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FULL PAPER

Cervical lymph node tuberculosis and *TNF*, *IL8*, *IL10*, *IL12B* and *IFNG* polymorphisms

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SUMMARY

Cervical lymph node tuberculosis (LNTB) is the most common manifestation of extrapulmonary tuberculosis, resulting from the interaction of environmental and genetic factors. The immune response against TB is regulated by several cytokines, which have single nucleotide polymorphisms (SNPs), leading to different levels of expression. The aim of this study was to evaluate the association of LNTB with the *TNF*, *IL8*, *IL10*, *IL12B* and *IFNG* gene polymorphisms in Mexican patients. We investigated the association of ten SNPs in 14 patients with LNTB and 138 healthy controls. Significant differences were found for the allele *TNF*–238A (P=0.03) and the genotypes *TNF*–238GA (P=0.03), *IL8*+396GG (P=0.01) and *IL12B*+1188CC (P=0.04). Allele *IL8*+781C showed some association trend (P=0.08). Haplotypes *TNF*-AA and *IL10*-GTA were of susceptibility, whereas haplotype *IL8*-ATT was of protection. No association was found with *IFNG*. The association of these polymorphisms with extrapulmonary TB was compared in different populations. Our results suggest that these cytokine SNPs may influence the manifestation of LNTB in Mexican patients; however, we are aware of the limitations of our study, so it is necessary to make a replica using a larger sample of patients, as well as an increased number of cytokines with SNPs.

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INTRODUCTION

Tuberculosis (TB) is the second cause of death from infectious diseases. In 2016, TB killed 1.3 million non-HIV-infected and 374,000 HIV-infected individuals, and there were an estimated 10.4 million new TB cases. Every year, 40 million persons get infected with *Mycobacterium tuberculosis* (*Mtb*) all over the world, only 10.4 million those having clinical disease (WHO, 2018). Each infection has a risk of 5% to 10% for the disease to progress, if not treated (CDC, 2013). In Mexico, TB is a significant health problem, since in 2012 the incidence was 16.8 cases per 100,000 habitants, and an estimated 25,000 cases were documented in 2013 (SINAVE/DGE/SALUD, 2012; PAHO/WHO, 2015). Some studies suggest that from 30% to 40% of the Mexican adult population is infected by *Mtb* and, therefore, are at risk of developing TB by re-activation (García-Sancho *et al*., 2006). In addition, in both developed and developing countries, including Mexico, the frequency of TB transmission with rapid progression to the disease is around 28%-40% (García-García *et al*., 2000).

Cervical lymph node tuberculosis (LNTB) is considered the most common extrapulmonary manifestation of the disease in developing countries. Lymph nodes may be affected in primary or secondary tuberculosis due to dissemination of the adjacent circulation (Ilgazli *et al*., 2004; Golden & Vikram, 2005). Usually, LNTB appears in young adults between 20 and 40 years old, with manifestation around 6 to 9 months after the initial infection (Popescu *et al*., 2014), and a preference for posterior cervical and supraclavicular ganglion chains (Ilgazli *et al*., 2004; Singh *et al*., 2016). Diagnosis could be established based on patient's anamnesis, complete physical examination, tuberculin test and acid-fast staining, but must be confirmed by histopathology (Zaatar *et al*., 2009).

Key words:
Cytokine polymorphism, disease susceptibility, extrapulmonary TB, cervical lymph node tuberculosis, SNPs.

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The immune response against TB is regulated by the interaction of lymphocytes, antigen-presenting cells, and cytokines. During infection, active phagocytes secrete pro-inflammatory cytokines such as IL (interleukin) 12 and TNF (tumor necrosis factor). IL12 induces the production of T helper (Th) type 1 cytokines by natural killer (NK) and T cells, for example IFN (interferon)-γ, to activate infected macrophages and eliminate *Mtb* (Flynn & Chan, 2001). Expression *in vivo* or *in vitro* of TNF activates IL10 production and this interaction (IL10/TNF) is involved in pulmonary tuberculosis (PTB) progression (Bean *et al*., 1999). IFNγ is a key Th1 cytokine produced primarily by NK and T cells; its production plays a pivotal role in macrophage activation for controlling *Mtb* infection (Collins & Kaufmann, 2001). Mice with a disrupted *IFNG* gene cannot produce reactive nitrogen intermediates when challenged, which restricts the increase in bacilli (Cooper *et al*., 1993). Humans with an inherited complete or partial IFNγ receptor deficiency are highly susceptible to infection by atypical mycobacteria (Casanova & Abel, 2004).

Elevated levels of IL8 have been described in several fluids of patients with TB, as well as in granulomas (Dlugovitzky *et al.,* 1997; Mastroianni *et al*., 1994; Bergeron *et al*., 1997); this chemokine is produced by leukocytes and endothelial cells in response to *Mtb* or its components (Zhang *et al*., 1995; Lin *et al*., 1998). Fibroblasts are also potent secretors either *in vivo* or *in vitro* of IL8, being the major potential source of this chemokine in TB. Its production restricts the intracellular growth, and enhances the macrophage killing of *Mtb*, suggesting a key role for fibroblasts in the immune response to *Mtb* infection (O'Kane *et al*., 2007). Although genes that code for cytokines have a relative low frequency of genetic variation, a growing number of association studies have involved single nucleotide polymorphisms (SNPs) as host factors influencing susceptibility to infectious diseases. These SNPs may be located on the promoter; coding and non-coding regions of the cytokine genes and some of them may influence their expression (Wilkinson, *et al*. 1999; Oral *et al*., 2006).

Reports on the association of cytokine polymorphisms with EPTB are limited and generally make no distinction in the type of EPTB. Therefore, the objective of this study is to determine if cervical lymph node tuberculosis is associated with any of the 10 selected polymorphisms of the *TNF, IL8, IL10, IL12B* and *IFNG* genes in a sample of the Mexican population.

MATERIALS AND METHODS

Study population

Fourteen patients with histopathology-confirmed LNTB attending the Otolaryngology Service of the General Hospital "Dr. Manuel Gea Gonzalez" in Mexico City were included. The control group was comprised of 138 subjects, with no current evidence or history of LNTB, paired by age and sex with patients. This work complies with the current health laws of Mexico and with the 1964 Helsinki Declaration and its later amendments, and was approved by the Ethics in Research and Research Committees of the General Hospital "Dr. Manuel Gea Gonzalez". Written informed consent was obtained from each participant before sampling.

Genotyping

DNA was obtained from 20 ml of EDTA-peripheral blood using proteinase K and phenol/chloroform extraction. Ten SNPs of the genes *TNF, IL8, IL10*, *IL12B* and *IFNG* were amplified by polymerase chain reac-

Table 1 - *Sequences of the primers and probes used for genotyping cytokine polymorphisms.*

	Primers	*Probes*	
TNF–308 (rs1800629)	F: 5'-AGGCAATAGGTTTTGAGGGCCAT-3' R: 5'-TCCTCCCTGCTCCGATTCCG-3'	Endonuclease: *NcoI*	Allele G: 87 + 20 bp Allele A: 107 bp
TNF–238 (rs361525)	F: 5'-GCCCCTCCCAGTTCTAGTTCTATC-3' R: 5'-AAGCGGTAGTGGGCCCTGCACCTT-3'	TNF–238-G TNF–238-A	5'-GGAATCGGAGCAGGGA-3' 5'-GGAATCAGAGCAGGGA-3'
IL8–251 (rs4073)	F: 5'-TCTAACACCTGCCACTCTAG-3' R: 5'-CCTGAGTCATCACACTTCCT-3'	IL8(–251)A IL8(–251)T	5'-CATACATTTGATAATTCA-3' 5-'CATACAATTGATAATTCA-3'
IL8+396 (rs2227307), +781 (rs2227306)	F: 5'-CTCTGTGTGAAGGTAAGCAC-3' R: 5'-TATCAACAGGCACAGCTCTG-3'	IL8(+396)G IL8(+396)T IL8(+781)C IL8(+781)T	5'-ATGCATGCTACATGGTATAA-3' 5'-ATGCATGCTAAATGGTATAA-3' 5'-ACATTGAACGACTTCCTAT-3' 5'-ACATTGAACAACTTCCTAT-3'
IL10–1082 (rs1800896), –819, (rs1800871), –592 (rs1800872)	F: 5'-ATCCAAGACAACACTACTAA-3' R: 5'-TAAATATCCTCAAAGTTCC-3'	IL-10(–1082)1A IL-10(–1082)1G IL-10(–819)2C IL-10(–819)2T IL-10(–592)3C IL-10(–592)3A	5'-CTTTGGGAAGGGGAAGTA-3' 5'-CTTTGGGAGGGGGAAGT-3' 5'-GTGATGTAACATCTCTGTG-3' 5'-GTGATGTAATATCTCTGTG-3' 5'-CGCCTGTCCTGTAGGAAG-3' 5'-CGCCTGTACTGTAGGAAG-3'
IL12B +1188 (rs3212227)	F: 5'-TTCTATCTGATTTGCTTTA-3' R: 5'-TGAAACATTCCATACATCC-3'	IL12B(+1188)A IL12B(+1188)C	5'-TGTATAGTTAGATGCTAAATG-3' 5'-GTATAGTTCGATGCTAAATG-3'
IFNG +874 (rs2430561)	F: 5'-TATGATTCTGGCTAAGGAATGT-3' R: 5'-ACGAGCTTTAAAAGATAGTTCC-3'	IFNG (+874)T IFNG (+874)A	5'-AAATCAAATCTCACACACACAC-3' 5'-AAATCAAATATCACACACACAC-3'

tion (PCR): *TNF* –308 G>A (rs1800629), –238 G>A (rs361525); *IL8* –251 A>T (rs4073), +396 G>T (rs2227307), +781 C>T(rs2227306); *IL10* –1082 A>G (rs1800896), –819 C>T (rs1800871), –592 C>A (rs1800872), *IL12B* +1188 A>C (rs3212227), and *IFNG* +874 T>A (rs2430561), with the primers and probes described in *Table 1*. PCR was performed in 100 μl total volume, containing 50 mM KCl; 10 mM Tris–HCl, pH 8.3; 2 mM $MgCl_2$; 200 mM of each dNTP; 0.25 mM of each primer (Eurofins MWG Operon, USA); two units of Taq polymerase (Epicentre Biotechnologies, USA); and 200 ng of DNA. PCR amplified DNA was blotted onto nylon membranes and hybridized with digoxigenin-11-ddUTP labeled oligonucleotide probes and visualized by chemiluminescence (Bignon & Fernandez-Vina, 1996).

Statistical analysis

Allele (AFs) and genotype (GFs) frequencies were calculated by direct counting. Differences in frequencies between groups were compared using a Chi-squared test with Yate's correction, considering $P \leq 0.05$ as the minimum level of significance. Two-tailed Fisher's exact test was used when the expected frequency in at least one cell was less than 5. Relative risk was calculated as an odds ratio (OR). Ninety-five percent confidence intervals (95% CI) were obtained by using Cornfield's approximation. Haplotypes and linkage disequilibrium (LD) blocks were determined by confidence interval method using Haploview 4.2 software (http://www. broadinstitute.org) (Barrett *et al.*, 2005). To establish the most informative model of Mendelian association (dominant, co-dominant, recessive, over-dominant, or log additive), the online SNPStats program was used (http://bioinfo.iconcologia.net/es/SNPStats_ web) (Solé *et al.*, 2006).

RESULTS

The polymorphisms of three cytokine genes (*TNF*, *IL10* and *IFNG*) that are known to be closely related to pulmonary tuberculosis, as well as those of two cytokine genes (*IL8* and *IL12B*) that have not been extensively investigated in TB patients but might be relevant to the disease, were selected and are described below. The *TNF* poly morphisms rs1800629 (-308G>A) and rs361525 (-238G>A) are known to be directly related to the expression levels of this cytokine, which is also important in susceptibility to TB (Higuchi *et al.*, 1998; Sharma *et al.*, 2008). Three polymorphisms of the *IL10* gene have been implicated on susceptibility to TB: rs1800896 (-1082 A>G), rs1800871 (-819 C>T), and rs1800872 (-592 C>A) (Liu *et al.*, 2015; Silva *et al.*, 2020). IFN-γ is a pro-inflammatory cytokine that plays an important role in resistance to TB, and the polymorphism rs2430561 (+874 A>T) of the *IFNG* gene is related to different levels of expression of this cytokine and susceptibility to TB (Wu *et al.*, 2019). *IL12B* rs3212227 (+1188 A>C) is one of the key polymorphisms that have functional effects and are associated with susceptibility to TB (Liu *et al.*, 2014). Some studies suggest that the IL8 polymor-

Table 2 - *Allele frequencies of the TNF, IL8, IL10, IL12B and IFNG polymorphisms in CLNTB[#].*

Gene	*Alleles*	*Cases (%)*	*Controls (%)*	*P*	*OR(95%CI)**
TNF	***–238G***	***88.46***	***98.06***	***0.03***	***0.14 (0.03-0.71)***
	–238A	***11.54***	***1.94***	***0.03***	***6.70 (1.41-31.82)***
	–308G	88.46	94.66	0.41	0.39 (0.10-1.52)
	–308A	11.54	5.34	0.41	2.53 (0.64-9.74)
IL8	–251A	73.08	63.04	0.42	1.53 (0.62-3.75)
	–251T	26.92	36.96	0.42	0.65 (0.27-1.61)
	+396G	57.69	42.81	0.21	1.79 (0.79-4.06)
	+396T	42.31	57.19	0.21	0.56 (0.25-1.25)
	+781C	***80.77***	***61.38***	***0.08***	***2.46 (0.89-6.76)***
	+781T	19.23	38.62	0.08	0.41 (0.15-1.11)
IL10	–1082G	38.46	45.31	0.64	0.76 (0.34-1.76)
	–1082A	61.54	54.69	0.64	1.30 (0.57-2.98)
	–819C	57.69	54.65	0.93	1.12 (0.49-2.53)
	–819T	42.31	45.35	0.93	0.89 (0.39-2.02)
	–592C	46.15	61.02	0.21	0.55 (0.25-1.24)
	–592A	53.85	38.98	0.21	1.81 (0.81-4.08)
IL12B	+1188C	83.33	66.54	0.14	2.29 (0.76-6.92)
	+1188A	16.67	33.46	0.14	0.43 (0.14-1.31)
IFNG	+874A	61.54	60.92	0.88	1.00 (0.44-2.32)
	+874T	38.46	39.08	0.88	0.99 (0.43-2.27)

[#]Cervical lymph node tuberculosis. *Odds ratio (95% confidence interval). Characters in bold and italics indicate association. Characters only in bold indicate trend of association.

Table 3 - *Genotype frequencies of the TNF, IL8, IL10, IL12B and IFNG polymorphisms in CLNTB[#].*

Gene	*Genotypes*	*Cases (%)*	*Controls (%)*	*P*	*OR (95%CI)**
TNFα	***–238GG***	***76.92***	***96.12***	***0.03***	***0.13 (0.02-0.69)***
	–238GA	***23.08***	***3.88***	***0.03***	***7.37 (1.44-37.69)***
	–308GG	76.92	89.32	0.40	0.37 (0.09-1.56)
	–308GA	23.08	10.68	0.40	2.68 (0.64-11.24)
IL8	–251AA	46.15	34.06	0.57	1.67 (0.53-5.25)
	–251AT	53.85	57.97	0.99	0.84 (0.26-2.63)
	+396GG	***23.08***	***3.60***	***0.01***	***8.2 (1.69-39.14)***
	+396TT	7.69	17.99	0.58	0.54 (0.07-4.34)
	+396GT	69.23	78.42	0.68	0.59 (0.17-2.04)
	+781CC	61.54	42.28	0.29	2.10 (0.65-6.80)
	+781CT	38.46	38.21	0.78	1.04 (0.32-3.37)
IL10	–1082GG	7.69	10.94	0.91	0.95 (0.11-7.85)
	–1082AA	30.77	20.31	0.60	1.83 (0.52-6.42)
	–1082GA	61.54	68.75	0.82	0.71 (0.22-2.29)
	–819CC	30.77	20.16	0.59	1.85 (0.53-6.48)
	–819TT	15.38	10.85	0.97	1.73 (0.35-8.62)
	–819CT	53.85	68.99	0.42	0.52 (0.16-1.65)
	–592CC	0.00	25.98	0.15	0.22 (0.01-3.77)
	–592AA	7.69	3.94	0.93	2.67 (0.29-24.79)
	–592CA	92.31	70.08	0.17	3.58 (0.45-28.55)
IL12B	+1188CC	66.67	33.09	0.04	3.79 (1.09-13.29)
	+1188CA	33.33	66.91	0.04	0.26 (0.08-0.92)
IFNG	+874AA	23.08	21.85	0.80	1.17 (0.30-4.59)
	+874AT	76.92	78.15	0.80	0.85 (0.22-3.32)

[#]Cervical lymph node tuberculosis. *Odds ratio (95% confidence interval). Characters in bold and italics indicate association. Characters only in bold indicate trend of association.

Table 4 - *Haplotype frequency of the TNF, IL8 and IL10 polymorphisms in CLNTB[#].*

Gene	*Haplotypes*	*Cases (%)*	*Controls (%)*	*P*	*OR (95%CI)d*
TNF[a]	GG	88.10	93.20	0.34	0.54 (0.15-1.99)
	AG	0.40	4.90	0.29	0.08 (0.00-38.90)
	AA	***11.10***	***0.50***	***<0.001***	***15.82 (2.54-260.13)***
	GA	0.40	1.40	0.65	0.26 (0.00-144.25)
IL8[b]	AGC	41.40	27.00	0.32	1.91 (1.05-3.46)
	TTC	9.00	15.70	0.15	0.53 (0.22-1.27)
	TTT	7.30	14.50	0.10	0.46 (0.18-1.18)
	ATC	21.70	13.00	0.10	1.85 (0.87-3.93)
	ATT	***4.30***	***14.10***	***0.01***	***0.27 (0.09-0.83)***
	AGT	5.60	9.10	0.34	0.59 (0.19-1.76)
	TGC	8.70	5.50	0.38	1.63 (0.54-4.95)
	TGT	2.00	1.30	0.69	1.55 (0.17-14.35)
IL10[c]	ACA	34.90	26.80	0.37	1.47 (0.63-3.45)
	GTC	18.90	25.70	0.45	0.67 (0.24-1.86)
	ATC	11.20	17.10	0.44	0.61 (0.17-2.16)
	GCA	6.70	10.10	0.56	0.62 (0.13-3.12)
	ACC	10.40	9.60	0.89	1.09 (0.29-4.13)
	GCC	5.70	8.70	0.61	0.64 (0.12-3.54)
	GTA	***7.20***	***1.20***	***0.02***	***6.59 (1.02-42.57)***
	ATA	5.10	1.00	0.09	5.29 (0.61-45.88)

[#]Cervical lymph node tuberculosis. [a]TNF haplotypes: -308, -238; [b]IL8 haplotypes: -251, +396, +781; [c]IL10 haplotypes: -1082, -819, -592; [d]Odds ratio (95% confidence interval). Characters in bold and italics indicate association.

phisms rs4073 (- 251A>T), rs2227307 (+396 G>T) and rs2227306 (+781T>C) are involved in respiratory diseases, including TB, and we wanted to determine their participation, if any, on LNTB.

The male to female ratio in the case group was 1:1 and the average age was 29.28±17.5 years. Only one patient presented arterial hypertension as comorbidity. The average age was 28.16±13.6 years in the control group and the male to female ratio was 1.2:08.

AFs and GFs of the *TNF, IL8, IL10*, *IL12B* and *IFNG* polymorphisms are presented in *Tables 2* and *3*, respectively. In every instance, the co-dominant model is shown. Only the allele *TNF*–238A (*P*=0.03; OR [CI95%] 6.7 [1.41-31.82]) was associated with susceptibility to LNTB, and reciprocally the allele *TNF*–238G is related with protection. A trend of association with the allele *IL8*+781C (*P*=0.08; OR [CI95%] 2.46 [0.89-6.76]) was observed. Genotypes *TNF*–238GA, (*P*= 0.03; OR [CI95%] 7.37 [1.44-37.69]), *IL8*+396GG (*P*=0.01; OR [CI95%] 8.2 [1.69-39.14]) and *IL12B*+1188CC (*P*=0.04; OR [CI95%] 3.79 [1.09-13.29]) were significantly increased in LNTB patients, whereas genotypes *TNF*–238GG, (*P*=0.03; OR [CI95%] 0.13 [0.02-0.69]) and *IL12B*+1188CA were protective. No association was found with *IL10* and *IFNG* alleles or genotypes.

Haplotype frequency is shown in *Table 4*. Haplotypes *TNF*AA (*P*<0.001; OR [CI95%] 15.82 [2.54-260.13]) and *IL10*GTA (*P*=0.02; OR [CI95%] 6.59 [1.02-42.57]) were associated with susceptibility, and the haplotype *IL8*ATT (*P*=0.01; OR [CI95%] 0.27 [0.09-0.83]) with protection.

DISCUSSION

Several features of the host, the pathogen and the environment are involved in TB pathogenesis. The goal of the present study was to relate 10 SNPs of the genes *TNF, IL8, IL10, IL12B* and *IFNG* with LNTB in a sample of the Mexican population.

To compare the association of these polymorphisms with EPTB in different populations, we carried out an exhaustive review of the literature (summarized in *Table 5*). Twelve studies have been conducted on the participation of *TNF* and EPTB polymorphisms, of which only six show association (Ben-Selma *et al*., 2011b; Zhu *et al*., 2012¸ Lv *et al*., 2016; Zhou *et al*., 2017; Zheng *et al*., 2018; Qiu *et al*., 2018). A risk allele was found in a Tunisian population (*TNF*–308A) (*P*=0.024) (Ben-Selma *et al*., 2011b). In the Chinese population, one study showed an association between the risk allele *TNF*–308A (*P*=0.006) and the genotype *TNF*–308GA (*P*=0.003) with osteoarticular TB (Lv *et al*., 2016). Two studies investigated the effect of *TNF* gene polymorphisms in spinal TB: one found a protective effect of the allele *TNF*–238A (*P*=0.04) and the dominant model *TNF*–238AA+AG (*P*=0.05) (Zhou *et al.,* 2017); the other did not found any significant association for *TNF*–238 or *TNF*–308 but did show an association with the genotype *TNF*–857CT (*P*<0.001) and the recessive model *TNF*–857CT+TT (*P*<0.001) (Zheng *et al.,* 2018)

One meta-analysis showed the association between the risk of PTB+EPTB and the genotypes *TNF*–857CT (*P*<0.001) and *TNF*–857CC (*P*=0.01), while no association was found with *TNF*–308 (Zhu *et al.,* 2012). On the contrary, another meta-analysis reported an association with the dominant and the allele model of *TNF*–238 (*P*=0.004, *P*=0.006, respectively) and *TNF*–308 (*P*<0.001, *P*<0.001, respectively) polymorphisms (Qiu et al., 2018). Our results differ from those previously reported indicating that susceptibility to LNTB is associated with the *TNF*–238A allele and the *TNF*–

Table 5 - *Studies of polymorphisms related to the pathogenesis of EPTB.*

Gene	*Polymorphism*	*P value*	*Population*	*Reference*
TNF	-308A	*P*=0.024	Tunisian	Ben-Selma *et al.*, (2011)
	-857CT -857CC	*P*<0.001 *P*=0.01	Analysis of all populations	Zhu *et al.*, (2012) Meta-analysis
	-308A -308GA	*P*=0.006 *P*=0.003	Chinese	Lv *et al.*, (2016)
	-238A -238AA+GA	*P*=0.04* *P*=0.05*	Chinese	Zhou *et al.*, (2017)
	-857CT -857CT+TT	*P*<0.001 *P*<0.001	Chinese	Zheng *et al.*, (2018)
	-238GG -238G -308GG -308G	*P*=0.004 *P*=0.006 *P*<0.001 *P*<0.001	Analysis of all populations	Qiu *et al.*, (2018) Meta-analysis
	-238A -238GA	*P*=0.03 *P*=0.03	Mexican	This study
IL8	-251TA -251AA -251TA -251AA	*P*=0.07 *P*<0.01 *P*<0.01 *P*<0.01	Caucasian African-American	Ma *et al.*, (2003)

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Gene	*Polymorphism*	*P value*	*Population*	*Reference*
	-251TA+AA	*P*=0.02	Analysis of all populations	Yu *et al.*, (2019) Meta-analysis
	+781C +396GG ATT haplotype	*P*=0.08* *P*=0.01 *P*=0.01*	Mexican	This study
IL10	-1082AA	*P*=0.02	Colombian	Henao *et al.*, (2006)
	-1082G	P_c=0.028	Turkish	Oral *et al.*, (2006)
	-1082G GCC haplotype	P_c<0.001 P_c<0.001	Turkish	Ates *et al.*, (2008)
	-592CA -592AA	*P*=0.003* *P*=0.003*	Peruvian	Taype *et al.*, (2010)
	-1082GG	P_c=0.003	Egyptian	Mosaad *et al.*, (2010)
	-1082G -1082AG	P_c=0.0002 P_c=0.0003	Tunisian	Ben-Selma *et al.*, (2011)
	-819T -592A	P_c=0.042 P_c=0.042	Chinese	Liang *et al.*, (2011)
	-1082AA	*P*=0.009	Indian	Ramaseri Sunder *et al.*, (2012)
	-1082GG -819C -819CC -592C -592CC	*P*=0.001 *P*=0.004* *P*=0.007* *P*=0.025* *P*=0.033* *P*<0.001* *P*=0.033 *P*=0.005 *P*=0.004 *P*<0.001* *P*=0.033	European Asian Asian Asian European Caucasian Asian European	Liu *et al.*, (2015) Meta-analysis
	-1082GG -819CT vs CC -819TT vs CC -592A -592AA	*P*=0.003 *P*=0.035 *P*=0.043 *P*=0.002 *P*=0.02	European Asian	Ke *et al.*, (2015) Meta-analysis
	-819T vs C -819TT vs CC -819TT+CT vs CC, -819TT vs CT+CC -592A -592AA ACC haplotype ACC haplotype GCC haplotype	*P*=0.003 *P*=0.006 *P*=0.006 *P*=0.03 *P*=0.03* *P*=0.03* *P*=0.04* *P*=0.002* *P*<0.0001	Asian European Asian European	Gao *et al.*, (2015) Meta-analysis
	GTA haplotype	*P*=0.02	Mexican	This study
IL12	+1188	NS	Caucasian, American and African	Ma *et al.*, (2003)
	+1188AC+CC vs AA +1188A	*P*=0.015 *P*=0.019	Caucasian	Liu *et al.*, (2014) Meta-analysis
	+1188CC	*P*=0.04	Mexican	This study
IFNG	+874AA +874A	*P*= 0.017 *P*=0.0055	South African	Rossow *et al.*, (2003)
	+874AA	*P*=<0.001	Chinese	Tso *et al.*, (2005)
	+874T +874AA	NA NA	Colombian	Henao *et al.*, (2006)
	+874T +874TT	*P*=0.0008* *P*=0.0002*	Analysis of all populations	Pacheco *et al.*, (2008) Meta-analysis
	+874A +874AA	*P*=<0.0009 *P*=<0.0019	Brazilian	Vallinoto *et al.*, (2010)
	+874AA, +874A	P_c=0.015	Egyptian	Mosaad *et al.*, (2010)
	+874AA	P_c=0.001	Tunisian	Ben-Selma *et al.*, (2011)
	+874AA	*P*=0.039	Chinese	Shen *et al.*, (2013)
	+874	NS	Mexican	This study

*Protective effect; P_c: Bonferroni correction of the p values; NS: Not Significant; NA: Not Available.

238GA genotype, contrary to the observations of Zhou *et al.* (2017), who showed a protective effect of these polymorphisms.

There are six studies in the literature on TB and *IL8* polymorphisms, of which only two include some patients with EPTB. One is by Ma *et al*. (2003b), who integrated a case-control study in adults in which they found that the *IL8*–251TA and *IL8*–251AA genotypes were associated with an increased risk of TB+EPTB for Caucasian (P=0.07 and P<0.01, respectively) and African American (P<0.01 and P<0.01, respectively) populations (Ma *et al*., 2003b). The other is by Yu *et al*. (2019), who found an association with the risk of TB+EPTB in the recessive model *IL8*–251TA+AA (P=0.02) (Yu *et al*., 2019). Our results showed an association with *IL8*+781C allele, *IL8*+396GG genotype with the risk of EPTB, while the *IL8*ATT haplotype (P=0.01) suggests an association with protection. However, no comparison could be made with the previous reports because none included these polymorphisms.

In 16 articles that analyzed *IL10* polymorphisms, samples of EPTB were included, finding significant associations in 11 studies. In the Turkish population the *IL10*–1082G allele was associated with risk of TB+EPTB (P_c=0.028 and P_c<0.001) (Oral *et al*., 2006; Ates *et al*., 2008), as well as the GCC haplotype (*IL10*–1082, –819, –592) (P_c<0.001) (Ates *et al*., 2008). In South America, two populations were studied, one of which showed an association of the *IL10*–1082AA genotype with risk of pleural tuberculosis (P=0.02) (Henao *et al*., 2006), while in the second, the *IL10*–592CA and *IL10*–592AA genotypes were associated with protection for EPTB (P=0.003) (Taype *et al*., 2010). One study in the Egyptian population showed that the high producer *IL10*–1082GG genotype was significantly higher in patients with PTB when compared with patients with EPTB (P_c=0.003) (Mosaad *et al*., 2010). In the Tunisian population the *IL10*–1082G allele was significantly over-represented in patients with PTB and EPTB (P_c=0.0002), however the *IL10*–1082AG genotype was associated with increased susceptibility to EPTB (P_c=0.0003) (Ben-Selma *et al*., 2011b). No association was found in the Chinese population with the polymorphism *IL10*–1082, but an association with pleural TB was found with the *IL10*–819T (P_c=0.042) and *IL10*–592A alleles (P_c=0.042) (Liang *et al.*, 2011). Contrary to previous studies, in the Indian population the risk genotype associated with PTB+EPTB was *IL10*–1082AA (P=0.009) (Ramaseri Sunder *et al.*, 2012). On the other hand, two meta-analyses showed risk association with the *IL10*–1082GG genotype in Europeans (P=0.001 and P=0.003) (Liu *et al*., 2015; Ke *et al*., 2015) and a third meta-analysis showed no association with this polymorphism (Gao *et al*., 2015). The *IL10*–819TC vs CC (P=0.035) and *IL10*–819TT+TC vs CC (P=0.043) models were associated with the risk in the Asian population in the meta-analysis performed by Ke, *et al*. (2015), finding similar results by Gao *et al*. (2015), T vs C (P=0.003), TT vs CC (P=0.006), TT+TC vs CC (P=0.006), TT vs TC+CC (P=0.03) related with risk in the Asian population. This is consistent with a third meta-analysis in which the *IL10*–819C allele (P=0.004 and P=0.007) and *IL10*–819CC genotype (P=0.025 and P=0.033) were associated with protection in the same population (Liu *et al*., 2015).

As for the *IL10*–592 polymorphism, Ke, *et al*. (2015) found that the *IL10*–592A allele (p=0.002) and the *IL10*–592AA genotype (p=0.02) were associated with the risk of PTB+EPTB in Asian populations, while Gao *et al*. (2015), found the same allele and genotype associated with protection in Europeans with PTB+EPTB (P=0.03 in both). Similarly, the haplotype ACC was associated with protection in Asians (P=0.04) and Europeans (P=0.002), while the GCC haplotype was significantly related with risk in Europeans (P<0.0001). Complementary to the findings reported by Gao *et al*. (2015), Liu *et al*. (2015) described the *IL10*–592C allele and the *IL10*–592CC genotype of risk in Europeans (P=0.033) and Caucasian (P=0.005 and P=0.004) (Liu *et al*., 2015). Regarding these SNPs, our results showed a risk association only with the *IL10*GTA haplotype.

For the *IL12B* 3'UTR+1188A>C polymorphism and PTB+EPTB, only two studies have been published. In one of them there was no association in both Caucasian and African-American populations (Ma *et al.*, 2003a); the other is a meta-analysis by Liu *et al*. (2014), in which a significant association with protection was found in Caucasians in the dominant model AC+CC vs AA (P=0.015), while the 3'UTR+1188A allele was associated with risk (P=0.019). Contrary to these results, the 3'UTR+1188CC genotype was associated with the risk of EPTB in our population.

In nine studies of the *IFNG*+874T>A (rs2430561) polymorphism and EPTB, only eight had a significant association. Two studies in the Chinese population showed the *IFNG*+874AA genotype associated with risk (P<0.001 and P=0.039) (Tso *et al*., 2005; Shen *et al*., 2013). Two studies describe associations of South American populations and EPTB: in Brazilians the *IFNG* +874A (P<0.0009) allele and *IFNG*+874AA (P<0.0019) genotype are related to risk, whereas in Colombians the *IFNG*+874T allele was increased in patients with pleural and miliary TB (P not available) (Henao *et al*., 2006; Vallinoto *et al*., 2010). In the Egyptian, South African and Tunisian populations, the *IFNG*+874AA genotype is also associated with risk of PTB+EPTB (P_c=0.015, P=0.017, P_c=0.001, respectively) (Mosaad *et al*., 2010; Rossouw *et al*., 2003; Ben-Selma *et al*., 2011a), while *IFNG*+874A allele is associated only with the South African population (P=0.0055) (Rossouw *et al*., 2003). Furthermore, in a meta-analysis, the *IFNG*+874T allele (P=0.0008) and the *IFNG*+874TT genotype (P=0.0002) indicated a

protective effect, considering the total analysis (Pacheco *et al*., 2008). In our study, this polymorphism did not reach statistical significance.

The discrepancies between our results and the results of the studies presented above may be due to the size of the sample and/or to differences in the genetic background of the populations analyzed. Additional studies with larger sample size will help identify the most important polymorphisms linked to extrapulmonary TB risk.

Finally, in this study we described for the first time the association of LNTB with the genotype *IL8*+396GG and the haplotype *IL8*ATT, as well as a trend of association *IL8*+781C. However, the small sample size might represent a source of bias for the results obtained, as well as an explanation of the high confidence intervals found. Therefore, to confirm these associations the study must be replicated with a larger sample size, as well as a larger number of cytokine polymorphisms included, in order to unravel possible immune mechanisms involved in the pathogenesis of this disease.

Our findings suggest the contribution of the *TNF*, *IL8* and *IL10* polymorphisms in the etiology of cervical lymph node tuberculosis in a sample of the Mexican population.

Conflicts of interest

No conflicts of interest are declared.

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References

Ates O., Musellim B., Ongen G., Topal-Sarikaya A. (2008). Interleukin-10 and tumor necrosis factor- alpha gene polymorphisms in tuberculosis. *J Clin Immunol.* **28**, 232-236.

Barrett J.C., Fry B., Maller J., Daly M.J. (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. **21**, 263-265.

Bean A.G., Roach D.R., Briscoe H., France M.P., Korner H., et al. (1999) Structural deficiencies in granuloma formation in TNF gene-targeted mice underlie the heightened susceptibility to aerosol *Mycobacterium tuberculosis* infection, which is not compensated for by lymphotoxin. *J Immunol.* **162**, 3504-3511.

Ben-Selma W., Harizi H., Bougmiza I., Hannachi N., Ben Kahla I., et al. (2011a). Interferon gamma +874T/A polymorphism is associated with susceptibility to active pulmonary tuberculosis development in Tunisian patients. *DNA Cell Biol.* **30**, 379-387.

Ben-Selma W., Harizi H., Boukadida J. (2011b). Association of TNF-α and IL-10 polymorphisms with tuberculosis in Tunisian populations. *Microbes Infect.* **13**, 837-843.

Bergeron A., Bonay M., Kambouchner M., Lecossier D., Riquet M., et al. (1997). Cytokine patterns in tuberculous and sarcoid granulomas: correlations with histopathologic features of the granulomatous response. *J Immunol.* **159**, 3034-3043.

Bignon J.D., Fernandez-Vina M. (1996). PCR-SSO protocol for the HLA-DNA typing technique. In: Charron D, Fauchet R (eds). Twelfth International Histocompatibility Workshop Reference Protocol Addendum: Technical Handbook. EDK, Paris, France.

Casanova J.L., Abel L. (2004). The human model: a genetic dissection of immunity to infection in natural conditions. Nat Rev Immunol. **4**, 55-66.

CDC. (2013). Latent tuberculosis infection: a guide for primary health care providers. Atlanta: Centers for Disease Control and Prevention. Available: www.cdc.gov/tb/ publications/ltbi/pdf/targetedltbi.pdf. Accessed July 25th, 2019.

Collins H.L., Kaufmann S.H. (2001). The many faces of host responses to tuberculosis. *Immunology.* **103**, 1-9.

Cooper A.M., Dalton D.K., Stewart T.A., Griffin J.P., Russell D.G., et al. (1993). Disseminated tuberculosis in interferon gamma gene disrupted mice. *J Exp Med.* **178**, 2243-2247.

Dlugovitzky D., Rateni L., Torres-Morales A., Ruiz-Silva J., Piñesky R., et al. (1997). Levels of interleukin-8 in tuberculous pleurisy and the profile of immunocompetent cells in pleural and peripheral compartments. *Immunol Lett.* **55**, 35-39.

Flynn J.L., Chan J. (2001). Immunology of tuberculosis. *Annu Rev Immunol.* **19**, 93-129.

Gao X., Chen J., Tong Z., Yang G., Yao Y., et al. (2015). Interleukin-10 promoter gene polymorphisms and susceptibility to tuberculosis: a meta-analysis. *PLoS One.* **10**, e0127496.

García-García M., Palacios-Martínez M., Ponce-de-León A., Jiménez-Corona M.E., Jiménez-Corona A., et al. (2000). The role of core groups in transmitting *Mycobacterium tuberculosis* in a high prevalence community in Southern Mexico. *Int J Tuberc Lung Dis.* **4**, 12-17.

García-Sancho FMC, García-García L, Jiménez-Corona ME, Palacios-Martínez M, Ferreyra-Reyes L.D., et al. (2006). Is tuberculin skin testing useful to diagnose latent tuberculosis in BCG-vaccinated children? *Int J Epidemiol.* **35**, 1447-1454.

Golden M.P., Vikram H.R. (2005). Extrapulmonary tuberculosis: an overview. *Am Fam Physician*. **72**, 1761-1768.

Henao M.I., Montes C., París S.C., García L.F. (2006). Cytokine gene polymorphisms in Colombian patients with different clinical presentations of tuberculosis. *Tuberculosis* (Edinb) **86**, 11-19.

Higuchi T., Seki N, Kamizono S., Yamada A, Kimura A, et al. (1998). Polymorphism of the 5'-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. *Tissue Antigens.* **51**, 605-612.

Hull J., Ackerman H., Isles K., Usen S., Pinder M., et al. (2001). Unusual haplotypic structure of IL8, a susceptibility locus for a common respiratory virus. *Am J Hum Genet.* **69**, 413-419.

Ilgazli A., Boyaci H., Basyigit I., Yildiz F. (2004). Extrapulmonary tuberculosis: clinical and epidemiologic spectrum of 636 cases. *Arch Med Res.* **35**, 435-441.

Ke Z., Yuan L., Ma J., Zhang X., Guo Y., et al. (2015). IL-10 Polymorphisms and Tuberculosis Susceptibility: An Updated Meta-Analysis. *Yonsei Med J.* **56**, 1274-1287.

Liang L., Zhao Y.L., Yue J., Liu J.F., Han M., et al. (2011). Interleukin-10 gene promoter polymorphisms and their protein production in pleural fluid in patients with tuberculosis. *FEMS Immunol Med Microbiol.* **62**, 84-90.

Lin Y., Zhang M., Barnes P.F. (1998). Chemokine production by a human alveolar epithelial cell line in response to *Mycobacterium tuberculosis*. *Infect Immun*. **66**, 1121-1126.

Liu G., Li G., Xu Y., Song N., Shen S., et al. (2014). Association between IL12B polymorphisms and tuberculosis risk: a meta-analysis. *Infect Genet Evol.* **2**, 401-407.

Liu Q., Li W., Li D, Feng Y., Tao C. (2015). The association of interleukin-10 -1082, -819, -592 polymorphisms and tuberculosis risk. *Saudi Med J.* **36**, 407-417.

Lv Y.J., Liu S.J., Hu W.N., Zhang G.P., Ren Q.Y., et al. (2016). Association of tumor necrosis factor-α gene polymorphism with osteoarticular tuberculosis prognosis in a Hebei population. *Genet Mol Res.* 15.

Ma X., Reich R.A., Gonzalez O., Pan X., Fothergill A.K, et al. (2003a). No evidence for association between the polymorphism in the 3' untranslated region of interleukin-12B and human susceptibility to tuberculosis. *J Infect Dis.* **188**, 1116-1118.

Ma X., Reich R.A., Wright J.A., Tooker H.R., Teeter L.D., et al. (2003b). Association between interleukin-8 gene alleles and human susceptibility to tuberculosis disease. *J Infect Dis.* **188**, 349-355.

Mastroianni C.M., Paoletti F., Rivosecchi R.M., Lancella L., Ticca F., et al. (1994). Cerebrospinal fluid interleukin 8 in children with purulent bacterial and tuberculous meningitis. *Pediatr Infect Dis J.* **13**, 1008-1010.

Mosaad Y.M., Soliman O.E., Tawhid Z.E., Sherif D.M. (2010). Interferon-gamma +874 T/A and interleukin-10 -1082 A/G single nu-

cleotide polymorphism in Egyptian children with tuberculosis. *Scand J Immunol*. **72**, 358-364.

O'Kane C.M., Boyle J.J., Horncastle D.E., Elkington P.T., Friedland J.S. (2007). Monocyte-dependent fibroblast CXCL8 secretion occurs in tuberculosis and limits survival of mycobacteria within macrophages. *J Immunol.* **178**, 3767-3776.

Oral H.B., Budak F., Uzaslan E.K., Baştürk B., Bekar A., et al. (2006). Interleukin-10 (IL-10) gene polymorphism as a potential host susceptibility factor in tuberculosis. *Cytokine*. **35**, 143-147.

Pacheco A.G., Cardoso C.C., Moraes M.O. (2008). *IFNG* +874T/A, *IL10* -1082G/A and *TNF*α -308G/A polymorphisms in association with tuberculosis susceptibility: a meta-analysis study. *Hum Genet*. **123**, 477-484.

PAHO/WHO. (2015). Tuberculosis in the Americas. Regional report 2014. Epidemiology, Control, and Financing. Available: http://www.paho.org/hq/index.php?option= com_topics&view=article&id=59&Itemid=40776/2015-cha-regional-report-americas-tb.pdf. Accessed July 25th, 2019.

Popescu M.R., Cžlin G., Strâmbu I., Olaru M., Bžlžşoiu M., et al. (2014). Lymph node tuberculosis - an attempt of clinico-morphological study and review of the literature. *Rom J Morphol Embryol.* **55**, 553-567.

Qiu Y, Cao S, Gou C, Yue Y, Jiang S, et al. (2018). Associations of tumor necrosis factor-α polymorphisms with the risk of tuberculosis: a meta-analysis. Scand J Immunol. e12719

Ramaseri Sunder S., Hanumanth S.R., Nagaraju R.T., Venkata S.K., Suryadevara N.C. (2012). IL-10 high producing genotype predisposes HIV infected individuals to TB infection. *Hum Immunol.* **73**, 605-611.

Rossouw M., Nel H.J., Cooke G.S., van Helden P.D., Hoal E.G. (2003). Association between tuberculosis and a polymorphic NFkappaB binding site in the interferon gamma gene. *Lancet*. **361**, 1871-1872.

Sharma S., Ghosh B., Sharma S.K. (2008). Association of TNF polymorphisms with sarcoidosis, its prognosis and tumour necrosis factor (TNF)-alpha levels in Asian Indians. *Clin Exp Immunol.* **151**, 251-259.

Shen C., Jiao W.W., Feng W.X.., Wu XR., Xiao J., et al. (2013). IFNG polymorphisms are associated with tuberculosis in Han Chinese pediatric female population. *Mol Biol Rep.* **40**, 5477-5482.

Silva C.A., Fernandes D.C.R.O., Braga A.C.O., Cavalcante G.C., Sortica V.A., et al. (2020). Investigation of genetic susceptibility to Mycobacterium tuberculosis (VDR and IL10 genes) in a population with a high level of substructure in the Brazilian Amazon region. *Int J Infect Dis.* **98**, 447-453

SINAVE/DGE/SALUD/ Perfil Epidemiológico de la Tuberculosis en México [online monograph]. (2012) México. Sistema Nacional de Vigilancia Epidemiológica. Available: http://www.dgepi.salud.gob.mx/2010/PDFS/PUBLICACIONES/2012/Monografias5_Tuberculosis_Mex_junio12.pdf. Accessed July 25th, 2019.

Singh S.K., Tiwari K.K. (2016). Tuberculous lymphadenopathy: Experience from the referral center of Northern India. *Niger Med J.* **57**, 134-138.

Solé X., Guinó E., Valls J., Iniesta R., Moreno V. (2006). SNPStats: a web tool for the analysis of association studies. *Bioinformatics*. **22**, 1928-1929.

Taype C.A., Shamsuzzaman S., Accinelli R.A., Espinoza J.R., Shaw M.A. (2010). Genetic susceptibility to different clinical forms of tuberculosis in the Peruvian population. *Infect Genet Evol.* **10**, 495-504.

Tso H.W., Ip W.K., Chong W.P., Tam C.M., Chiang A.K., et al. (2005). Association of interferon gamma and interleukin 10 genes with tuberculosis in Hong Kong Chinese. *Genes Immun*. **6**, 358-363.

Vallinoto A.C., Graça E.S., Araújo M.S., Azevedo V.N., Cayres-Vallinoto I., et al. (2010). IFNG +874T/A polymorphism and cytokine plasma levels are associated with susceptibility to Mycobacterium tuberculosis infection and clinical manifestation of tuberculosis. *Hum Immunol.* **71**, 692-696.

WHO Global tuberculosis report 2018 (2018). Geneva: World Health Organization. Available: https://apps.who.int/iris/bitstream/handle/10665/274453/9789241565646-eng.pdf. Accessed July 25th, 2019.

Wilkinson R.J., Patel P., Llewelyn M., Hirsch C.S., Pasvol G., et al. (1999). Influence of polymorphism in the genes for the interleukin (IL)-1 receptor antagonist and IL-1beta on tuberculosis. *J Exp Med.* **189**, 1863-1874.

Wu S., Wang Y., Zhang M., Wang M., He J.Q. (2019) Genetic variants in IFNG and IFNGR1 and tuberculosis susceptibility. *Cytokine.* **123**, 154775.

Yu Z., Wit W., Xiong L., Cheng Y. (2019). Associations of six common functional polymorphisms in interleukins with tuberculosis: evidence from a meta-analysis. *Pathog Dis.* **77**, pii: ftz053.

Zaatar R., Biet A., Smail A., Strunski V., Page C. (2009). Cervical lymph node tuberculosis: diagnosis and treatment. *Ann Otolaryngol Chir Cervicofac.* **126**, 250-255.

Zhang Y., Broser M., Cohen H., Bodkin M., Law K., et al. (1995). Enhanced interleukin-8 release and gene expression in macrophages after exposure to *Mycobacterium tuberculosis* and its components. *J Clin Invest*. **95**, 586-592.

Zheng M., Shi S., Wei W., Zheng Q., Wang Y., et al. (2018). Correlation between MBL2/CD14/TNF-α gene polymorphisms and susceptibility to spinal tuberculosis in Chinese population. *Biosci Rep.* 38: BSR20171140.

Zhou Y., Tan C.Y., Mo Z.J., Gao Q.L., He D., et al. (2017). Polymorphisms in the SP110 and TNF-α gene and susceptibility to pulmonary and spinal tuberculosis among southern Chinese population. *Dis Markers.* 2017, 4590235

Zhu H., Zhang Z., Lei X., Feng J., Zhang F., et al. (2012). Tumor necrosis factor alpha -308G>A, -863C>A, -857C>T gene polymorphisms and tuberculosis susceptibility: a meta-analysis. *Gene.* **509**, 206-214.