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IL-1 β gene *(+3954 C/T,* exon 5, rs*1143634*) and *NOS2 (exon 22)* polymorphisms associate with early aseptic loosening of hip and knee arthroplasties but not with implants infection

Esteban López-Anglada [a], Julio Collazos [b], A. Hugo Montes [c,f], Laura Pérez-Is [c,f], Imanol Pérez-Hevia [a], Sergio Jiménez-Tostado [a], Tomás Suárez-Zarracina [d,f], Victoria Alvarez [e,f], Eulalia Valle-Garay [c,f], Víctor Asensi [d,f]

[a]Traumatology Department, Hospital Universitario Central de Asturias, Oviedo, Spain [b]Infectious Diseases Section, Hospital de Galdacano, Vizcaya, Spain. [c]Biochemistry and Molecular Biology Department, University of Oviedo School of Medicine, Oviedo, Spain
[d] Infectious Diseases Unit, Hospital Universitario Central de Asturias, University of Oviedo School of Medicine, Oviedo, Spain [e]Molecular Genetics Section, Hospital Universitario Central de Asturias, Oviedo, Spain
[f]Group of Translational Research in Infectious Diseases, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain

Contact:vasensia@gmail.com

Abstract

- Aseptic prosthetic loosening (APL) and prosthetic joint infections (PJI) are frequent complications of hip and knee implants. Polymorphisms of cytokines and nitric oxide (NO), key inflammatory molecules in APL and PJI pathogenesis, could explain individual susceptibility to these complications. Three cytokines (*IL-1-α, IL-1-β, TNF-α)* and two nitric oxide synthase (*NOS2, NOS3*) genes polymorphisms were genotyped in 77 APL and 117 PJI patients and 145 controls with aseptic hip or knee implants lasting > 16 years. Plasma cytokines and nitrate-nitrite (NOx) levels were measured.
- The *TT* genotype and *T* allele of the *(+3954 C/T, exon 5, rs1143634) IL-1β* polymorphism were more frequent in APL patients compared to controls (P=0.03 and P=0.02, respectively). No genotypic associations in PJI patients were observed. *Staphylococcus epidermidis* was their most frequently isolated microorganism (39.3%). Plasma IL-6, TNF-α and NOx were significantly different between APL and controls (P<0.0001). Plasma IL-1β and IL-6 were significantly higher in APL *T* allele carriers vs. non-carriers (P<0.03). Knee implant (HR 2.488, 95% CI 1.307-4.739, P=0.005), male gender (HR 2.252, 95% CI 1.121-4.525, P=0.023), carriages of the *TT* genotype of the *(+3954 C/T) IL-1β* polymorphism (HR 3.704, 95% CI 1.274-10.753, P=0.016) and *AA* genotype of the *(exon 22) NOS2* polymorphism (HR 3.509, 95% CI 1.266-9.709, P=0.016) were independently associated with a shorter implant survival by Cox regression.
- Genotyping of *IL-1β (+3954 C/T, exon 5, rs1143634*) and *NOS2 (exon 22)* polymorphisms could be useful as predictors of early hip or knee APL.

Introduction

- Aseptic prosthetic loosening (APL) is a major clinical problem of unclear pathophysiology causing postsurgical pain and revision surgery.
- Proinflammatory cytokines are critical in bone remodelling. Interleukin-1 isoforms (IL-1α and β) and tumor necrosis factor-α (TNF- α) are potent stimulators of bone resorption by inducing osteoclastogenesis.
- Genes encoding *IL-1α, IL-1β, TNF-α* and other cytokines are polymorphic and the various alleles may have different translational efficiency, affecting cytokines production. Associations between several cytokines polymorphisms and risk of osteolysis after hip arthroplasty have been reported.
- Nitric oxide (NO) is crucial in bone metabolism. Some *NOS3* polymorphisms have been linked to differences in NO blood levels or in protein expression in response to several stimuli. These *NOS3* and other *NOS2* polymorphisms from are associated with osteoarticular diseases.
- Prosthetic joint infections (PJI) can develop in up to 1.7% of primary hip and 2.5% of knee arthroplasties. Several polymorphisms of *IL-1β* , of the mannose-binding lectin (MBL), toll-like receptor 9 (*TLR9*), and vitamin D receptor (VDR) might contribute to the susceptibility to PJI.
- The aim of this study was to analyze a potential association between hip and knee APL and some polymorphisms of cytokines and NOS, previously associated with inflammatory bone diseases: *-889 C/T IL-1α, +3954 C/T, exon 5 IL-1β, -308 G/A TNF-α)], -786 T/C NOS3* and *exon 22, NOS2* in a group of APL and PJI patients and in **patients with hip or knee implants that did not develop APL or PJI after ≥16 years of follow-up as controls** . A secondary aim was to study the expression of cytokines and *NOS* in the different genotypes by measuring IL-1β, IL-6, TNF-α and nitrate-nitrite (NOx) plasma levels in APL and PJI patients and controls.

Material and Methods

- Patients admitted to the HUCA between January 2003 and April 2021 with APL of hip or knee for second arthroplasty or with PJI for IV antimicrobial treatment were included in this study. Surgical confirmation of the APL and a negative bacteriological culture of surgical samples to exclude PJI were required for inclusion as APL. PJI patients were included if they fulfilled the 2013 Infectious Diseases Society of America PJI diagnostic criteria. Cultures of surgical and sinus tract samples from PJI patients were collected.
- The main diagnosis before undergoing the primary arthroplasty was degenerative osteoarthritis in all the APL and PJI patients and controls. Other diseases which could affect the bone metabolism were excluded from the study.
- Controls were individuals admitted to the HUCA to undergo a primary hip or knee arthroplasty since January 2003 and were followed ≥ 16 years until April 2022 or implant–unrelated death without developing APL or PJI.

Genotypic analysis

- Polymorphisms of cytokines *([-889 C/T] IL-1α, rs1800587, [+3954 C/T] exon 5 IL-1β , rs1143634, and [–308 G/A]), TNF-α, rs1800629], NOS3 [-786 T/C], rs2070744, and NOS2 [exon 22*]) were determined by PCR. The PCR results were confirmed by sequencing representative samples for each genotype of each polymorphism.

Cytokines levels

- Plasma cytokine levels (IL-1β, IL-6, TNF-α) were measured by ELISA kits from R&D Systems (R&D Systems Inc., 614 McKinley Place, MN, USA).

Plasma nitrate and nitrite levels

- Plasma nitrate and nitrite (NO_x) determinations were performed using the Griess reaction. Results were expressed as μM of NO_x /sample.

Table 1 Demographic and clinical features of patients with prosthesis loosening, infection and controls.

		Loosening (n=77)	Infection (n=117)	Controls (n=145)	P value
Gender	Female	50 (64.9%)	59 (50.4%)	96 (66.2%)	0.02
	Male	27 (35.1%)	58 (49.6%)	49 (33.8%)	
Age	Years	63.33 (58.40-69.52)	69.06 (61.15-76.00)	70.12 (63.74-75.30)	0.0001
Prosthesis location	Hip	58 (75.3%)	63 (53.8%)	80 (55.2%)	0.001
	Knee	19 (24.7%)	49 (41.9%)	65 (44.8%)	
	Other	0 (0.0%)	5 (4.3%)	0 (0.0%)	
Side	Right	39 (50.6%)	56 (49.6%)	86 (59.3%)	0.2
	Left	38 (49.4%)	57 (50.4%)	59 (40.7%)	
Prosthesis change	Yes	69 (97.2%)	89 (71.1%)	0 (0.0%)	<0.0001
	No	2 (2.8%)	28 (23.9%)	145 (100%)	
Time to prosthesis change		10.08 (4.128-15.04)	4.82 (1.71-9.77)		0.0003

Table 2. Cytokine and nitric oxide (NOx) plasma levels in patients with prosthesis loosening, infection and controls.

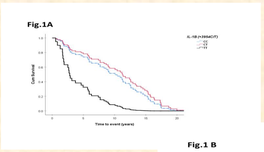
	Loosening (n=58)	Infection (n=99)	Controls (n=108)	P value
IL-1β (pg/ml)	2.66 (1.77-12.82)	15.58 (2.41-30.17)	2.0 (0.85-3.65)	0.13
IL-6 (pg/ml)	27.94 (8.59-71.53)	30.60 (14.78-65.07)	131.71 (31.00-216.85)	<0.0001
TNF-α (pg/ml)	30.66 (21.06-45.66)	3.91 (1.66-12.57)	11.96 (0.00-24.61)	<0.0001
NOx (μM/ml)	36.8 (26.8-51.6)	Not done	98.0 (70-156)	<0.0001

Table 3. Polymorphisms of *IL-1α, IL-β, TNF-α, NOS3 and NOS2* in patients with aseptic loosening, infection and controls

Gene polymorphism	Geno type	Loosening n (%)	Infection n (%)	Control n (%)	P value	Alleles	Loosening n (%)	Infection (5)	Control n(%)	P value
IL1-α (-889 C/T)	*CC*	28 (45.2)	54 (52.4)	61 (48.8)	0.2	*C*	85 (68.5)	146 (70.9)	181 (72.4)	0.7
	CT	29 (46.8)	38 (36.9)	59 (47.2)		*T*	39 (31.5)	60 (29.1)	69 (27.6)	
	TT	5 (8.0)	11 (10.5)	5 (4.0)						
IL1-β (+3954 C/T)	*CC*	34 (44.2)	66 (56.9)	81 (55.9)	0.1	*C*	102 (66.2)	174 (75.0)	221 (76.2)	0.06
	CT	34 (44.2)	42 (36.2)	59 (40.7)		*T*				
	TT	9 (11.6)	8 (6.9)	5 (3.4)						
TNF-α (-308 G/A)	*GG*	47 (75.8)	86 (74.1)	83 (66.4)	0.4	*G*	109 (87.9)	200 (86.2)	204 (81.6)	0.2
	GA	15 (24.2)	28 (24.1)	38 (30.4)		*A*	15 (12.1)	32 (13.8)	46 (18.4)	
	AA	0 (0)	2 (1.7)	4 (3.2)						
NOS3 (-786 T/C)	*TT*	21 (33.9)	29 (28.2)	46 (36.8)	0.3	*T*	73 (58.9)	114 855.3)	143 (57.2)	0.8
	TC	31 (50.0)	56 (54.4)	51 (40.8)		*C*	51 (41.1)	92 (44.7)	107 (42.8)	
	CC	10 (16.1)	18 (17.5)	28 (22.4)						
NOS2 (exon 22)	*GG*	18 (29.0)	34 (33.0)	47 (37.6)	0.7	*G*	72 (58.1)	121 (58.7)	155 (62.0)	0.7
	GA	36 (58.1)	53 (51.5)	61 (48.8)		*A*	52 (41.9)	85 (41.3)	95 (38-0)	
	AA	8 (12.9)	16 (15.5)	17 (13.6)						

Results

- Demographic and clinical features of patients with APL, PJI and controls are shown in Table 1.
- There were no differences in IL-1β levels among the groups, but controls had substantially higher levels of IL-6 than the APL and PJI groups, whereas APL patients had higher TNF-α serum levels than the other two groups. Plasma NOx levels were significantly higher in the controls compared to APL patients (Table 2).
- The *TT* genotype and *T* allele of the *(+3954 C/T, exon 5, rs1143634) IL-1β* polymorphism were more frequent in APL patients compared to controls (P=0.03 and P=0.02, respectively). No genotypic associations in PJI patients were observed (Table 3; overall but not individual comparison data are shown in the Table).
- Plasma IL-1β and IL-6 were significantly higher in APL *T* allele carriers vs. non-carriers (IL-1 β: 11.79 [IQR 3.41-21.14] vs. 2.11 [IQR 1.61-5.5], p<0.03); IL-6: 43.59 [IQR 16.21-146.47 vs. 38.12 (IQR 15.0-128.7, P=0.006]). Plasma levels of NOx did not differ in carriers of the different *NOS3* and *NOS2* genotypes.
- Knee implant, male gender, carriages of the *TT* genotype of the *(+3954 C/T) IL-1β* polymorphism and *AA* genotype of the *(exon 22) NOS2* polymorphism were independently associated with a shorter implant survival by Cox regression (P<0.016) (Table 4).
- **Patients carrying the respective variant homozygous genotypes of the *IL-1β (+3954 C/T)* and *NOS2 (exon 22)* polymorphisms experienced earlier prosthesis loosening than those carrying the heterozygous and homozygous wild genotypes, an effect that was mainly noted during the first 5 years after surgery (Fig.1 A and B).**



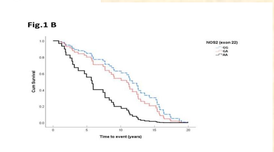


Fig.1. Cox regression curves comparing the development over time of aseptic prosthetic loosening (APL), according to the *IL-1β (+3954 C/T)* (A) and *NOS2 (exon22)* (B) genotypes.

	Factor	HR (95%CI)	P value
IL-1β (+3954 C/T) model	Knee prosthesis	2.488 (1.307-4.739)	0.005
	IL-1β (+3954 C/T) SNP		0.012
	TT vs. *CC* genotype	3.704 (1.274-10.753)	0.016
	TT vs. *CT* genotype	4.587 (1.675-12.500)	0.003
NOS2 (*exon 22)* model	Knee prosthesis	4.367 (1.972-9.709)	0.0003
	Male gender	2.252 (1.121-4.525)	0.023
	NOS2 *(exon 22)* SNP		
	AA. vs. *GG* genotype	3.509 (1.266-9.709)	0.016
	AA vs. *GA* genotype	2.639 (1.072-6.494)	0.035

Table 4. Factors independently associated with prosthesis loosening according to the multivariate Cox regression

Conclusions

- **Genotyping of *IL-1β (+3954 C/T, exon 5, rs1143634*) and *NOS2 (exon 22)* polymorphisms could be useful as predictors of early hip or knee APL.**

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