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EVALUATION OF INTERLEUKIN -10 (1082G/A) POLYMORPHISM AMONG SUDANESE PATIENTS WITH SICKLE CELL ANEMIA

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ABSTRACT

Background: Interleukin-10 (IL-10) is a multifunctional cytokine with both immunosuppressive and anti-angiogenic functions and may have both tumor-promoting and -inhibiting properties. We examined the association between a single nucleotide polymorphism (SNP) in IL-10 -1082G/A (rs1800896) among Sudanese sickle cell patients and to assess the association between polymorphisms in IL-10 -1082G/A (rs1800896) and according to age and sex in Sudanese patients with SCA. **Methods:** A total of 40 patients with sickle cell and 40 control subjects were enrolled in this study. Blood samples were collected from all patients in EDTA containing tubes. Genomic DNA was extracted from all blood samples using salting out method. The genotypic variants of IL-10 (-1082G/A) polymorphism were detected by allele specific-PCR. **Results:** We found that (70%) of patients have heterogeneous AG genotype, and (17.5%) have homozygous AA genotype and (12.5%) have homozygous GG genotype. GA genotype was significantly associated with higher risk of SCA compared with the homozygous Genotypes (GG and AA), there is no association between IL-10 (-1082G/A) polymorphism and gender and age group. **Conclusion:** Our study concluded that GA genotype of IL-10 -1082G/A (rs1800896) polymorphism is a risk factor for SCA and G allele is insignificantly higher than a allele in SCA patient.

KEYWORDS: *Interleukin-10- polymorphism- Sickle cell anemia*

INTRODUCTION

Sickle cell disease (SCD) is an inherited disorder of hemoglobin (Hb) structure and synthesis, which is characterized by chronic hemolysis, frequent infections and recurrent occlusions of the microcirculation. These comp locations cause painful crises and lead to chronic organ damage, disability and ultimately, premature death (Bunn HF.*et al.*, 1997; Stuart MJ *et al.*, 2004).

Direct adherence of sickle red blood cells to the endothelium plays a major role in vaso-occlusion and the process is the main cause of morbidity and mortality international society for clinical densitometry. (Platt *et al.*, 1995; Setty bny, *etal.*, 1996).

Vascular lumen obstruction in SCD result from interaction of erythrocyte, leukocytes, platelets, plasma proteins and vessel wall. The disease phenotype is a product of various genes and environmental factors acting in concert with the portion lesion underlying the res cell anomaly the severity of SCD increase with leukocytes count. The biological basis and therapeutic implications of this relationship are discussed. Leukocytes contribute to SCD by adhering.

To blood vessel walls and obstructing the lumen aggregating with other blood cell with more effecting blockage of the lumen stimulating the vascular endothelium to increase its expression of ligands for adhesion molecules on blood cells and inflammatory reaction which predispose to vaso – occlusion. Patients with impaired ability of leukocytes to kill microbes are more prone to infections which precipitate.

Sickle cell crisis Reduction of leukocytes count ameliorates SCD.

Similarly targeted blocked or reduced synthesis of specific leukocytes adhesion molecules and their ligands might confer clinical benefit SCD (okpala 2004)

Interleukins

Interleukins (ILs) are a diverse set of small cell signaling protein molecules, or cytokines, that function to alter the immune system in humans (Lippitz, 2013; Tsai *et al.*, 2013). ILs are predominantly produced by antigen-presenting cells, monocytes, macrophages, and endothelial cells, which are involved in the regulation of immune cell responses against infections, as well as

governing the inflammation, differentiation, proliferation, and secretion of antibodies for tumor development (Salazar-Onfray *et al.,* 2007; Gounaris *et al.,* 2009).

Several cytokines are also involved in the chronic inflammatory state that is present in SCA the involvement of several other cytokines, such as IL-18, IL-17, IL-23, IL-12 and IL10, in inflammatory responses in SCA patients (Levy, D.E. *et al.,* 2002).

IL-10 also known as human cytokine synthesis inhibitory factor (CSIF) is an anti- inflammatory cytokine. In human interleukin 10 is encoded by the il10 gene (Eskadale *et al.,* 1997).

Signals through receptor complex consisting of two IL-10 receptor-1 and two IL-10 receptor-2 proteins consequently, the functional receptor consists of four il10 binding induces state signaling via the phosphorylation of the cytoplasm tails of IL-10 receptor 1+ IL-10 receptor 2 by the JAK1 and TYK2 respectively (Mosser DM, zhang x, 2008).

MATERIALS AND METHODS

Study Population

In this is case-control study a total of 40 newly diagnosed sickle cell anemia patients attending Gafar Ibnaoaf hospital-Sudan in the period from January to May 2017 were recruited in this study.

Additionally, 40 age and gender-matched apparently healthy control subjects were included in the study.

Samples collection

Blood samples (3ml) were collected from patients and control- in ethylene diamine tetra acetic acid (EDTA).

DNA extraction

DNA extraction will be done by salting out method through protein precipitation at high salt concentration. The traditional protocol involves initial cell disruption and digestion with (SDS) sodium dodecyl sulfate-proteinase K, followed by the addition of high concentration of salts usually 6M sodium chloride.

The mixture is then centrifuged to allow proteins to precipitate to the bottom with the supernatant containing DNA then transferred to a new vial. DNA is then precipitated using ethanol or isopropanol (Carpi FM *et al.,* 2011; Miller SA *et al.,* 1988; Brimberg *et al.,* 1989).

Genotyping of IL-10(1082G/A)

Genotyping was carried out by using the polymerase chain reaction with allele specific primers as described by (Newton *et al.,* 1989). The primer sequences for genotyping are shown in (Table1). Two separated PCR reaction mixtures of 20 µl was prepared for each sample. PCR was performed by using Maxime PCR Premix Kit (i-Taq), (iNtRON BIOTECHNOLOGY, South Korea), Cat. No. 25025), 4µl of genomic DNA, 0.5 µl of each primer, and 14 µl distilled water. Beta globin gene was used as the internal control. PCR started at 94°C for 5 min, followed by denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 40 s, with a final extension at 72°C for 5 min. Thermo cycling was using TECHNE Tc-412-UK PCR Thermal Cycler 96 well.

The amplified products were run on 1.5% agarose gel, and then stained with ethidium bromide for visualization under ultraviolet gel documentation system (Figure 1).

Table 1: Allele Specific Primers for IL-10 -1082A/G Polymorphism.

Primers	Sequence	Product size (bp)
IL-10 A Allele	5'-CTACTAAGGCTTCTTTGGGAA-3'	550 bp
IL-10 G Allele	5'-TACTAAGGCTTCTTTGGGAG-3'	
IL-10 common	5'-CAGCCCTTCCATTTTACTTTC-3'	
β globin-GH20 (Forward)	5'-GAAGAGCCAAGGACAGGTAC-3'	268 bp
β globin-PC04 (Reverse)	5'-CAACTTCATCCACGTTCACC-3	

Statistical analysis

Data was analyzed using SPSS version 25. Desrciptive statistics was used to describe the study variable age and sex. Chi square was obtained to study the association between the gene and disease as well as with study variables. P value less than 0.05 considered as relation and significant difference.

RESULTS AND DISCUSSION

Results revealed that (70%) of patients have heterogeneous AG genotype, and (17.5%) have homozygous AA genotype and (12.5%) have homozygous GG genotype. GA genotype was significantly associated with higher risk of SCA compared with the homozygous Genotypes (GG and AA), there is no association between IL-10 (-1082G/A) polymorphism and SCA gender, age group.

The genotype and allele frequencies for both patients and controls are listed in (Table 2), showing a highly statistically significant difference in the genotype distribution between cases and controls. GA genotype prevalence Were significantly higher in cases than in controls P – value less than 5%. On the other hand, AA genotype prevalence Were higher among controls than in SCA patients than GG.

Genotype according to age AA *P. value* (0.072) and GG *P. value* (0.515).

Genotype according to Sex AA *P. value* (0.475) and GG *P. value* (0.386).

This is insignificantly and no association between age and sex and polymorphism of Il- 10 G/A (-1082G/A) in SCA.

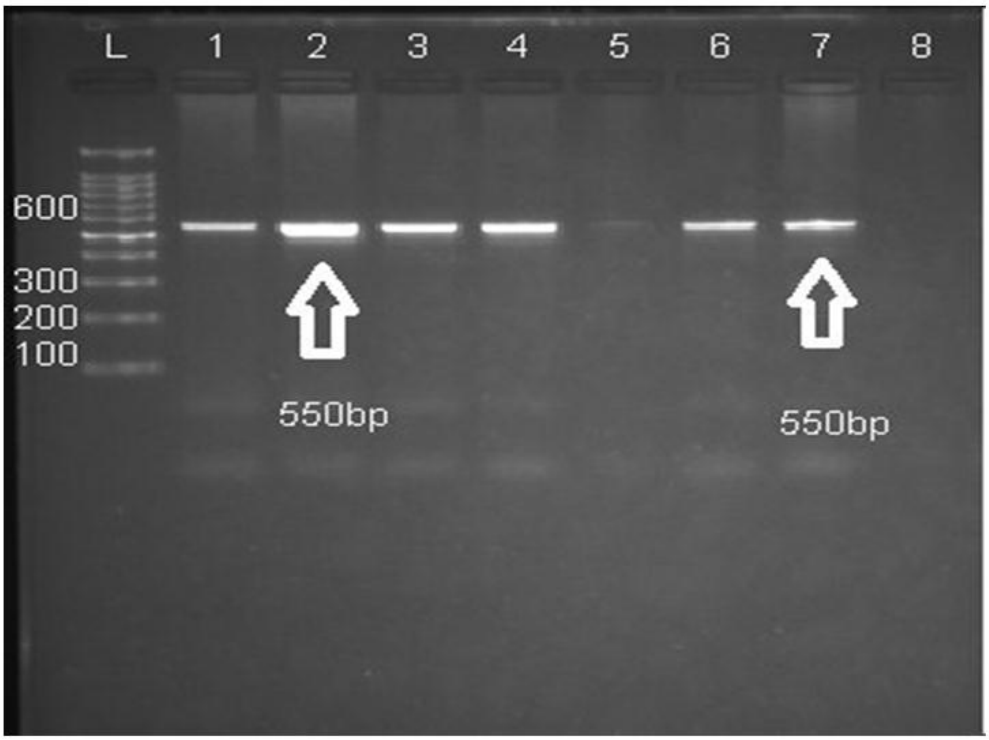

Table 2: Genotypes and Allele Frequencies of IL-10 (A-1082 G) Polymorphism in SCA Patients and Control.

Genotype	Group		Total	P-value	OR (CI Lower-CI Upper)
	Patient	Control			
AA	7 (17.5%)	23 (57.5%)	30 (37.5%)	0.000	30.667 (7.117-132.134)
GG	5 (12.5%)	14 (35.0%)	19 (23.8%)	0.000	26.133 (5.445-125.432)
AG	28 (70.0%)	3 (7.5%)	31 (38.8%)		
Total	40 (100.0%)	40 (100.0%)	80 (100.0%)		

Table 3: Genotypes and Allele Frequencies of IL-10 (A-1082 G) Polymorphism in different age groups.

Genotype	Age		Total	P-value	OR (CI Lower-CI Upper)
	5-10 Years	11-16 Years			
AA	5 (33.3%)	2 (8.0%)	7 (17.5%)	0.072	0.189 (0.031-1.171)
GG	1 (6.7%)	4 (16.0%)	5 (12.5%)	0.515	1.895 (0.184-19.482)
AG	9 (60.0%)	19 (76.0%)	28 (70.0%)		
Total	15 (100.0%)	25 (100.0%)	40 (100.0%)		

Table 4: Genotypes and Allele Frequencies of IL-10 (A-1082 G) Polymorphism according to gender.

Genotype	Gender		Total	P-value	OR (CI Lower-CI Upper)
	Male	Female			
AA	5 (19.2%)	2 (14.3%)	7 (17.5%)	0.475	0.618 (0.102-3.765)
GG	4 (15.4%)	1 (7.1%)	5 (12.5%)	0.388	0.386 (0.038-3.927)
AG	17 (65.4%)	11 (78.6%)	28 (70.0%)		
Total	26 (100.0%)	14 (100.0%)	40 (100.0%)		

CONCLUSION

In summary the study concluded that GA genotype of IL-10 -1082G/A (rs1800896) polymorphism is a risk factor for SCA.

REFERENCES

1. André Rolim Belisário a,b,∗, Rahyssa Rodrigues Salesb, Nayara Evelin Toledoc,Cibele Velloso-Rodriguesd, Célia Maria Silvac, Marcos Borato Vianab. (2017) Interleukin-10 haplotypes are not associated with acute cerebral ischemia or high-risk transcranial Doppler in a newborn cohort of 395 children with sickle cell anemia. *rev bras hematol hemoter*., 2017; 39(2): 108–114
2. Bunn HF Pathogenesis and treatment of sickle cell disease. N EnglJ Med., 1997; 337: 762-9.
3. Carpi FM, Di pretro F, vincenzettis,migninif, napolioni V, human DNA ertration methods: patents and applications recent pat DNA gene seq., 2011; 5(1): 1-7
4. Frenette, P.S. Sickle cell vaso-occlusion: Multi- step and multicellular paradigm. Current Opinion Hematology., 2002; 9: 101-106. doi:10.1097/00062752-200203000-00003
5. Frenette P. Sickle cell adhesion: heterotypic, multicellular aggregations driven by leukocyte adhesion. Microcirc., 2004; 11: 167-77.
6. Gee BE, Platt OS. Sickle reticulocytes adhere to VCAM-1. *Blood*, 1995; 85: 268-74.
7. Gounaris E, Blatner NR, Dennis K, Magnusson F, et al. T-regulatory cells shift from a protective anti-inflammatory to a cancer-promoting proinflammatory phenotype in polyposis. *Cancer Res*., 2009; 69: 5490-5497.
8. Grimberg j, nawoschik s, bellusciol, mckee r, turck A, Eisenb A, Asimple and efficient non organic procedure for the isolation of genomic DNA from blood. *nucleic Acid res.*, 1989; 17(20): 8390.
9. Hebbel RP, Osarogiagbon R, Kaul D. The endothelial biology of sickle cell disease: inflammation and a chronic vasculopathy. Microcirc., 2004; 11: 129-51.
10. John R Krimm,Do,Faaem Thoma Rebbecchi,MD,FAAEM, Overview of The Mangment and Prognosis of Sickle Cell Disease.
11. Lippitz BE Cytokine patterns in patients with cancer: a systematic review. *Lancet Oncol*., 2013; 14: e218-e228.
12. Levy, D.E. and Darnell Jr., J.E. Stats: Transcriptional control and biological impact. Nature Reviews, 2002.
13. Miller SA, dykes DD, polesky HF, Asimple salting out procedure for entraetion DNA from human rueleated cells nueleic Acid res., 1988; 16(3):1215.
14. Mullis, kary B. et al.”Process for amplifying,detecting,and/or-cloning nucleic acid sequences” U.S. Patent 4,683,195.
15. Opkala I The intriguing contribution of white blood cells to sickle cell disease: a red cell disorder. *Blood Rev*., 2004; 18: 65-73.
16. Rother, R.P., Bell, L., Hillmen, P. and Gladwin, M.T. 2004 The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin.
17. Stuart MJ, Nagel RL. Sickle cell disease. *Lancet*., 2005; 364: 1343-60.
18. Salazar-Onfray F, López MN and Mendoza-Naranjo A Paradoxical effects of cytokines in tumor immune surveillance and tumor immune escape. Cytokine Growth Factor Rev., 2007; 18: 171-182.
19. Saiki,RK,”Amplification of a Short DNA Stretch by Repeated Cycles of In Vitro DNA Polymerization” (PDF). American Association for the advancement of Science.
20. Setty BNY, Stuart MJ. Vascular cell adhesion molecule-1 is involved in mediating hypoxia-induced sickle red blood cell adherence to endothelium: Potential role in sickle cell disease. *Blood*, 1996; 88: 2311-20.
21. Steinberg, M.H. and Rodgers, G.P. Pathophysiology of sickle cell disease: Role of cellular and genetic modifiers. Seminars in Hematology, 2001; 38: 299-306. doi:10.1016/S0037-1963(01)90023-X
22. Tsai CW, Tsai MH, Shih LC, Chang WS, et al. Association of interleukin-10 (IL10) promoter genotypes with nasopharyngeal carcinoma risk in Taiwan. Anticancer Res., 2013; 33: 3391-3396.