NOS3 27-bp and *IL4* 70-bp VNTR Polymorphisms Do Not Contribute to the Risk of Sickle Cell Crisis

NOS3 27-bp ve *IL4* 70-bp VNTR Polimorfizmleri Orak Hücreli Anemide Kriz Riskine Katkıda Bulunmaz

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To the Editor,

A great deal of data support the direct involvement of the vascular endothelium, complex cellular interactions, and global inflammation-mediated cell activation in triggering vaso-occlusive crisis (VOC) in sickle cell disease (SCD) [1]. In the transgenic mice model for SCD, it has been shown that nitric oxide (NO) protects the mice from VOC [2]. Elevated plasma levels of certain proinflammatory cytokines support a role for cytokine-driven inflammation in SCD. The aim of the present study was to evaluate the role of the *NOS3* 27-bp variable number tandem repeat (VNTR) and *IL4* intron-3 VNTR functional polymorphisms in the development of crisis in Indian SCD patients. The study protocol was approved by the Institutional Ethics Committee of the Sickle Cell Institute Chhattisgarh, Raipur, India. Written informed consent was obtained from the study participants. A total of 256 individuals with SCD (55.5% men) were divided into two groups based on the history of VOC. The patients hospitalized with recurrent VOC were considered as the frequent crisis (FC) group (n=140; 54.7%) and patients who had not experienced any VOC during the past 1 year were considered as the infrequent crisis (IFC) group (n=116; 45.3%). Genotyping of the *NOS3* 27-bp VNTR [3] and *IL4* intron-3 VNTR [4] functional polymorphisms was performed and results were compared between the FC and IFC groups.

The genotype frequencies were in agreement with Hardy-Weinberg equilibrium for both the *NOS3* 27-bp (p=0.063) and the *IL4* 70-bp (p=0.614) VNTR. The genotype frequencies were not significantly different between the FC and IFC groups (Table 1). Similarly, the risk of frequent crisis was not found to be different between male and female SCD patients or between SCD patients with different HbF levels or different age groups (Table 1). Several lines of evidence suggest that there is vascular dysfunction and impaired NO bioactivity in SCD. Although no significant differences were observed in plasma NO metabolites between controls and SCD patients in the steady state, a significant reduction was noticed during VOC or acute chest syndrome [5]. Analysis of three *NOS3* gene polymorphisms did not reveal a significant association with severe clinical manifestations in Brazilian SCD patients [6]. In contrast to this, in another study a significant association of *NOS3* variants with VOC in SCD patients was reported [7]. However, our results indicate that the *NOS3* 27-bp VNTR polymorphism is not associated with the risk of frequent crises. Although the role of IL4 in SCD is controversial, increased serum IL4 levels were found in steady-state SCD patients compared to normal healthy controls [8]. Remarkably elevated levels of IL4 were noted in a VOC group compared to steady-state SCD patients and healthy controls [9]. IL4 levels correlated well with SCD status in Jamaicans, while exhibiting an ethnic difference between British and Jamaican children [10]. So far there are no published studies concerning *IL4* SNPs and SCD or its complications. As these results conflict with the biological plausibility that NO and interleukin levels modulate SCD, they deserve careful interpretation and further exploration.

Keywords: Sickle cell disease, Crisis, *NOS3, IL4*

Anahtar Sözcükler: Orak hücre hastalığı, Kriz, *NOS3, IL4*

Ethics

Ethics Committee Approval: The study protocol was approved by the Institutional Ethics Committee of the Sickle Cell Institute Chhattisgarh, Raipur, India, Informed Consent: Written informed consent was obtained from the study participants.

Authorship Contributions

Concept: L. V. K. S. Bhaskar, P. K. Patra; Design: L. V. K. S. Bhaskar, P. K. Patra; Data Collection or Processing: Henu Verma, L. V. K. S. Bhaskar; Analysis or Interpretation: L. V. K. S. Bhaskar; Literature Search: P. K. Khodiar, Henu Verma, Hrishikesh Mishra; Writing: Henu Verma, L. V. K. S. Bhaskar.

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Table 1. Association between *NOS3* 27-bp and *IL4* 70-bp VNTR polymorphisms and development of vaso-occlusive crisis in sickle cell disease.

	Vaso-Occlusive Crisis		Unadjusted		Adjusted for Age and Sex	
Genotype	**FC**	**IFC**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
***NOS3* 27-bp VNTR**						
4bb	101 (72.1)	89 (76.7)	Reference			
4ab	39 (27.9)	26 (22.4)	1.32 (0.75-2.34)	0.339	1.32 (0.75-2.35)	0.338
4aa	0 (0)	1 (0.9)	-	-	-	-
***IL4* 70-bp VNTR**						
3R3R	83 (59.3)	67 (57.8)	Reference			
2R3R	51 (36.4)	39 (33.6)	1.06 (0.62-1.79)	0.840	1.04 (0.61-1.76)	0.897
2R2R	6 (4.3)	10 (8.6)	0.48 (0.17-1.40)	0.181	0.49 (0.17-1.41)	0.184
Sex						
Male	78 (55.7)	64 (55.2)	Reference			
Female	62 (44.3)	52 (44.8)	0.98 (0.60-1.61)	0.931	0.97 (0.59-1.59)	0.896
HbF						
>20.1%	66 (47.1)	60 (51.7)	Reference			
10.1%-20%	59 (42.1)	43 (37.1)	1.25 (0.74-2.11)	0.140	1.27 (0.75-2.16)	0.374
<10%	15 (10.7)	13 (11.2)	0.105 (0.46-2.38)	0.909	1.05 (0.46-2.40)	0.916
Age						
<10 years	32 (22.9)	20 (17.2)	Reference			
10.1-20 years	65 (46.41)	76 (65.5)	0.54 (0.28-1.02)	0.059	0.53 (0.27-1.02)	0.056
>20.1 years	43 (30.7)	20 (17.2)	1.34 (0.62-2.90)	0.452	1.33 (0.61-2.88)	0.457

4b: *NOS3* VNTR wild-type allele, 4a: *NOS3* VNTR mutant allele, 2R: *IL4* VNTR 2 repeats, 3R: *IL4* VNTR 3 repeats, HbF: fetal hemoglobin, FC: frequent crisis, IFC: infrequent crisis, VNTR: variable number tandem repeat.

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