compared to female. Neopterin level was significantly correlated with the age of subjects. In spite of adjustment for age and gender, the geometric mean of Neopterin among TB patients (19.88 nmol/l), subjects with lung cancer (18.92 nmol/l), and pneumonia (20.70 nmol/l) were significantly higher than that of healthy individuals (4.57 nmol/l). Comparing TB patients' group with the patients suffering from lung cancer and pneumonia, the area under curve reduced to 55%. For the optimal cut-off-point of 8.05, the estimated sensitivity and specificity were 80.05 and 86.5%, respectively. Finally, the serum levels of Neopterin with good diagnostic accuracy was able to differentiate between TB patients and healthy individuals, though it was not able to differentiate between TB patients and lung cancer or pneumonia patients. It seems the biomarker with other clinical symptoms and biological tests can play a valuable role in shortening the duration of TB diagnosis.

A definitive diagnosis of TB is made when Mycobacterium tuberculosis is detected in a clinical sample (*e.g.* mucus, pus, or a biopsy from a tissue). To detect and diagnose latent TB infection, so far various methods have been introduced, the most important of which include tuberculin skin test and tests based on measuring interferon gamma.

The cornerstone of TB treatment is pharmacotherapy, and the established solution for TB treatment is the DOTS strategy. In most cases, the risk of disease transmission is eliminated after two weeks of initiating effective treatment. With the completion of the treatment course, the infection source would be eliminated from the target population. Therefore, identifying and treating contagious TB patients is the only essential step to prevent the spread of the disease, and for this reason, this treatment is freely provided to TB patients.

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Neopterin, as one of the indicators of the cellular immune system, is secreted in response to the cytokines secreted from lymphocytes and natural stimulated killer cells. Use of the mentioned diagnostic methods has a direct relationship with the experience of the tester and necessitates a great deal of focus and time for staining and accurate observation of all parts of the prepared smear (Eisenhut, 2013). Sometimes it may develop some error, and even it is not available everywhere. Therefore, the existence of other methods capable of diagnosing the disease within a shorter time and yet with acceptable specificity and sensitivity highlights the necessity of availability of a noninvasive diagnostic method for diagnosing pulmonary TB is necessary. It requires especially in such cases when the patient is not able to give mucus or for cases where it is not possible to perform a smear test or mucous culture. The present study was performed with the aim of assessing the diagnostic value of serum Neopterin level in diagnosing pulmonary TB.

Methodology:

The study included groups of healthy individual patients suffering from pulmonary TB, malignant pulmonary disease, and pneumonia. Tuberculosis patients included 36 individual visiting healthcare centers in Ahvaz city who were diagnosed as definitive TB based on the national TB protocol (at least two positive mucus smear or a positive smear test and clinical symptoms, or a positive smear test and radiographic evidence corresponding pulmonary TB or a positive smear test along with positive Mycobacterium tuberculosis culture) (Pedersen *et al.*, 2011). The control group consisted of 37 healthy individuals accompanying the patients visiting the clinic of Razi hospital, for whom the tuberculin skin test was negative. We have also included patients with lung cancer, chosen from patients visiting the clinic of Shafa hospital before receiving the first therapeutic dose of the new course of chemotherapy. The fourth group was patients with pneumonia for whom the disease was properly diagnosed, chosen from Razi hospital. A trained person collected 5 ml of blood from each individual under aseptic conditions. Free anti-coagulant tubes contain blood samples were transferred to Ahvaz Pasteur laboratory under constant temperature (i.e., 0-4°C) within an hour, using a cool box. In the laboratory, samples centrifuged and the blood serum separated. We used ELISA method (Brahms ELItest Neopterin kit) and coated plate technique; serum levels of Neopterin were measured. All the measurement rechecked for quality control.

Mean±SD, frequency (%), and median (25th & 75th percentile) were calculated. The distribution and variance of data were examined, and skewed variable, logarithmically transformed (Ln). The mean difference of Neopterin serum level across different groups was calculated by one-way ANCOVA along with Bonferroni post hoc test. Thus, it was adjusted for gender and age. To

determine the sensitivity and specificity, positive and negative predictive value, and the accuracy of the Neopterin serum level test, ROC analysis was used. Data management and analysis were performed by SPSS 22. The significance threshold level was considered as 0.05.

Results:

The characteristics of the subjects and Neopterin serum levels are presented in Table-1. In total, 55% of the studied population were male, and the age range of the subjects was 19-96 years old (mean=52.35). Also, the mean age of the patient groups was 14-22 years greater than that of healthy individuals.

Table 1: Characteristics of the subjects & Neopterin serum levels

Parameters	TB n= 36	Healthy n= 37	LungCan. n= 25	Pneumonia n= 23
Male, n(%)	28 (77)	11 (30)	15 (66)	13 (56)
Age, mean±SD	57.53±18	39.59±12	54.88±13	62.00±23
Neopterin*	28.25	5.90	14.10	21.00
*Med. (25th, 75th)	(11.77, 41.32)	(3.55, 7.65)	(10.25, 28.75)	(8.90, 56.20)

The range of Neopterin serum level was revealed as 0.3-104. Since Neopterin variable did not show a normal distribution, logarithm transformation was used, and after adjusting for age and gender variables, the difference of means was investigated. The geometric mean of Neopterin among the patients with TB (19.88 nmol/l), lung cancer (18.92 nmol/l), and pneumonia (20.70 nmol/l) was significantly higher than that of healthy individuals (4.57 nmol/l) ($F_{(3,115)}=17.90$, $p<0.00$). In this study, 38% of the variance of Neopterin variable was group dependent (Adjusted R squared=0.384).

Figure-1: The ROC curve for Neopterin level in differentiating TB and healthy individuals

The accuracy of the present test (area under the curve) in differentiating between TB and healthy individuals was estimated as 0.87 (95% CI, 0.777-0.959) (Fig.-1). Point 8.05 was chosen as the optimal cut-off to differentiate between TB patients and healthy subjects. The sensitivity and specificity estimated for this point were 80.05 and 86.5%, respectively. In other words, our data suggest that at this point, the test properly diagnoses 80.05% of the positive

Figure-2: The ROC curve for Neopterin level in differentiating TB and other patients (i.e. lung cancer and pneumonia)

cases reported, and 13.5% of patients will be incorrectly reported as negative (Table-2). Further, the positive and negative predictive values were calculated as 85.29 and 82.05%, respectively. Our data also indicated that (Fig.-2) clinically, Neopterin level had no diagnostic value ($AUC < 0.56$) to differentiate between TB and other patients' groups (lung cancer & pneumonia).

Table 2: Reliability of Neopterin in Differentiating disease and Control (Non-diseased)

Studied Subjects	Test result Cut-point 8.05		Total
	Positive (N)	Negative (N)	
Tuberculosis	29	7	36
Cancer	24	1	25
Pneumonia	20	3	23
Control	5	32	37

Discussion:

In this study, 73% of patients with TB, lung cancer, and pneumonia were with abnormal serum Neopterin levels (< 10 nmol/l), as compared with only one case (2.7%) which was reported as abnormal among the healthy individuals. In this study, the serum Neopterin level above 8.05 to differentiate between TB patients and healthy individuals had a high sensitivity (80.05%) and specificity (86%). However, when the groups of patients with lung cancer or pneumonia were considered as the comparative group, the diagnostic accuracy of this biomarker was minimized ($AUC < 0.56$). It seems that this biomarker alongside other clinical symptoms and biological tests can play a valuable role in reducing the time for diagnosing tuberculosis (Bhatt *et al.*, 2012).

Alveolar macrophages and T-helper lymphocytes constitute the main components of the immune response to mycobacterial infection (Rothchild *et al.*, 2017). Neopterin (NPT) (6-D-erythro-trihydroxypropyl pteridin) is one of the indicators of cellular immune system activity in the body, whose serum levels rise in infections that cause stimulation of the cellular immune system (Plata-Nazar *et al.*, 2015). NPT is produced in 'T' lymphocytes stimulated by interferon-gamma and to a lesser extent by interferon-alpha and beta as well as in macrophages, dendritic cells,

endothelial cells, and to a lesser extent in fibroblasts, smooth muscles of the vascular wall, epithelial cells of kidneys, and from converting guanosine triphosphate to NPT (Kozłowska-Murawska & Obuchowicz, 2008). Since NPT is secreted with the mediation of cytokines secreted from stimulated lymphocytes and natural killer cells, this substance is considered as a marker of cellular immune system activity (Pedersen *et al.*, 2011). Activation of the cellular immune system is observed in conditions such as infections, malignancies, autoimmune diseases, transplant rejection, and atherosclerosis (Pedersen *et al.*, 2011; Arshadi *et al.*, 2013).

Elevated serum levels of NPT were observed in viral infections such as measles, rubella, varicella, viral infections of the respiratory system, viral hepatitis, and HIV, whose level is associated with the activity of the virus (Michalak *et al.*, 2017). Considering non-mycobacterial bacterial infections, reports are available that the urinary excretion of NPT is less compared to viral infections (Eisenhut, 2017). However, in Mycobacterium tuberculosis-induced pneumonia, the urinary NPT level was clearly higher than NPT levels in bacterial pneumonia and cancer (Yuksekol *et al.*, 2003; Ozdemir *et al.*, 2006; Mohamed *et al.*, 2006). The previous study stated that the serum levels of ADA and NPT were considerably higher in pulmonary TB patients compared to other patients, and with treating TB at the end of the two months of treatment, the level of these two biomarkers diminished (Salmanzadeh *et al.*, 2015). Cesur *et al.* (2014) observed that serum levels of NPT in patients with TB were significantly higher than those of healthy individuals. They concluded that the serum NPT level is a helpful marker for diagnosing patients with pulmonary TB. We did not observe a significant difference between serum NPT levels in patients with pulmonary TB and those with lung cancer and pneumonia. Accordingly, the test was not able to differentiate between pulmonary TB patients and lung cancer or pneumonia cases. On the other hand, study shows that the serum levels of NPT were significantly higher in pulmonary TB patients (20.6 ± 12) when compared to lung cancer (14.3 ± 4.7) and pneumonia (10.7 ± 3.7) (Yuksekol *et al.*, 2003).

The results of the present study estimated the diagnostic value of neopterin as large as 86%, which is comparable to 89% obtained in the study by Mohamed *et al.* (2001). In this study, the sensitivity, specificity, positive predictive value, and negative predictive value had been reported as 85%, 93%, 94%, and 84%, respectively, though other studies had presented different results (sensitivity 51% and specificity 92%) (Cok *et al.*, 2007). The reason for this difference could be related to the composition and number of participants in the study, the different measurement accuracy levels of NPT kits utilized, and selection of the studied groups across different stages of the course of TB disease.

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Similar studies have evaluated on different age groups, and none of them had followed a single protocol to select patient and control groups. Possibly, the difference in the studied age groups arose from the rate of incidence of the disease across different regions and different contraction ages. In regions where the disease is endemic and its incidence is high, the disease is observed more at younger ages. For example, in the present study, the mean age in the TB and control groups was 57 ± 18.3 and 39 ± 12.2 years, respectively. However, other studies investigated patients with different age groups (21-41 years), in which the individuals in control groups were often significantly younger (Yuksekol *et al.*, 2003; Ozdemir *et al.*, 2006). Since the age of variable can exert a significant effect on the clinical status of individuals especially serum NPT levels, it is recommended that future studies should consider age factor (equal range) when selecting the groups, or take different age results into account in their analysis. Nevertheless, the advantage of the present study is that in addition to the healthy control group, we also included other groups such as those with lung cancer and pneumonia, in order to have a more suitable setting for comparing and analyzing the diagnostic use of serum NPT levels.

Conclusively, the results of the present study showed that serum NPT levels could differentiate between TB patients and healthy individuals with good accuracy. Nevertheless, this test was not able to differentiate between TB and other conditions including lung cancer and pneumonia. Based on the results of previous studies and the present research, the necessity of developing a standard and international guideline is further highlighted to employ NPT as an auxiliary tool for faster and more effective diagnosis of TB and especially in line with the call by WHO for action to terminate TB disease.

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