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Association of Interleukin-6 and Interleukin-1 Family Gene Polymorphisms in Autoimmune Hepatitis

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ABSTRACT

Introduction and aim. Autoimmune hepatitis (AIH) is an immune-mediated destruction of liver cells, in recognition of interface hepatitis, seropositivity for autoantibodies, and interface hepatitis in histology sections. Hepatocyte destruction in AIH is the direct result of $CD4^+$ T-cell destruction. Yet, Th17 mediated immune attach and a diversity of cytokine networks, including pro-inflammatory cytokines such as Interleukin 1 (IL-1) and Interleukin 6 (IL-6), set the stage for the destructive liver damage. **Material and method.** Peripheral blood samples from 57 patients, with AIH, recruited from referrals to the main pediatric hospital in Tehran. Single nucleotide polymorphisms for the following cytokines genes, were evaluated through, polymerase chain reaction with sequence-specific primers (PCR-SSP) assay: IL-1a (C/T -889), IL-1α (C/T -511), IL-1β (C/T +3962), IL-1 receptor (IL-1R; C/T Pst-I 1970), IL-1RA (C/T Mspa-I 11100), and IL-6 (C/G -174 and A/G nt565). **Results.** Significant higher frequency of genotype AA was detected in patients in IL-6 at position nt565 (15.8% in AIH patients *vs.* 2.9% in controls, $p = 0.003$). The haplotype GA of IL-6 at -174 and nt565, was significantly overrepresented in the AIH group, compared to (20.9% of AIH *vs.* 1.4% in controls $p < 0.0001$). **Conclusion.** Results of our study, indicate significant deviation toward high yield IL-6 polymorphisms, in AIH patients. These data could bring new insights in pathophysiology of disease, which could contribute to developing novel treatments for AIH.

Key words. Chronic autoimmune hepatitis. Single nucleotide polymorphism. Proinflammatory cytokines. Genetic predisposition. Hepatocyte destruction.

INTRODUCTION

A chronic hepatitis, affecting children and adults of all ages, auto-immune hepatitis (AIH) can subsume a variety of symptoms from benign elevation of liver enzymes, to acute or fulminant hepatitis. Features supporting an autoimmune etiology to AIH, are high total serum concentrations, seropositivity for a spectrum of autoantibodies, including anti-nuclear antibody (ANA) and anti-smooth muscle antibodies (ASMA), and evidence of adaptive immune attack directed toward hepatocytes in histology.[1] Although auto-antibodies are not believed to be involved in AIH pathogenesis and do not predict individuals prognosis, together they yield a high predictive value, based on clinical presentations, for diagnosing AIH.[1-3] In fact there are two types of AIH in children, based on the presence of ANA and ASMA (type 1 AIH), or, antibodies to liver-kidney microsome type 1 (anti-LKM1), in AIH type 2. There is a girl to boy predominance in both AIH types, and both types respond tremendously to immunosuppressive therapy.[4]

AIH has a "complex trait" pattern of inheritance, with multiple genes seem to be responsible for the pathogenesis of disease. Environmental factors in a genetically predisposed person could lead to loss of tolerance to liver cells in patients with AIH.[5,6] Defects in liver cells antigen presentation, helper-T-cells antigen recognition and a consequent cascade of cytokine production and cellular/

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humoral immune cells activation, could each play a role in formation of this autoimmune condition.[2,7-10]

Helper T-cells directly target hepatocytes, and along with Th17 conduct the adaptive immune response, to form a full blown immune attack. Pro-inflammatory cytokines, including members of interleukin 1 (IL-1) family and IL-6, produced by activated T-cells and monocytes, play an essential role in further activating autoreactive effector cells and advancing the immune reaction.[10,11] IL-17, IL-23 producing cells infiltrating the liver, enhance hepatic IL-6 and IL-1 production, and further stimulate Th17 and Th1 formation.[12]

Polymorphisms both in translated or non-translated regions of genes, directly correlate and control transcription level of IL-1 and IL-6.[13,14] Association of certain single nucleotide polymorphisms (SNPs) of the genes with some immune related diseases have already been reported.[15-24] Herein, we investigated the pro-inflammatory cytokines gene polymorphisms in a group of pediatric patients with AIH compared to healthy individuals.

MATERIAL AND METHODS

Study design

This study was conducted on 57 pediatric patients with AIH, who were referred to the Children's Medical Center Hospital, the Pediatrics Center of Excellence in Tehran, Iran. The diagnosis of AIH was made by two pediatric gastroenterologists, based on standard criteria.[25] Also a number of 140 healthy, age and sex matched individuals,[26] without evidence of autoimmune disorders, or chronic liver disease, were included as control group from the Iranian blood bank data base.

The study was approved by the Ethical Committee of Tehran University of Medical Sciences. All individuals signed the informed consent before sampling. Parents of under aged patients, were provided with information on the basis of the study and signed informed consent form.

DNA sampling and analysis

Five milliliters of whole blood was obtained from each patients and control, preserved with EDTA (Ethylene-diamine-tetra-acetic acid), and was used to extract genomic DNA, by Phenol-Chloroform method.[27] The extracted DNA was amplified, using the thermal cycler PCR Techne Flexigene apparatus (Rosche, Cambridge, UK) under the following conditions: initial denaturation 94 °C, 2 min; denaturation 94 °C, 10 sec; annealing extension 65 °C, 1 min (10 cycles); denaturation 94 °C, 10 sec; annealing 61 °C, 50 sec; extension 72 °C, 30 sec (20 cycles).[28] Polymerase chain reaction with sequence-specific primers (PCR-SSP) assay, was implemented to identify cytokine gene polymorphisms, for IL-1α (C/T -889), IL-1β (C/T -511), IL-1β (C/T +3962), IL-1 receptor (IL-1R; C/T Pst-I 1970), IL-1RA (C/T Mspa-I 11100), and IL-6 (C/G -174 and A/G nt565), using cytokine gene polymorphism kit (Heidelberg University, Heidelberg, Germany).[26] PCR products were visualized on 2% agarose gel, using an ultraviolet transilluminator.[18]

Statistical analysis

Allelic and genotype frequencies were counted in patients and controls by direct gene counting. Frequencies in patients and controls were compared using chi-square or fisher exact test, when applicable. The p-value, odds ratio, and 95% confidence intervals (CI) were calculated for each allele, genotype and haplotype. p value of less than 0.05 was considered significant.

RESULTS

Allelic frequencies

Allelic frequencies of proinflammatory cytokines in AIH patients and the controls are shown in table 1. IL-6, A allele at position nt565 was more frequent in patients compared to controls (35.1% in AIH patients *vs.* 18% in controls, p = 0.00042). None of the investigated polymorphisms in IL-1 gene family, were displayed differently between the two groups.

Genotype frequencies

Significant higher frequency of genotypes AA and GG were detected in patients with AIH, in IL-6 at position nt565 (15.8% in AIH patients *vs.* 2.9% in controls, p = 0.003 for the AA genotype, and 45.6% in AIH patients *vs.* 66.9% in controls, p = 0.009, for the GG genotype).

Haplotype frequencies

The haplotypes GA and GG of IL-6 at -174 and nt565, were significantly overrepresented in the AIH group, compared to (OR(95%CI) = 0.34(0.21-0.53), p < 0.0001 for GA, and OR (95%CI) = 18.13(5.84-62.75), p < 0.0001 for the GG haplotype) (Table 2).

DISCUSSION

Presentation of a self-antigen in hepatocytes by macrophages and activation of autoreactive helper T-cells, is believed to be the primary step in formation the inflammatory cellular reaction seen in AIH. Macrophages facilitate

Table 1. Frequencies of alleles and genotypes in patients with AIH and control individuals.

Cytokine	Position	Alleles/ Genotypes	AIH (n = 57)	Controls (n = 140) N(%)	*p* value	OR (95%CI)
IL-1α	-889	C	70 (62.5)	186 (68.4)	0.32	0.77 (0.47-1.25)
		T	42 (37.5)	86 (31.6)	0.32	1.30 (0.00-2.11)
		CC	19 (33.9)	62 (45.6)	0.18	0.61 (0.30-1.23)
		TC	32 (57.2)	62 (45.6)	0.19	1.59 (0.81-3.13)
		TT	5 (8.9)	12 (8.8)	0.8	1.01 (0.29-3.31)
IL-1β	-511	C	68 (59.7)	154 (55.4)	0.51	1.19 (0.75-1.9)
		T	46 (40.3)	124 (44.6)	0.51	0.84 (0.53-1.34)
		CC	19 (33.3)	36 (25.8)	0.38	1.43 (0.69-2.94)
		TC	30 (52.6)	82 (59)	0.51	0.77 (0.4-1.5)
		TT	8 (14.1)	21 (15.2)	0.98	0.92 (0.35-2.37)
IL-1β	+3962	C	76 (67.9)	198 (70.7)	0.66	0.87 (0.53-1.44)
		T	36 (32.1)	82 (29.3)	0.66	1.14 (0.69-1.88)
		CC	25 (44.7)	70 (50)	0.6	0.81 (0.41-1.57)
		TC	26 (46.4)	58 (41.4)	0.63	1.23 (0.63-2.39)
		TT	5 (8.9)	12 (8.6)	0.84	0.92 (0.35-2.37)
IL-1R	Pst-I 1970	C	71 (62.3)	174 (62.1)	0.93	1.01 (0.63-1.62)
		T	43 (37.7)	106 (44.2)	0.93	0.99 (0.62-1.6)
		CC	22 (38.6)	54 (38.6)	0.87	1.00 (0.51-1.97)
		TC	27 (47.4)	66 (47.1)	0.9	1.01 (0.52-1.96)
		TT	8 (14)	20 (14.3)	0.86	0.98 (0.37-2.55)
IL-1RA	Mspa-I 11100	C	19 (17.3)	64 (22.9)	0.28	0.7 (0.38-1.29)
		T	91 (82.7)	216 (77.1)	0.28	1.42 (0.78-2.61)
		CC	0 (0)	4 (2.9)	0.48	0 (0-3.93)
		CT	19 (34.5)	56 (40)	0.59	0.79 (0.39-1.59)
		TT	36 (65.5)	80 (57.1)	0.37	1.42 (0.71-2.86)
IL-6	-174	C	40 (35.1)	101 (36.3)	0.91	0.95 (0.59-1.53)
		G	74 (64.9)	177 (63.7)	0.91	1.06 (0.65-1.71)
		CC	5 (8.8)	4 (2.9)	0.16	3.25 (0.72-15.09)
		CG	30 (52.6)	93 (66.9)	0.09	0.55 (0.28-1.08)
		GG	22 (38.6)	42 (30.2)	0.33	1.45 (0.72-2.9)
IL-6	nt565	A	40 (35.1)	50 (18)	**0.0004**	2.46 (1.46-4.15)
		G	74 (64.9)	228 (82)	**0.0004**	0.41 (0.24-0.68)
		A A	9 (15.8)	4 (2.9)	**0.003**	6.33 (1.68-25.77)
		GA	22 (38.6)	42 (30.2)	0.33	1.45 (0.72-2.9)
		GG	26 (45.6)	93 (66.9)	**0.009**	0.41 (0.21-0.82)

Table 2. Frequencies of haplotypes in patients with AIH and control individuals.

Cytokine	Haplotype	AIH (n = 58)	Controls (140 subjects) N(%)	P-value	OR (95%CI)
IL-6	GG	46 (35.7)	173 (62.2)	**< 0.00001**	0.34 (0.21-0.53)
(-174,nt565)	CG	28 (21.7)	55 (19.8)	0.75	1.12 (0.65-1.93)
	CA	28 (21.7)	46 (16.6)	0.26	1.40 (080-2.44)
	GA	27 (20.9)	**< 0.00001**		18.13 (5.84-62.75)
		4 (1.4)			

polarization of Naïve T-cells into Th1, by their selective production of IL-2, IL-12, and IFN-γ. These cytokines perpetuate the destructive inflammatory cascade by enhancing the expression of HLA class I and class II on hepatocytes, leaving them vulnerable to cell mediated cytotoxicity and further recognition by activated T-cells. Later in disease course, IL-6 along with TGF-β set the stage for development of Th17 cells.[28] Liver damage in then, thought to be orchestrated by a Th17 and Th1 populations and a dysregulated/suppressed population of CD4+CD25+ regulatory T-cells.[29]

Circulating levels of IL-6, TNF-α and IL-1 are increased in sera of patients with AIH.[30,31] The proinflmmatory cytokines, are direct products of activated hepatocytes, as well as the infiltrating mononuclear cells in portal space and surrounding parenchyma. IL-1 and IL-6 are thus considered as two major contributing factors to hepatocyte destruction and their levels directly correlate with transaminase levels, markers of cell death and markers of disease severity.[29,32]

A leading study in 1999, failed to report any significant difference in the frequency of polymorphisms, IL-1β (at +3962) and IL-1RA at intron 2,[33] in agreement with our results demonstrating no significant association in these sites.

Level of IL-6 expression is increased in pediatric patients with AIH[31] and in type 2 AIH,[30] compared to healthy individuals, and in autoimmune chronic active hepatitis,[34] compared to viral forms of chronic hepatitis. Our findings suggest a higher frequency of IL-6 A allele, and AA genotype, at position nt565, in AIH patients. We have previously found in our region, a genetic predisposition to cardiovascular events, and impaired vascular pressure response, conferred by AA genotype at IL-6 nt565.[35] This genotype also confers higher serum IL-6 concentrations and susceptibility to mental retardation in adolescent patients with intellectual disability (36), while the GG genotype conferred a protection against chronic idiopathic urticarial.[37]

The haplotype GA at IL-6 (-174, nt565), was associated with an 18-fold predisposition toward AIH (OR (95%CI) = 18.13(5.84-62.75), while the GG haplotype at the same position, was associated with lower AIH risk (OR (95%CI) = 0.34(0.21-0.53)). This was in line with previous finding on higher frequency of the GG haplotype, in patients with irritable bowel syndrome, atopic dermatitis, and Behçet's disease.[15,16]

Results of our study, support a significant and powerful association between polymorphic variant's of IL-6 in terms of allele frequencies and genotypes of IL-6 at nt565, and haplotypic variant's of IL-6 at (-174, nt565). These results suggest a possible predictive value for IL-6 polymorphism in Iranian patients with AIH.

ABBREVIATIONS

- **AIH:** autoimmune hepatitis.
- **ANA:** anti-nuclear antibody.
- **anti-LKM1:** antibodies to liver-kidney microsome type 1.
- **ASMA:** anti-smooth muscle antibodies.
- **CI:** confidence intervals.
- **IL-1:** interleukin 1.
- **IL-6:** interleukin 6.
- **SNPs:** single nucleotide polymorphisms.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest regarding the publication of this article.

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