THESIS ABSTRACT

Study of Herd Immunity Associated with Tuberculosis Infections in Basra Province, South of Iraq

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Background: In Iraq, tuberculosis is a real problem worth to be studied carefully to determine all its dimensions.
Aim: To study the herd immunity in tuberculosis infection in Basra population.
Materials and methods: The study conducted in Institute of Tuberculosis and Chest Disease in Basra province during the period from February to September, 2015. The study included 74 cases that were recruited from 392 (age range 4-65 years) subjects attending to the clinic for consultation. Tuberculosis was diagnosed using conventional laboratory test and diagnosis confirmed using Gene Xpert system.
Results: The total number of subjects enrolled in this study was 148, of them 74 patients (33 male and 41 female) with average age of 38.73±23 years and 74 healthy controls (36 male and 38 female) mean age of 35.19±7.86 years. According to the laboratory primary investigation, out of 74 sputa of 74 patients, 66 (89.2%) sputa were positive and eight sputa were negative. However, all 74 sputa were positive when cultured on Lowenstein-Jensen media. Out of 74 diagnosed as TB cases the diagnosis of 32 (43.2%) cases was confirmed by Gene Xpert system and all gave positive diagnosis, of them 7 specimens were positive as rifampicin resistant.

Classification and identification of Mycobacterial cultures depend on *16SrRNA* and accordingly the 74 isolates subdivided into four groups: twenty (27%) isolates were with identity percentage of 100% compared with BCG Iraq, Tokyo strain (172) and Beijing strain. Thirty-one (41.9%) isolates possessed frame shift mutation due of deletion of two nucleotides at positions (+5) and (+11), while addition mutation occurred at (+16) position leading to change three amino acid valine, glutamic acid and arginine into serine, threonine and glutamic acid. Twenty-one (28.4%) isolates had mutation in position (+27) which was same sense mutation in amino acid lead to transversion of guanine into thymine. Two (2.7%) isolates with transversion mutation in the position +857 and +891, the two mutations are same sense, so the arrangement of amino acids in 16S rRNA was not changed. Eight New alleles submitted to Genebank (NCBI) and accepted as new reference alleles from Iraq depends on classification and identification by *16SrRNA*. Further identification for local Mycobacterial isolates was done depending on RD-4 region (*Rv1510*) and RD-1 region (*Rv3877/8*) revealed that the 74 local Mycobacterial isolates consist of 65(88%) isolates as MTB, 6(8%) isolates as *M.bovis* and 3(4%) isolates as *M. bovis* (BCG). Study of the virulence factors associated with MTB depending on insertion region (IS1081) revealed that there is single nucleotide polymorphism (SNP) in the position (+72) downstream the first amplified nucleotide, this SNP leads to transversion mutation converting cytosine C into adenine. Statistical comparison explained that (A)

allele is more dominant than (C) allele with percentage of 62.3% and 27.7% respectively and with significant difference *(P=0.0001)*.

Study of the polymorphisms in *Cfp-10* gene demonstrated that the difference with universal reference strains found in two positions 33 and 170 with C and A allele instead of G and G allele respectively for all MTB isolates. New cfp-10 allele submitted to Genebank (NCBI) and accepted as new reference allele from Iraq.

Detection of polymorphisms in *ESAT-6* show a results indicating that Iraqi isolates differ from low *ESAT-6* secreted strains in four positions while differ from highly secreted strain in two positions, Iraqi strains in position closer to hypervirulence strains. New ESAT-6 allele submitted to Genebank (NCBI) and accepted as new reference allele from Iraq.

The results of genetic analysis of mycolic acid gene (whiB7) revealed that there are differences between wt1 region of Iraqi isolates and MTB reference strains sequences in NCBI in two positions, two mutations in the promoter region and ORF of whiB7 gene at -191 and +35(change Proline into Glutamine). Phylogenetic analysis referred that Iraqi isolates in subject of whiB7 are more closely relatedness to Korean strain than Beijing strain and XDR strains. New wt1 allele submitted to Genebank (NCBI) and accepted as new reference allele from Iraq. Study of predisposing factors associated with host showed that there are nine single nucleotide polymorphisms (SNPs) in IFN-γ promotor of patients but not control, (G→T) SNP at locus (-714) was the most frequency. Three IFN-γ new alleles registered in NCBI at the first time, found in Iraqi community. Polymorphism gene was found in *INFGR1* by present of various numbers of CA motifs, the genetic analysis showed that the individuals which had CA17 and CA22 appeared to be the most susceptible to infection with TB while CA13 ,CA14 and CA19 repeats of are responsible for protective against tuberculosis. One hundred eighty SNPs were recorded in TNF promoter of patients. (G→T) was the most dominance SNP with frequency of 52(28%), two new TNF alleles registered in ENA at the first time which found in Iraqi community. The results revealed that there is no mutation was found in the studied regions of IL-12.Genotyping of IL-10 promoter displayed that range from 19 to 25 motifs but without 23-motif repeat. Individuals with CA21, CA22 and CA24 appeared to be the most susceptible to infection with (TB) with PIC larger than (0.5). CA20 is responsible for protective against tuberculosis, the t-value showed that there were significant differences between the patients and controls groups, with PIC larger than (0.5). Genotyping analysis of IL-4 determined that patients divided in subject of IL-4 into two carriers alleles C and T, of them C allele is predominant (73%), while T allele is recessive (24%). The results of present study appeared that there are two SNPs recorded in the amplified region of promoter of IL-2, which are A→C and T→G with frequencies of 0.1 and 0.19 respectively. While Genotyping of TLR-2 revealed shows that out of 74 patients with TB 38 (51.3%) patients found to possess C allele (T→C), while 36 (48.7%) possessed T allele.

The evaluation of cytokines IFN-γ, TNF-α, IL-2 and IL-12 are measured in TB patients serum. The mean IFN-g serum level was significantly depressed in patients with TB (26.92 pg/ml) compared with healthy control (28.40 pg/ml), with standard deviation (5.29) and (10.73) respectively. The results of evaluation of TNF-α concentration showed significant elevation in patients compared to healthy control. The mean concentrations were 43.969 ± 28.72 pg / ml for patients and 35.60 ± 8.64 f pg / ml or healthy controls with significant difference ($P \leq 0.05$). Significant elevation also recorded in serum level of IL-2 of patients compared to healthy control, the mean

of concentrations was 96.35± 23.39 pg/ ml for patients and 89.08 ± 13.96 pg / ml or healthy controls with significant difference ($P \leq 0.05$). The results indicate that there is no significant difference ($P \leq 0.05$) in the level of IL-12, the mean of concentrations was 12.7± 3.98 pg/ml for patients and 12.21 ± 6.01 pg/ ml for healthy controls.

Conclusion: New cfp-10 allele submitted to Genebank (NCBI) and accepted as new reference allele from Iraq. Eight New alleles submitted to Genebank (NCBI) and accepted as new reference alleles from Iraq depends on classification and identification by *16SrRNA*. Phylogenetic analysis referred that Iraqi isolates in subject of whiB7 are more closely relatedness to Korean strain than Beijing strain and XDR strains. New wt1 allele submitted to Genebank (NCBI) and accepted as new reference allele from Iraq.