Pentraxin 3 Genetic Variants and The Risk of Active Pulmonary Tuberculosis

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Tuberculosis is a major health problem worldwide. Genetic factors are considered important determinants of the host susceptibility to *Mycobacterium tuberculosis*. The aim of the current study was to evaluate the association between pentraxin 3 genetic variants and the susceptibility and severity of active pulmonary tuberculosis. The study included 100 patients with newly diagnosed pulmonary TB and 50 healthy controls. PTX3 plasma level was assayed using ELISA and PTX3 genotypes (rs2305619, rs3816527, rs1840680) were detected by real time PCR in all participants. PTX3 rs1840680 genotype (AA) and allele (A) were significantly higher in the study group while the genotype (GG) was higher in the control group. The plasma level of PTX3 was higher in the patients than controls (*P*<0.0001). There is a strong association between PTX3 plasma level and the activity and severity of pulmonary TB. PTX3 rs1840680 genotype AA is associated with increased risk of active pulmonary TB.

Tuberculosis (TB) is a devastating public health problem with high morbidity and mortality particularly in the developing countries [1]. In Egypt, TB is the third most challenged public health problem following schistosomiasis and hepatitis C [2].

The causative organism of the disease is *Mycobacterium tuberculosis* (MTB) which is an intracellular pathogen, usually transmitted via aerosols and establishes a stable infectious state in the respiratory system. Therefore, the organism is engulfed by macrophages and dendritic cells (DCs) which serve as host cells for survival and propagation of MTB. This pathway highlights the defensive role of the innate immune system against MTB [3].

Pentraxins (PTX) are pivotal components of the innate immune system. They are divided into short pentraxins such as the C-reactive protein and the serum amyloid P and long pentraxins such as PTX3. Pentraxin 3 or Tumor necrosis factor-Stimulated Gene 14 (TSG-14) acts as a soluble pattern recognition receptor (PRR) with an important protective role against selected pathogens [4]. It is expressed by several cell types, including mononuclear phagocytes, dendritic cells and non-immune cells in response to pro-inflammatory cytokines, Toll-like Receptor engagement, microbial components or intact microorganisms [5]. The blood level of PTX3 is low (< 2 ng/ml) in healthy individuals, increases rapidly (maximally at 6–8 hours) and dramatically (200–800 ng/ml) in patients with endotoxic shock, sepsis, inflammatory and infectious conditions [6-8]. It was previously reported that mycobacterial lipoarabinomannan; a major cell wall-associated glycolipid, and whole mycobacteria strongly induce PTX3 production by human mononuclear phagocytes ex vivo [9].

The human PTX3 gene is located on chromosome 3 band q25 and is organized in

three exons separated by two introns. The three exons code for the leader peptide, the N-terminal domain of the protein and the pentraxin domain, respectively [10]. Certain single nucleotide polymorphisms (SNPs) spanning the 6.7 kb length of PTX3 gene were reported. Most of these genetic variations were found in the non-coding regions; rs2305619, rs3816527, rs1840680, rs3845978, rs2614 and rs6788044 while only two described in exons1 and 2 (rs35948036 and rs3816527 respectively) [11]. Interestingly, some of these polymorphisms (rs2305619, rs3816527, rs1840680), especially when associated in specific haplotypes (e.g. GAG haplotype) seem to be protective against some infections as *M. tuberculosis* and *P. aeruginosa* [12,13].

It has been shown that the innate immunity represents the first and main defensive strategy of the body against MTB. Herein; it has been assumed that the innate immunity genes are crucial for modulating the host susceptibility to MTBs. The PTX3 gene was reported to be a potentially relevant gene for host susceptibility to pulmonary TB [12]. Moreover, it was reported that the plasma level of PTX3 correlated well with the disease activity in West Africans patients (14). These findings could be considered as a foundation for studying the role of genetic susceptibility to TB. The aim of the present study was to explore the correlation between the plasma level of PTX3 and the three common SNPs of PTX3 gene (rs2305619, rs3816527 and rs1840680) with the risk of active pulmonary tuberculosis infection.

Patients and Methods

Study Design and study population

This is a case-control study which was conducted during the period from September 2015 to August 2016 at the Central Research Laboratory in collaboration with the Department of Chest Diseases, Sohag University Hospital, Egypt. A total of 100 patients with newly diagnosed pulmonary TB (study group) and 50 healthy individuals without symptoms or signs suggestive of active TB or contact with TB patients (control group) were enrolled into the study. The sample size of the study group was calculated using a program at (www.openepi.com/SampleSize/SSCC.htm), adjusted to achieve 80% power and 5% confidence of significance (type I error). Pulmonary TB was defined by the presence of acid-fast bacilli in Ziehl Neelsen stained sputum smears. The exclusion criteria of the patients were age < 18 years at the time of recruitment, co-existing extra-pulmonary TB, miliary TB, past history of TB, pregnancy, lactation and coexisting medical conditions (diabetes, HIV infection, hepatic failure, malignancy or autoimmune diseases). The study was reviewed and approved by the ethical committee of Sohag Faculty of Medicine, Sohag University and written informed consents were obtained from all participants.

Full medical history was obtained from all participants followed by thorough clinical examination. The severity of the disease was assessed clinically using the Karnofsky score (KS) [15]. Briefly, this score classifies the patients into mild TB (KV ≥ 80) or moderate/severe TB (KS < 80). All participants were then underwent chest X-rays which were evaluated and scored by 2 independent pulmonologists. The chest X-ray cavitation classification (CXR classification) was used for describing the lung pathology in all patients. In summary, this system categorizes the lung pathology into 3 classes according to the presence and the size of cavitations; class 1 (no cavitation), class 2 (< 4 cm in size), and class 3 (> 4 cm in size) [16]. Depending upon the extent of the lung lesions in relation to the corresponding lung field, the severity of TB was then consequently graded into 2 grades; limited (lesions involving < 1/3 of the lung field) or extended (lesions involving > 1/3 of the lung field) [17].

Blood sampling

A total of 5 ml venous blood sample from each participant was collected in ethylene-diamine-tetraacetic acid (EDTA) tubes, centrifuged at 1000 ×g for 15 minutes. Plasma samples were separated in microcentrifuge tubes, stored at -80 for future PTX3 level measurement and the mononuclear cell layer was immediately used for DNA extraction.

Measurement of PTX3 plasma level

The plasma level of PTX3 was measured by an enzyme–linked immunosorbent assay technique using Quantikine® ELISA kit (R&D Systems, Inc. Ca & USA, Cat. Number DPTX30), according to the manufacturer's instructions, and results were determined by the Stat fax 2600 microplate reader (Awareness Technologies, Palm City, USA).

DNA extraction and Genotyping

Genomic DNA was extracted from the mononuclear cell layer using QIAamp DNA Blood Mini Kit (Qiagen, Germany), according to the manufacturer's protocol and stored at −80°C for further genotyping. DNA purity and concentration was evaluated using NanoDrop® Spectrophotometery (wavelength 200-850 nm) (Quawell Q5000, USA). It measures DNA in a 2ul sample in 5-10 seconds with a high degree of accuracy and reproducibility. PTX3 (rs2305619, rs3816527 and rs1840680) SNPs were assessed by the allelic discrimination assay using StepOne real time- PCR system (Applied Biosystem, Ca, USA). Information about the studied SNPs is shown in Table 1. Real-time PCR was done in 25 µl reaction volume containing 12.5 µl TaqMan® Genotyping Master Mix, 1.25 µl specific TaqMan® SNP genotyping assays (ABI, Foster, CA, Cat. # 4351379) and 5 µl (20 ng) of genomic DNA, according to the manufacturer's instructions. The reaction mixture was held at 95°C for 10 min for AmpliTaq Gold enzyme activation, followed by 40 amplification cycles. Each cycle consisted of denaturation at 95°C for 15 s, primer annealing and primer extension at 60°C for 60 s. The study data were analyzed by The TaqMan® Genotyper™ Software.

Statistical Analysis

All statistical calculations were performed using the computer program SPSS (Statistical Package for the Social Science; SPSS, Chicago, IL, USA) version 16 for Microsoft Windows, USA). Data were expressed as mean ± SD or number and percent. Continuous data were compared using Mann-Whitney and ANOVA tests. Genotype distribution was tested for deviation from Hardy–Weinberg equilibrium (HWE) by x^2 analysis. Chi-square test was used to compare the genotypes frequencies in the studied groups. P-value less than 0.05 was considered significant. Graphpad Prism was used to illustrate the PTX3 plasma level according to the 3 SNP genotypes.

Table 1. PTX3 gene SNPs information

SNP ID (rs)	Position (bp)	Localization	Alleles	Peptide shift	Peptide location
rs2305619	157154861	Intron 1	G/A		
rs3816527	157155314	Exon 2	A/C	Ala–Asp	48
rs1840680	157156029	Intron 2	G/A		

Results

The characteristics and the clinical presentations of the participants' data are displayed in table 2.

The PTX3 rs1840680 genotype revealed significant differences between the cases and the controls for both genotypic (AA in cases) (GG in controls) and allelic (A) distribution while the difference of the other two SNPs between the 2 groups was insignificant (table 3).

The plasma level of PTX3 was higher in the study group than the control group (3.78 ± 3.28 Vs 1.4 ± 1.8, $P < 0.0001$). The PTX3 was higher in patients with moderate or severe disease compared to those with mild disease (4.8±3.7 Vs 2.9±2.7, $P<0.05$). The plasma level of PTX3 was higher in CXR class 3 (6.0±3.6, $P<0.05$) and in patients with extended disease (4.7±3.8, $P<0.05$). However, no statistical relationship was found between PTX3 plasma level and age, gender, smoking or history of BCG vaccination (table 4). None of the studied genotypes or the alleles was significantly associated with increased PTX3 plasma level (table 4 & figure 1).

Table 2. Characteristics of patients and controls.

Parameter	Patients (n=100) N (%)	Control (n=50) N (%)
Age		
18-41 y	56 (56%)	35 (70%)
42-65 y	44 (44%)	15 (30%)
Gender		
Male	68 (68%)	33 (66%)
female	32(32%)	17 (34 %)
Smoking history	52 (52%)	30 (60%)
BCG scar	69 (69%)	36 (72%)
Clinical presentation		
mild	55 (55%)	
moderate/severe	45 (45%)	
CXR class		
Class 1	29 (29%)	
Class 2	37 (37%)	
Class 3	34 (34%)	
Lung involvement		
Limited diseases	65 (65%)	
Extended disease	35 (35%)	

CXR; chest x-

Table 3. Distribution of PTX3 SNP genotypes among study participants.

SNP	Patients (n=100)	controls (n=50)	*P-value*
rs2305619			
AA, n (%)	32 (32)	15 (30)	NS
AG, n (%)	48 (48)	27 (54)	NS
GG, n (%)	20 (20)	8 (16)	NS
A-allele, n (%)	112 (56)	57 (57)	NS
G-allele, n (%)	88 (44)	43 (43)	----------
HWE-*P value*	0.8	0.47	----------
rs3816527			
AA, n (%)	30 (30)	10 (20)	NS
AC, n (%)	53 (53)	25 (50)	NS
CC, n (%)	17 (17)	15 (30)	NS
A-allele, n (%)	113 (56.5)	45 (45)	NS
C-allele, n (%)	87 (43.5)	55 (55)	----------
HWE-P value	0.43	0.94	----------
rs1840680			
AA, n (%)	35 (35)	8 (16)	0.015*
AG, n (%)	55 (55)	26 (52)	NS
GG, n (%)	10 (10)	16 (32)	0.0008*
A-allele, n (%)	125 (62.5)	42 (42)	0.015*
G-allele, n (%)	75 (37.5)	58 (58)	----------
HWE-P value	0.083	0.63	----------

*$P > 0.05$ is not significant (NS)

Table 4. Correlation between PTX3 plasma level and patients' studied parameters

Parameter	PTX3 level (ng/ml) Mean (SD)
Age	
18-41 y (n=56)	3.7 (3.2)
42-65 y (n=44)	3.7 (3.7)
Gender	
males (n=68)	3.8 (3.3)
females (n= 32)	3.7 (3.3)
Smoking history	
Present (n =52)	3.6 (3.2)
Absent (n= 48)	3.9 (3.3)
BCG Scar	
Present (n=69)	4.0 (3.3)
Absent (n=31)	3.7 (3.3)
Clinical presentation	
Mild (n=55)	2.9 (2.7)
moderate/severe (n=45)	4.8 (3.7)*
CXR	
Class 1 (n=29)	1.3 (0.8)
Class 2 (n=37)	4.1 (3.5)
Class 3 (n=34)	6.0 (3.6)*
Lung involvement	
Limited (n=65)	2.5 (1.8)
Extended (n=35)	4.7 (3.8)*
PTX3 SNP genotypes	
rs2305619	
AA (n= 32)	3.5 (2.9)
AG (n= 48)	4.0 (3.4)
GG (n= 20)	3.6 (2.4)
rs3816527	
AA (n= 30)	3.8 (2.8)
AC (n= 53)	4.2 (3.1)
CC (n= 17)	3.7 (2.5)
rs1840680	
AA (n= 35)	3.2 (2.4)
AG (n= 55)	3.9 (3.1)
GG (n= 10)	3.8 (2.9)

*$P < 0.05$

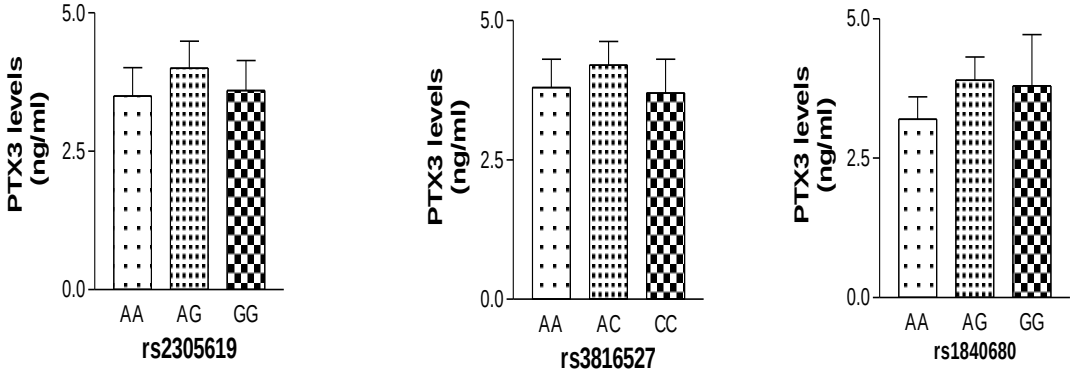

Figure 1. PTX3 plasma level (ng/ml) according to 3 SNP genotypes

Discussion

The present study analyzed the genotypic and allelic distribution of the three common SNPs of PTX3 gene (rs2305619, rs3816527 and rs1840680) in 100 patients with active pulmonary tuberculosis and in 50 control individuals. Only the AA- genotype and A-allele of PTX3 gene SNP (rs1840680), but not the other PTX3 gene SNPs (rs2305619 and rs3816527) or allele frequencies, were associated with increased risk of active pulmonary TB. This finding not only reinforces the concept of the genetic susceptibility to TB but also establishes the role of PTX 3 gene in modulating the immune response to TB. In 2007, Olesen and his colleagues (2007) analyzed five SNPs of PTX3 in patients with pulmonary TB. They reported that 2 SNPs (rs2305619 and rs1840680) were more linked to TB, while the remaining 3(rs3816527, rs3845978 and rs2614) were found to be statistically insignificant [12]. Another study of PTX3 gene SNPs but with a different pathogen, revealed an association of G-allele of rs2305619 and rs1840680 with lung colonization with *Pseudomonas aeruginosa* in cystic fibrosis patients [13].

The plasma level of PTX3, in the present study was significantly higher in the infected than the healthy individuals. Moreover, a significant positive correlation was found between the plasma level of PTX3 and both the disease severity and the degree of lung involvement. These findings extrapolate the value of PTX3 as potentially appropriate indicator of the stage of pulmonary TB. These data perfectly coincides with that reported by Azzuri and his associates (2005) [14].

Although the host susceptibility to TB has been previously reported to be influenced by human genetic factors, very little consistency on the candidate gene has been reported. This could be attributed to the complex phenotype of MTB, genetic heterogeneity and variation of the population as well as to socio-demographic factors [18].

The present study provided additional evidence about the role of genetic factors for modulating the individual susceptibility to pulmonary TB. Moreover, to the best of our knowledge, this is the first study which evaluated both of PTX3 genotypic variations and its plasma level in patients with active TB. Nevertheless, the study has many limitations. Firstly; the small sample size of the patients, which could be attributed to the financial constrains. Secondly; recruiting the patients from the same locality, would render evaluating the effect of the genetic heterogeneity practically invalid. Despite these shortcomings, the current study may open the door for more researches to study the effect of the genetic susceptibility to TB in the Egyptian population.

In conclusion, there is a strong association between the activity and severity of pulmonary tuberculosis with PTX3 plasma level. PTX3 rs1840680 genotype AA is associated with active pulmonary tuberculosis risk.

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