

KIR genotype distribution among symptomatic patients with and without *Helicobacter pylori* infection: is there any role for the B haplotype?

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Received 8 September 2014
 Accepted 9 October 2014
 Published Online First 28 October 2014

ABSTRACT

Contact of peripheral blood lymphocytes with *Helicobacter pylori* was proved to induce non-major histocompatibility complex-restricted cytotoxicity and natural killer cells are thought to play an important role in the immunity against *H. pylori*.

Aims In this research, we investigated any possible association between killer immunoglobulin-like receptors (KIR) genotypes and *H. pylori* infection.

Methods KIR genotype was analysed in 101 Lebanese symptomatic patients (51 *H. pylori* positive and 50 *H. pylori*-negative) using the *KIR Genotyping SSP kit*.

Results Among the *H. pylori*-positive patients, the AA, AB and BB genotypical frequencies were, respectively, 43.14%, 41.18% and 15.68% with an A:B ratio of 1.76:1. The AA, AB and BB genotypes frequencies for *H. pylori*-negative individuals were 18%, 62% and 20%, respectively, with an A:B ratio of 0.96:1. No significant difference between patients and controls was detected.

Conclusions We noticed a reduced distribution of A haplotype among the '*H. pylori*-negative' patients as compared with the '*H. pylori*-positive' group. This is the first study in the international literature that targets the correlation between KIR genotypes and *H. pylori*.

INTRODUCTION

Helicobacter pylori is a Gram-negative bacterium which colonises the duodenal and gastric mucosa and elicits a chronic infection,¹ which is the main cause of active gastritis and duodenal ulceration in the stomach. The inflammation caused by *H. pylori* is also closely associated with gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphomas.²

Natural killer (NK) cells comprise 5%–15% of peripheral blood lymphocytes as part of the innate immunity system and form an important first line of defence against invading foreign antigens. The main role of NK cells is the effective destruction of virus-infected cells early in the course of infection before the killer CD8-positive T cells are generated by the immune system. Moreover, NK cells can suppress cancer development by killing certain tumour cells including intestinal tumours.^{3–4} Certain reports also provide evidence that NK cells can be activated due to bacterial infection.^{5–6}

Killer immunoglobulin-like (KIR) receptors, which are members of the immunoglobulin superfamily, are receptors found on NK cells. KIR interaction with human leucocyte antigen class I molecule protects healthy cells from destruction through the inhibition of NK cell activity, whereas other KIR isotypes stimulate the activity of NK

cells. Therefore, KIR holds an important role in the control of the immune response.⁷

Contact of peripheral blood lymphocytes with *H. pylori* was proved to induce non-major histocompatibility complex (MHC) restricted cytotoxicity.⁸ Therefore, NK cells are thought to play an important role in the immunity against *H. pylori* and many previous reports have shown the ability of *H. pylori* components to activate NK cells stimulating them to release interferon (IFN)- γ .^{8–9} An increase in the infiltration of NK cells was observed in the gastric mucosa of *H. pylori* patients¹⁰ and in *H. pylori*-dependent high-grade MALT lymphomas.² It has also been shown that interaction between NK cells and dendritic cells (DCs) after *H. pylori* infection results in the release of interleukin-12 from DCs, which leads to the rapid activation of NK cells. This activation leads in turn to the secretion of IFN- γ by NK cells where IFN- γ increases MHC class II expression, thus facilitating the development of autoreactive T cells.¹

In this research, we studied KIR expression of NK cells in patients infected with *H. pylori* and in *H. pylori*-negative patients, to check for any association with KIR genotypes. This the first study in the international literature and Lebanon that targets the correlation between KIR genotypes and *H. pylori*. Previous unique studies were conducted among Lebanese patients to assess the association between KIR genotypes and several diseases in Lebanon including familial Mediterranean fever,¹¹ Behçet's disease¹² and tuberculosis¹³ as well as the distribution of the KIR genotypes in the general Lebanese population.¹⁴

MATERIALS AND METHODS

Study population

The study was conducted at the American University of Beirut Medical Center, a major tertiary-care centre in Lebanon. KIR genotype was analysed for 101 consented Lebanese patients presenting to the endoscopy unit and referred for *H. pylori* testing using the *KIR Genotyping SSP kit*. We identified 51 *H. pylori*-positive and 50 *H. pylori*-negative patients.

DNA extraction and KIR genotyping

PEL-FREEZ kits (Pel-Freez/Dynal, Norway) were used for DNA extraction from 2 to 3 mL of collected peripheral blood. The DNA material was properly labelled and stored at -80°C . Based on our institutional review board committee rules and study approval protocols, confidentiality was strictly observed for all analysed samples.



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To cite: Mahfouz R, Hoteit R, El Hajj N, et al. *J Clin Pathol* 2015;**68**: 40–43.

Profile	N	%	KIR genes	2DL1	2DL2	2DL3	2DL4	2DL5A	2DL5B	3DL1	2DS1	2DS2	2DS3	2DS4*001	2DS4*003	2DS5	3DS1	2DP1	3DP1*001	3DP1*003
AA	7	13.72	5																	
AA	7	13.72	6																	
AA	1	1.96	6																	
AB	1	1.96	6																	
AB	1	1.96	6																	
BB	1	1.96	6																	
BB	2	3.93	7																	
BB	1	1.96	7																	
AA	1	1.96	8																	
BB	2	3.93	8																	
AA	4	7.83	9																	
AA	1	1.96	9																	
AA	1	1.96	9																	
AB	1	1.96	9																	
AB	2	3.93	9																	
AB	3	5.88	10																	
AB	1	1.96	10																	
BB	1	1.96	10																	
AB	2	3.93	11																	
AB	1	1.96	11																	
AB	1	1.96	11																	
BB	1	1.96	12																	
AB	5	9.8	13																	
AB	2	3.93	13																	
AB	1	1.96	13																	

Figure 1 Killer immunoglobulin-like receptors (KIR) genotypical profile among 51 Lebanese patients with *Helicobacter pylori*.

Primer mixes were purchased from PEL-FREEZ/DYNAL company (Oslo, Norway) as part of the *KIR Genotyping SSP kit* which is a PCR-based method designed to detect the absence or presence of the following 16 gene loci of KIR (variants also tested): *2DL1*, *2DL2*, *2DL3*, *2DL4*, *2DL5*, *2DS1*, *2DS2*, *2DS3*, *2DS4*, *2DS5*, *3DL1*, *3DL2*, *3DL3*, *3DS1*, *2DP1* and *3DP1*. Two variants for *KIR2DL5* were typed *KIR2DL5A*001* and *KIR2DL5B*002/003/004* and two variants for *KIR2DS4* were tested and reported as *KIR2DS4*001/002* and *KIR2DS4*003-006*. In addition, two variants for the pseudo-gene *KIR3DP1* were tested: *KIR3DP1*001/002/004* and *KIR3DP1*003*.

KIR genotyping was performed as recommended by the manufacturer. Briefly, 25 μ L of DNA was added to 150 μ L of PCR buffer and 2.4 μ L of Taq DNA polymerase and dispensed as aliquots of 8 μ L into a supplied 96-well plate for a total reaction volume of 23 μ L in each well (reaction+paraffin oil). The thermocycling steps included an initial heating step at 95°C for 1 min, followed by 30 cycles of 94°C for 20 s, 63°C for 20 s and 72°C for 90 s. A final holding step was performed at 4°C. Electrophoresis of the 2% agarose gel was done in ethidium bromide and visualisation performed under ultra violet light transillumination.

Statistical analysis

We used direct counting for the observed KIR phenotype frequencies. SPSS V.15.0 was used to conduct statistical analysis. Genetic expression was expressed as number and frequency and χ^2 was used to test for its association with any of the two groups (*H. pylori* negative and *H. pylori* positive). Fisher's exact test was conducted when the expected count of at least one of the categories was <5 . p Value <0.05 was considered statistically significant.

RESULTS

KIR genotypical profile distribution among the 51 Lebanese patients with *H. pylori* is shown in figure 1. The content of KIR genes ranged from 5 to 13 and as per table 1, the AA, AB and BB genotypical frequencies were 43.14%, 41.18% and 15.68%, respectively, with an A:B ratio of 1.76:1.

Figure 2 shows the distribution of different KIR genotypes among the 50 *H. pylori*-negative Lebanese patients. The content

of KIR genes ranged from 7 to 13 and, as per table 1, the AA, AB and BB genotypes frequencies were 18%, 62%, and 20%, respectively, with an A:B ratio of 0.96:1.

Table 2 shows the distribution of different KIR genes among the 50 *H. pylori*-negative and the 51 *H. pylori*-positive patients. KIR 2DL4 was present in all individuals. No significant difference between patients and controls was detected.

DISCUSSION

NK cells are known to induce antiviral and antitumour immunity via production of proinflammatory cytokines and based on their KIR gene content, two groups of haplotypes are known in humans: A and B. Haplotype A encodes inhibitory receptors and consists of nine genes (3DL3, 2DL3, 2DP1, 2DL1, 3DP1, 2DL4, 3DL1, one activating (2DS4), 3DL2 and 2DL5), whereas haplotype B carries a variety of gene combinations and encodes more activating receptors as compared with haplotype A (3DL3, 2DS2, 2DL2, 2DL5B (inhibitory) 2DS3, 2DP1, 2DL1, 3DP1, 2DL4, 3DS1, 2DL5A (inhibitory), 2DS5, 2DS1 and 3DL2).¹⁵ It is the balance between the inhibitory and activating signals that regulates the function of NK cells and predisposes or protects an individual against microbes or diseases, according to the building international literature about KIR genotypical profiling.

KIR genotype has been recently reported to be a significant factor in the control of primary cytomegalovirus infection¹⁶ and

Table 1 Frequency distribution of KIR genotypes and haplotypes among *Helicobacter pylori*-positive and *H. pylori*-negative patients

Genotype	<i>H. pylori</i> negative		<i>H. pylori</i> positive	
	n	Per cent	n	Per cent
AA	9	18	22	43.14
AB	31	62	21	41.18
BB	10	20	8	15.68
Total	50	100	51	100

Haplotype	<i>H. pylori</i> negative		<i>H. pylori</i> positive	
	n	Per cent	n	Per cent
A	49	98	65	127.46
B	1	2	37	72.54
A:B ratio	0.96:1		1.76:1	

KIR, killer immunoglobulin-like receptors.

Profile	N	%	KIR genes	2DL1	2DL2	2DL3	2DL4	2DL5A	2DL5B	3DL1	2DS1	2DS2	2DS3	2DS4*001	2DS4*003	2DS5	3DS1	2DP1	3DP1*001	3DP1*003
AA	1	2	4																	
AA	2	4	5																	
BB	1	2	5																	
AA	1	2	6																	
AA	2	4	6																	
AB	1	2	7																	
AB	1	2	7																	
AA	1	2	8																	
AB	1	2	8																	
AB	1	2	8																	
BB	1	2	8																	
BB	1	2	8																	
BB	2	4	8																	
AA	2	4	9																	
AB	1	2	9																	
AB	1	2	9																	
AB	1	2	9																	
AB	1	2	9																	
AB	1	2	9																	
AB	2	4	9																	
AB	2	4	9																	
BB	1	2	9																	
BB	1	2	9																	
AB	2	4	10																	
AB	2	4	10																	
BB	1	2	10																	
AB	1	2	11																	
AB	1	2	11																	
AB	2	4	11																	
AB	3	6	12																	
BB	1	2	12																	
BB	1	2	12																	
AB	1	2	13																	
AB	6	12	13																	

Figure 2 Killer Immunoglobulin-like receptors (KIR) genotypical profile among 50 Lebanese individuals negative for *Helicobacter pylori*.

an interesting study also showed that KIR3DS1 is closely associated with hepatitis C virus (HCV) clearance and sustained virological response in HIV/HCV patients.¹⁷

Our study data are consistent with the presence of the two major KIR haplotypes, group A and group B, with predominance of the group A haplotype among the *H. pylori*-positive patients, whereas the group B haplotype is more prevalent among the *H. pylori*-negative patients. To our knowledge, this is the first study in the literature that compares KIR genotypical distribution in patients with and without positivity for *H. pylori*

infection. Although there was no statistical significance in the difference between the KIR gene distributions among the two compared groups, we noticed a reduced distribution of A haplotype among the *H. pylori*-negative patients as compared with the *H. pylori*-positive group. This means that more activating genes (through a B haplotype) are present in the former group which may explain the negativity for the organism in terms of 'more killing' induced against *H. pylori*, which is typically what the NK cells function is about in this context. This important finding will have to be validated through a much larger number of patients in another study to translate this important genetic observation into a clinically significant finding especially in terms of eradication of the organism upon treatment. Studies involving KIR genotyping in *H. pylori*-associated gastric lymphomas are also worth conducting in view of the results reported in this study.

Our study represents a template for future researchers to build on its results and will help in assessing the role of KIR genotypical profiling in its association with various clinical entities. Treatment strategies may be tailored based on the KIR genotypical profile in a patient investigated for *H. pylori* infection where KIR genotyping may become an important diagnostic asset in managing these cases

Table 2 KIR gene frequency difference between *Helicobacter pylori*-positive and *H. pylori*-negative patients

	Status		p Value
	Negative n (%)	Positive n (%)	
2DL1	48 (96.0)	49 (96.1)	1.00
2DL2	38 (76.0)	33 (64.7)	0.21
2DL3	39 (78.0)	44 (86.3)	0.28
2DL4	50 (100.0)	51 (100)	–
2DL5A	21 (42.0)	19 (37.3)	0.63
2DL5B	28 (56.0)	19 (37.3)	0.06
3DL1	45 (90.0)	47 (92.2)	0.74
2DS1	27 (54.0)	24 (47.1)	0.48
2DS2	37 (74.0)	33 (64.7)	0.31
2DS3	29 (58.0)	24 (47.1)	0.27
2DS4*001	12 (24.0)	18 (35.3)	0.21
2DS4*003	43 (86.0)	37 (72.5)	0.10
2DS5	22 (44.0)	21 (41.2)	0.77
3DS1	24 (48.0)	21 (41.2)	0.49
2DP1	48 (96.0)	48 (94.1)	1.00
3DP1*001	11 (22.0)	11 (22.0)	1.00

A p value <0.05 was considered significant.
KIR, killer immunoglobulin-like receptors.

Take home messages

- ▶ Killer Immunoglobulin-like receptors (KIR) genotyping has been implicated in a variety of clinical and immunological disorders.
- ▶ This study is the first international research paper that describes the prevalence of KIR genes among *Helicobacter pylori* patients.
- ▶ Compared with a control population sample, the *H. pylori*-negative patients were found to have a higher B haplotype profile.

Acknowledgements The authors would like to acknowledge and thank Dr Ihab El Hajj and Dr Lara El Zahabi for their support in this research work.

Contributors RM: principal investigator of the research and finalised analysis and manuscript editing. RH: PCR data analysis, writing of the manuscript and online submission handling. NEH: PCR data analysis and follow-up on consenting. DS: PCR data analysis and writing up of the manuscript. AS: co-principal investigator of the research and recruitment of patients.

Competing interests None.

Ethics approval Institutional Review Board.

Provenance and peer review Not commissioned; externally peer reviewed.

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