RESEARCH ARTICLE
DOI: 10.4274/tjh.galenos.2025.2025.0441

Exploration of Leucine Rich Alpha-2-Glycoprotein 1 (LRG1) and its Association with Proangiogenic Mediators in Sickle Cell Disease: A Potential Player in the Pathogenesis of the Disease

Özcan O. et al.: Role of LRG1 in Sickle Cell Pathogenesis

Oğuzhan Özcan[1], Murat Kaçmaz[2], Fatma Hazal Erdoğan[1], Lütfiye Seçil Deniz Balyen[3], Hamdi Oğuzman[1], Hasan Kaya[3], Abdullah Arpacı[1]
[1]Hatay Mustafa Kemal University Faculty of Medicine, Department of Biochemistry, Hatay, Türkiye
[2]Diyarbakır Gazi Yaşargil Training and Research Hospital, Clinic of Hematology, Diyarbakır, Türkiye
[3]Hatay Mustafa Kemal University Faculty of Medicine, Department of Hematology, Hatay, Türkiye

Oğuzhan Özcan, M.D., Hatay Mustafa Kemal University Faculty of Medicine, Department of Biochemistry, Hatay, Türkiye
drozan29@hotmail.com
https://orcid.org/0000-0001-7486-503X
November 24, 2024
April 14, 2025

ABSTRACT

Objective: Leucine rich alpha-2-glycoprotein 1 (LRG1) is a novel mediator involved in the abnormal angiogenesis. We aimed to investigate circulating LRG1 levels and their relationship with proangiogenic mediators in sickle cell disease (SCD).

Methods: A total of 50 patients with SCD, 25 in steady-state conditions (SCD-SS), 25 in painful VOC periods (SCD-VOC), and 25 healthy controls were included in the study. Demographical and clinical data were collected from hospital records. Serum LRG1, VEGFA, and HIF1A levels were measured by ELISA, and CRP by the nephelometric method. Routine biochemical parameters were assessed using an auto analyzer. Multinomial logistic regression was used to analyze ELISA parameters, and ROC curves were constructed to determine the optimal cut-off point for HIF1A to estimate VOC in SCD patients.

Results: LRG1 and VEGFA levels were significantly higher in SCD patients than controls ($p<0.001$), with no difference between SCD-SS and SCD-VOC groups. HIF1A, CRP, and LDH levels differed significantly across all groups, highest in SCD-VOC ($p<0.001$). After adjusting for age and gender, LRG1, HIF1A, and VEGFA remained elevated in SCD groups. HIF1A correlated with CRP ($r = 0.351$, $p = 0.024$), but LRG1 showed no correlation with proangiogenic mediators in SCD-VOC group. The area under the curve (AUC) was calculated as 0.694 (95% CI, 0.542-0.845, $p=0.021$) and the optimal cut-off point were 494.5 pg/ml for HIF1A parameter to estimate VOC in patients with SCD.

Conclusion: Circulating LRG1 levels may reflect neutrophil activation and contribute to the cross-talk between proangiogenic mediators released in SCD.

Keywords: Sickle cell disease, LRG1, Angiogenesis, VEGF, HIF1A

INTRODUCTION

Sickle cell diseases (SCD), a group of autosomal recessive hemoglobinopathies, are prevalent in the Cukurova region of Turkey, particularly in the Hatay province (1). It is a monogenetic disorder caused by a single base-pair mutation in the β-globin gene. This mutation results in the substitution of valine for glutamic acid at the 6th position of the β chain, forming abnormal hemoglobin (HbS) (2, 3). The polymerization of deoxygenated HbS results in the transformation of red blood cells (RBC) from biconcave into sickle cell shape under low-oxygen conditions, leading to vaso-occlusion, especially in small vessels. Continuous and intermittent microvascular blockages initiate complex pathophysiological mechanisms, encompassing ischemia-reperfusion injury, hypercoagulability, heightened leukocyte adhesion to the endothelium, and ultimately tissue damage (4).

While the ischemic events of SCD have been extensively investigated, the role of hypoxia-induced angiogenic responses has not been fully elucidated in patients with SCD. Hypoxia inducible factor 1 subunit alpha (HIF1A) plays a pivotal role in maintaining oxygen homeostasis and is upregulated during hypoxia (5). Under hypoxic or

ischemic conditions, HIF1A initiates the upregulation of gene transcription and associated mediators responsible for promoting angiogenesis, such as vascular endothelial growth factor A (VEGFA). There are limited studies showing increased HIF1A expression levels in SCD (6, 7). Elevated levels of VEGFA have also been observed in patients with SCD, attributed to factors including hypoxia, ischemia, and vascular damage (7-9). However, certain studies have reported inconsistent findings (10). While these observations suggest a correlation between hypoxia and angiogenesis, the presence of pro-angiogenic mechanisms in SCD remains unclear. Among the emerging factors, leucine rich alpha-2-glycoprotein 1 (LRG1) is a secreted glycoprotein belonging to the leucine-rich repeat (LRR) protein family (11). LRG1 is primarily secreted from neutrophils, but it is also released from other cell types, including endothelial cells, epithelial cells and fibroblasts (12). Its primary function involves modulating angiogenesis, specifically pathological angiogenesis, by promoting vascular remodeling through the TGF-β signaling pathway (13). Elevated levels of LRG1 have been documented in diseases characterized by abnormal angiogenesis, such as diabetic kidney disease (14), multiple myeloma (15) and osteoarthritis (16)
However, there have been no studies investigating LRG1 levels in SCD. Considering the unbalanced angiogenesis resulting from hypoxia in patients with SCD, investigating the role of LRG1 may contribute to elucidating the cross-talk between angiogenetic and inflammatory mediators, including VEGF and HIF1A.

This is the first study evaluating serum LRG1 levels in patients with SCD. The aim of this study is to examine serum LRG1 level and its relationship with VEGFA, HIF1A, and acute phase reactants in patients with SCD during steady-state (SS) conditions and painful vaso-occlusive crisis (VOC) episodes.

Materials and Methods

Study design

This is a prospective case-control study. A total of 50 patients with proven SCD by hemoglobin electrophoresis (homozygous sickle cell disease [HbSS] and sickle cell beta thalassemia [HbS/β-thal]) presenting to the Hematology Department of Mustafa Kemal University Hospital between December 2021 and January 2023 were included in the study. The control group consisted of 25 age- and sex-matched healthy subjects. All demographic and clinical features were obtained from the hospital information system and recorded. Out of 50 patients with SCD, 25 were in steady-state (SCD-SS) and 25 were in a painful vaso-occlusive crisis (SCD-VOC) condition. An acute VOC phase was clinically considered a painful episode lasting more than 2 hours, and the patient felt the pain was specific to vaso-occlusion. Doctors could not identify another etiology for the pain, and the patient sought treatment for pain in the emergency department. The patients in the SS period were clinically defined as patients who had not been in crisis for at least one month before the study inclusion (17).
Informed written consent was obtained from all patients and healthy control participants. This study was performed according to the principles of the Declaration of Helsinki Ethical Principles for Human Medical Research, and the study protocol was approved by the Mustafa Kemal University Ethics Committee (protocol number: 2021/47).

Exclusion criteria

Individuals with any hematological disorders other than SCD and patients with chronic pain that is unrelated to SCD. Patients who had acute chest syndrome or other SCD-related complications within the past year as well as those with chronic kidney disease, inflammatory or connective tissue disease, acute or chronic infections, diabetes, obesity, liver failure, coronary heart disease, smokers, alcohol consumers, those who had received transfusions in the last 3 months, individuals under 18 years of age, pregnant women, and breastfeeding women were excluded from the study.

Sample collection and measurement of biochemical parameters

Whole blood (8 cc) for clot-activator gel tubes (BD® Vacutainer Serum Separation Tubes, USA) and the blood with EDTA (4 cc) (BD Vacutainer K_2EDTA tubes, USA) samples were collected from SCD patients and healthy control group participants. In the VOC group, blood samples were collected within the first hour of presentation before administering any medication to the patient. After centrifugation at 1500 x g for 10 minutes (Nuve NF 1200, Turkey), samples were aliquoted for the measurement of routine biochemistry and enzyme-linked immunosorbent assay (ELISA) analyses and stored at -80°C. Serum albumin, blood urea nitrogen (BUN), creatinine levels, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities were measured by the spectrophotometric method using an autoanalyzer (Advia 2400, Siemens, Japan), and serum high sensitive C-reactive protein (hsCRP) levels were measured by the nephelometric method (Siemens ADVIA 1200, Japan). Hematological parameters were assayed using a whole blood count analyzer (Mindray BC-6800, Shenzhen, China) within two hours of collection.
Serum LRG1, HIF1A, and VEGFA levels of all samples were measured by the ELISA method using commercially available kits (Elabscience ELISA Kits, China) according to the manufacturer's protocol for the ELISA device (Thermo Fisher Scientific Multiscan Go, Finland). Concentrations were calculated using a 4P-logic calibration curve, and the performance characteristics of the kits are as below:

LRG1 ELSIA kit (LOT: E-EL-H1287), the analysis range was 7.81–500 ng/mL, with a sensitivity of 4.69 ng/mL. Intra-assay CV<% 8 and inter-assay CV<% 10. The final concentrations were calculated by multiplying the dilution factor and given as µg/mL.
HIF1A ELISA kit (LOT: E-EL-H6066), the analysis range was 62.5–4000 pg/mL, with a sensitivity of 37.5 pg/mL. Intra-assay CV<% 8 and inter-assay CV<% 10.
VEGFA ELISA kit (LOT: E-EL-H0111), the analytical range was 31.25–2000 pg/mL, with a sensitivity of 18.75 pg/mL. Intra-assay CV<% 8 and inter-assay CV<% 10.

Statistical Analysis

Statistical package IBM SPSS Statistics software (SPSS) for Windows, Version 21.0 (SPSS Inc., Chicago, IL) was used to analyze the data. The power analysis was performed using an effect size (Cohen's f) of 0.44, with an alpha level of 0.05 and a power of 0.80. The effect size was determined based on similar studies in the literature and pilot data previously obtained. The required sample size for each group was calculated to be at least 18 subjects. The normality of the data was evaluated using the Shapiro-Wilk test. Normally distributed variables were presented as mean ± standard deviation, whereas non-normally distributed variables were characterized by median (25th–75th) values. Categorical data were analyzed using the chi-square test. Continuous variables with a normal distribution were analyzed by one-way analysis of variance (ANOVA), followed by post-hoc Tukey or Tamhane's T2 tests as appropriate. For non-normally distributed continuous variables, the Kruskal-Wallis with post-hoc Bonferroni multiple comparison test was utilized. The relationships between variables were assessed using the Pearson correlation test for normally distributed data and the Spearman correlation test for non-normally distributed data. A multinomial logistic regression analysis was conducted to evaluate the relationship between SCD groups and ELISA parameters. In the regression model, the ELISA parameters were defined as independent variables, while the disease groups (SCD-SS and SCD-VOC) were defined as dependent variables, with the control group serving as the reference category. Adjustments were made for age and gender, and the resulting Odds Ratios (OR) and 95% confidence intervals (CI) were reported. We also constructed receiver-operating characteristic (ROC) curves and calculated the sensitivities and specificities of HIF1A. The optimal cut-off point was detected for HIF1A parameter to estimate VOC in patients with SCD. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS

The demographic distribution and laboratory results of participants were presented in Table 1. Of the 50 patients with SCD, 25 (50%) were males and 25 (50%) were females, with a mean age of 34±9 years. There were no statistically significant differences in age and gender among participants in all three groups. The majority of the patients had homozygous sickle cell anemia (HbSS). Of the patients, 47 (94%) had homozygous sickle cell anemia (HbSS), while only 3 (6%) had sickle/β-thalassemia (HbS/β+-thalassemia). Fifteen patients (60%) in the SCD-VOC group and 13 patients (52%) in the SCD-SS group were using hydroxyurea.
Serum CRP levels and LDH activities were significantly different for all three groups, but highest in the SCD-VOC group ($p<0.001$). White blood cell (WBC) and neutrophil counts and AST activities were found to be higher in SCD patients compared to the healthy controls ($p<0.001$), but there was no significant difference between the SCD-VOC and SCD-SS groups. Hemoglobin levels were significantly different for all three groups and lowest in the SCD-VOC group, but albumin levels were significantly lower only in the SCD-VOC group ($p<0.001$) (Table 1). Serum LRG1 levels were significantly higher in both the SCD-SS and SCD-VOC groups compared to the controls ($p=0.003$ and $p=0.001$, respectively). There was no significant difference observed in serum LRG1 levels between the SCD-SS and SCD-VOC groups (Figure 1A). Serum HIF1A levels were significantly higher in both the SCD-SS and SCD-VOC groups compared to the controls ($p<0.001$) (Figure 1B), and its highest levels were observed in the SCD-VOC group ($p=0.006$). VEGFA levels were significantly higher in both the SCD-SS and SCD-VOC groups compared to the controls ($p=0.004$ and $p<0.001$, respectively), but no significant difference was observed between the SCD-SS and SCD-VOC groups (Figure 1C). In the VOC-SCD group, serum HIF1A levels positively correlated with CRP levels ($r = 0.351$, $p = 0.024$). There were no significant correlations observed between LRG1 and VEGFA ($r = 0.047$, $p = 0.832$) and HIF1A ($r = 0.085$, $p = 0.699$) levels in the SCD-VOC and SCD-SS groups. According to the multinomial logistic regression analysis, after adjusting for age and gender, the SCD-SS and SCD-VOC groups were found to have significantly higher levels of LRG1, HIF1A, and VEGFA compared to the control group (Table 2). The area under the curve (AUC) was calculated as 0.694 (95% CI, 0.542-0.845, $p= 0.021$) and the optimal cut-off point were 494.5 pg/ml for HIF1A parameter to estimate VOC in patients with SCD (Figure 2). The HBS levels of SCD-SS and SCD-VOC groups were 76.5% (58.6-88.4) and 79.5% (76.5-84.4), respectively. There was no significant difference observed between groups ($p= 0.572$). HbS levels were not correlated with LRG1, HIF1A and VEGFA levels in SCD patients (respectively, $r = 0.099$, $p = 0.748$; $r = 0.421$, $p = 0.152$; $r = -0.022$, $p=0.940$)

DISCUSSION

In this study, serum levels of LRG1 along with proangiogenic mediators HIF1A and VEGFA were measured at different stages of SCD. Both LRG1 and VEGFA levels were significantly elevated in SCD patients compared to healthy controls, with no significant differences observed between the SS and VOC periods, displaying similar patterns. Serum HIF1A levels significantly higher in SCD patients, with the highest levels observed in the VOC group, differing from LRG1 and VEGFA. Additionally, a significant but low correlation was observed between serum HIF1A and CRP levels, while no correlation was found between LRG1 and proangiogenic mediators. HIF1A demonstrated better discriminatory ability between VOC and SS conditions in SCD patients.

In the present study, it was found that LRG1 levels were significantly elevated in both the SS and VOC groups compared to the healthy controls ($p=0.003$ and $p=0.001$, respectively) (Fig. 1A) (Table 1). Additionally, serum LRG1 levels remained significantly higher in SCD-SS and SCD-VOC groups in multivariable models adjusting for age and gender (Table 2). But there was no significant difference observed between the VOC and SS periods. A potential explanation for the elevated levels of LRG1 in SCD patients could be associated with its secretion kinetics. Because LRG1 is packaged into the granule compartment of human neutrophils and secreted upon neutrophil activation to modulate the microenvironment (18). Considering the increased granulopoiesis in SCD patients (19-21), we can say that LRG1 may be upregulated by the chronic systemic inflammation observed in SCD patients and contribute to the microcirculatory irregularities. The significantly elevated WBC and neutrophil counts in both the SCD-VOC and SCD-SS groups compared to the controls in this study further support this notion (Table 1).

We also found higher serum CRP levels in the patient group, but its levels were highest in the VOC group (Table 1). CRP is a well-known acute-phase reactant and a laboratory indicator of the severity of inflammation in SCD. But in the VOC group, there was no significant correlation between LRG1 levels and CRP, WBC, or neutrophil counts. However, the level of LRG1 did not vary with disease severity. We can say that LRG1 is not an effective biomarker for distinguishing between the crisis and remission periods. Therefore, further studies are needed to understand the role of LRG1 as an acute-phase protein in patients with SCD. However, the significantly elevated levels of circulating LRG1 in patient groups suggest its potential involvement in the angiogenic imbalance associated with SCD. LRG1 exerts these effects by activating the pro-angiogenic pathway, promoting endothelial cell proliferation, migration, and tubulogenesis (11). It has also been reported that LRG1 enhances TGF-β signaling in the endothelium, contributing to abnormal vascular growth in inflammatory and ischemic conditions (13). This mechanism may also play a role in the pathogenesis of SCD. In a study conducted on a cardiomyocyte cell line, it was demonstrated that LRG1 significantly enhanced the expression of HIF1A (22). Another study reported that LRG1 induces HIF1A and regulates epithelial-mesenchymal transition and angiogenesis in colorectal cancer (23). Moreover, LRG1 has been demonstrated to guide glomerular endothelial cells toward a pro-angiogenic pathway (24) and to enhance ocular neovascularization in diabetic retinopathy (13). In this study, serum VEGFA and HIF1 levels, proangiogenic mediators, were also measured. Serum HIF1A levels were significantly increased in both SCD-SS and SCD-VOC groups compared to healthy controls ($p<0.001$) (Fig. 1B). This difference was significant, after adjusted for age and gender (Table 2). HIF1A is a widely recognized key transcription factor that becomes activated in response to hypoxia and plays a vital role in restoring oxygen homeostasis (25, 26). Increased expression of HIF1A in SCD patients has been demonstrated in a limited number of studies (7, 27). Our findings were consistent with previous studies and support the idea of elevated HIF1A levels in SCD patients. Unlike LRG1, its levels were significantly higher in the SCD-VOC group compared to the SCD-SS group ($p=0.006$) (Fig. 1B) and showed a positive correlation with CRP levels ($r = 0.351$, $p = 0.024$). This is an anticipated result, whereby elevated levels of HIF1A in the SCD-VOC group may be associated with the severe hypoxia observed in these patients. We also performed ROC analysis to determine the optimal threshold value for HIF1A in patients with SCD. AUC was calculated as 0.694 (95% CI, 0.542-0.845, $p= 0.021$) (Fig. 2). We can say that a serum HIF1A cut-off value greater than 494.5 pg/ml is feasible to predict VOC in SCD patients.

However, the elevated HIF1A levels observed in patients during the SS period were likely a result of ongoing chronic inflammation, as suggested by a previous study (28). During hypoxia, it has been demonstrated that HIF1A rapidly binds to the regulatory region of the VEGFA-expressing gene, thereby initiating its transcription and translation (29, 30). In the present study, VEGFA levels were also found to be elevated in the patients at the SS and VOC periods compared to the controls ($p=0.004$ and $p<0.001$, respectively), similar to LRG1. However, no significant difference was observed between two periods (Fig. 1C). VEGFA has been previously investigated in patients with SCD; however, conflicting results have been reported. While some studies have found no differences in VEGFA levels between patients with SCD during VOC and SS periods (10, 31), other researchers have reported higher VEGFA levels during VOC episodes (32). A possible reason for the differences between studies could be related to the anti-angiogenic effects of hydroxyurea, a drug used in SCD treatment. One study has suggested that hydroxyurea reduces VEGFA and HIF1A expression under both in vivo and in vitro conditions (33). Although the underlying mechanism has not yet been elucidated, hydroxyurea treatment may have affected serum LRG1 and VEGF levels through novel vascular mechanisms in the patients at different stages of SCD. The use of hydroxyurea was comparable between both groups in patients during the SS (52%)

and VOC periods (60%). This may explain the lack of a significant difference between the two groups in our study. In the current study, serum VEGFA levels have demonstrated a similar pattern to serum LRG1 levels in patients with SCD. A similar relationship has been reported in another study conducted on tumor cells. They have shown that LRG1 directly induces VEGFA expression and promotes angiogenesis in colorectal cancer cells (23). Another study also showed that knockdown of LRG1 dramatically reduced VEGFA expression in mice retina (13). In the present study, elevated LRG1 and VEGFA levels in SCD patients may cause the formation of non-functional vessels, contributing to the unbalanced angiogenesis triggered by ischemic processes.
In conclusion, we observed elevated levels of LRG1 and along with other proangiogenic mediators, VEGF and HIF1A, in SCD patients. Additionally, HIF1A may prove to be more useful in distinguishing between VOC and steady-state periods. Circulating LRG1 levels may reflect neutrophil activation in SCD and contribute to the cross-talk between proangiogenic mediators released during hypoxia. However, this small cohort study does not support LRG1 as a biomarker for VOC. Larger studies with more extensive sample sizes are needed to validate its potential as a biomarker in patients during the VOC period.

Limitations of the study

The first limitation is the small sample size of the study because of the single-center design. Another limitation is drug use. More than half of the patients were receiving hydroxyurea treatment, which had previously been reported to affect the serum levels of some markers. Therefore, further studies with a larger case group considering drug usage are needed.

The authors contributions:
OO and MK designed the study, analyzed results and wrote the manuscript. FHE, HO and LSDB contributed to the data and sample collection and experimental/statistical analyses. AA and HK edited and revised the manuscript. AA was the mentor and participated in all steps of the study.
Conceptualization: OO and MK
Methodology: OO and HO
Formal analysis and investigation: OO, FHE and LSDB
Writing - original draft preparation: OO, MK and HO
Writing - review and editing: OO, MK, HK and AA
Supervision: HK and AA

The authors have no competing interests to declare that are relevant to the content of this article.
No funding was received for conducting this study.

References

1. Soylemez-Gokyer D, Kayaalti Z. Distribution of sickle cell anemia in Turkey, pathophysiology and iron toxicity. Marmara Pharm J 2016;20:092-099.
2. Inusa BPD, Hsu LL, Kohli N, Patel A, Ominu-Evbota A, Anie KA, Atoyebi W. Sickle cell disease-genetics, pathophysiology, clinical presentation and treatment. Int J Neonatal Screen 2019;5:20.
3. Ozcan O, Erdal H, Ilhan G, Demir D, Gurpinar AB, Neselioglu S, Erel O. Plasma ischemia-modified albumin levels and dynamic thiol/disulfide balance in sickle cell disease: a case-control study. Turk J Haematol 2018;35:265-270
4. Kato GJ, Steinberg MH, Gladwin MT. Intravascular hemolysis and the pathophysiology of sickle cell disease. J Clin Invest 2017;127:750-760.
5. Krock BL, Skuli N, Simon MC. Hypoxia-induced angiogenesis: good and evil. Genes Cancer 2011;2:1117-1133.
6. Kaul DK, Fabry ME, Suzuka SM, Zhang X. Antisickling fetal hemoglobin reduces hypoxia-inducible factor-1α expression in normoxic sickle mice: microvascular implications. Am J Physiol Heart Circ Physiol 2013;304:H42-50.
7. Pedrosa AM, Lemes RPG. Gene expression of HIF-1α and VEGF in response to hypoxia in sickle cell anaemia: influence of hydroxycarbamide. Br J Haematol 2020;190:e39-e42.
8. Antwi-Boasiako C, Frimpong E, Gyan B, Kyei-Baafour E, Sey F, Dzudzor B, Abdul-Rahman M, Dankwah GB, Out KH, Ndanu TA, Campbell AD, Ekem I, Donkor S. Elevated proangiogenic markers are associated with vascular complications within Ghanaian sickle cell disease patients. Med Sci (Basel) 2018;6:53.
9. Niu X, Nouraie M, Campbell M, Rana S, Minniti CP, Sable C, Darbari D, Dham N, Reading NS, Prchal JT, Kato GJ, Gladwin MT, Castro OL, Gordeuk VR. Angiogenic and inflammatory markers of cardiopulmonary changes in children and adolescents with sickle cell disease. PLoS One 2009;4:e7956.
10. Duits AJ, Rodriguez T, Schnog JJ. Serum levels of angiogenic factors indicate a pro-angiogenic state in adults with sickle cell disease. Br J Haematol 2006;134:116-9.

11. Camilli C, Hoeh AE, De Rossi G, Moss SE, Greenwood J. LRG1: an emerging player in disease pathogenesis. J Biomed Sci 2022;29:6.
12. Yang J, Yin GN, Kim DK, Han AR, Lee DS, Min KW, Kim HM. Crystal structure of LRG1 and the functional significance of LRG1 glycan for LPHN2 activation. *Exp Mol Med* 2023;55(5):1013-1022.
13. Wang X, Abraham S, McKenzie JAG, Jeffs N, Swire M, Tripathi VB, Luhmann UFO, Lange CAK, Zhai Z, Arthur HM, Bainbridge J, Moss SE, Greenwood J. LRG1 promotes angiogenesis by modulating endothelial TGF-β signalling. Nature 2013;499:306-311.
14. Hong Q, Zhang L, Fu J, Verghese DA, Chauhan K, Nadkarni GN, Li Z, Ju W, Kretzler M, Cai GY, Chen XM, D'Agati VD, Coca SG, Schlondorff D, He JC, Lee K. LRG1 promotes diabetic kidney disease progression by enhancing TGF-beta-induced angiogenesis. J Am Soc Nephrol 2019;30:546-562.
15. Kacmaz M, Oguzman H. The Leucine-Rich α2-Glycoprotein-1 Levels in patients with multiple myeloma. Oncol Res Treat 2023;46:415-423.
16. Wang Y, Xu J, Zhang X, Wang C, Huang Y, Dai K, Zhang X. TNF-α-induced LRG1 promotes angiogenesis and mesenchymal stem cell migration in the subchondral bone during osteoarthritis. Cell Death Dis 2017;8:e2715.
17. Lamarre Y, Romana M, Waltz X, Lalanne-Mistrih ML, Tressières B, Divialle-Doumdo L, Hardy-Dessources MD, Vent-Schmidt J, Petras M, Broquere C, Maillard F, Tarer V, Etienne-Julan M, Connes P. Hemorheological risk factors of acute chest syndrome and painful vaso-occlusive crisis in children with sickle cell disease. Haematologica 2012;97:1641-1647.
18. Druhan LJ, Lance A, Li S, Price AE, Emerson JT, Baxter SA, Gerber JM, Avalos BR. Leucine Rich α-2 Glycoprotein: A novel neutrophil granule protein and modulator of myelopoiesis. PLoS One 2017;12:e0170261.
19. Rees DC, Kilinc Y, Unal S, Dampier C, Pace BS, Kaya B, Trompeter S, Odame I, Mahlangu J, Unal S, Brent J, Grosse R, Fuh BR, Inusa BPD, Koren A, Leblebisatan G, Levin C, McNamara E, Meiser K, Hom D, Oliver SJ. A randomized, placebo-controlled, double-blind trial of canakinumab in children and young adults with sickle cell anemia. Blood 2022;139:2642-2652.
20. Krishnan S, Setty Y, Betal SG, Vijender V, Rao K, Dampier C, Stuart M. Increased levels of the inflammatory biomarker C-reactive protein at baseline are associated with childhood sickle cell vaso-occlusive crises. Br J Haematol 2010;148:797-804.
21. West MS, Wethers D, Smith J, Steinberg M. Laboratory profile of sickle cell disease: a cross-sectional analysis. The cooperative study of sickle cell disease. J Clin Epidemiol 1992;45:893-909.
22. Feng J, Zhan J, Ma S. LRG1 promotes hypoxia-induced cardiomyocyte apoptosis and autophagy by regulating hypoxia-inducible factor-1α. Bioengineered 2021;12:8897-907.
23. Zhang J, Zhu L, Fang J, Ge Z, Li X. LRG1 modulates epithelial-mesenchymal transition and angiogenesis in colorectal cancer via HIF-1α activation. J Exp Clin Cancer Res 2016;35:29.
24. Zhang A, Fang H, Chen J, He L, Chen Y. Role of VEGF-A and LRG1 in abnormal angiogenesis associated with diabetic nephropathy. Front Physiol 2020;11:1064.
25. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 1992;12:5447-5454.
26. Kenneth NS, Rocha S. Regulation of gene expression by hypoxia. Biochem J 2008;414:19-29.
27. Zhang X, Zhang W, Ma SF, Desai AA, Saraf S, Miasniakova G, Sergueeva A, Ammosova T, Xu M, Nekhai S, Abbasi T, Casanova NG, Steinberg MH, Baldwin CT, Sebastiani P, Prchal JT, Kittles R, Garcia JGN, Machado RF, Gordeuk VR. Hypoxic response contributes to altered gene expression and precapillary pulmonary hypertension in patients with sickle cell disease. Circulation 2014;129:1650-1658.
28. Kaul DK, Hebbel RP. Hypoxia/reoxygenation causes inflammatory response in transgenic sickle mice but not in normal mice. J Clin Invest 2000;106:411-20.
29. Xu Y, Kong X, Li J, Cui T, Wei Y, Xu J, Zhu Y, Zhu X. Mild hypoxia enhances the expression of HIF and VEGF and triggers the response to injury in rat kidneys. Front Physiol 2021;12:690496.
30. Milkiewicz M, Pugh CW, Egginton S. Inhibition of endogenous HIF inactivation induces angiogenesis in ischaemic skeletal muscles of mice. J Physiol 2004;560:21-26.
31. Al-Habboubi HH, Mahdi N, Abu-Hijleh TM, Abu-Hijleh FM, Sater MS, Almawi WY. The relation of vascular endothelial growth factor (VEGF) gene polymorphisms on VEGF levels and the risk of vasoocclusive crisis in sickle cell disease. Eur J Haematol 2012;89:403-409.
32. Gurkan E, Tanriverdi K, Baslamisli F. Clinical relevance of vascular endothelial growth factor levels in sickle cell disease. Ann Hematol 2005;84:71-75.
33. Lopes FCM, Ferreira R, Albuquerque DM, Silveira AAA, Costa R, Soares R, Costa FF, Conran N. In vitro and in vivo anti-angiogenic effects of hydroxyurea. Microvasc Res 2014;94:106-113.

Table 1. The comparison of age, gender and the levels of biochemical parameters in study groups.

Variables	Control (n=25) Mean ± SD Median (25th-75th)	SCD-SS (n=25) Mean ± SD Median (25th-75th)	SCD-VOC (n=25) Mean ± SD Median (25th-75th)	p-Values
Female, n (%)	17 (68)	11 (44)	14 (56)	0.232*
Age, yrs	33±7	35±9	33±9	0.715**
WBC, 10^3 /µL	7.2 (5.9-8.3)	11.4 (6.9-13.7)	13.4 (10.7-17.6)	0.014[a] <0.001[b] 0.164[c]
Neutrophil counts, 10^3 /µL	4.1 (3.4-5.2)	6.1 (4.1-8.9)	9.3 (6.3-13.1)	0.024[a] <0.001[b] 0.145[c]
Hemoglobin, g/dL**	14±1.6	9.3±2.1	7.7±1.1	<0.001[a] <0.001[b] 0.002[c]
Platelet, 10^3 /µL	243 (211-288)	361 (219-444)	317 (184-415)	0.065
hsCRP, mg/L	0.92 (0.64-1.71)	4.39 (3.13-7.73)	34.7 (10.5-61.5)	<0.001[a] <0.001[b] 0.003[c]
Albumin, g/dL	4.6 (4.4-4.7)	4.3 (4.2-4.7)	3.7 (3.6-3.9)	0.398[a] <0.001[b] <0.001[c]
ALT, U/L	17 (14-26)	18 (14-29)	25 (14-33)	0.416
AST, U/L	15 (13-20)	40 (27-62)	50 (36-60)	<0.001[a] <0.001[b] 0.791[c]
LDH, U/L	145 (89-201)	339 (238-542)	638 (491-680)	<0.001[a] <0.001[b] 0.016[c]
BUN, mg/dL	11 (10-14)	9 (8-13)	8 (6-11)	0.280[a] 0.252[b] 0.002[c]
Creatinine, mg/dL	0.69 (0.61-0.79)	0.49 (0.42-0.61)	0.42 (0.34-0.55)	<0.001[a] <0.001[b] 0.813[c]
LRG1 (µg/mL)**	17.5±7.5	29.5±9.4	31.3±18.3	0.003[a] 0.001[b] 0.862 [c]
HIF1A (pg/mL)**	174.8±44.7	413.5±169.3	572.1±241.5	<0.001[a] <0.001[b] 0.006[c]
VEGFA (pg/mL)	416 (374-484)	492 (417.5-630.5)	528 (446.5-589)	0.004[a] <0.001[b] 1.000[c]

* Chi-square test, **ANOVA test. Kruskal Wallis test were conducted for other parameters. SCD-SS, the patients with SCD in steady-state condition. SCD-VOC, The patients with SCD in painful VOC period. To demonstrate statistical significance in pairwise comparisons: [a], Control vs. SCD-SS; [b], Control vs. SCD-VOC groups; [c], SCD-SS vs. SCD-VOC groups. WBC, white blood cell; hsCRP, high-sensitivity C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; LRG1, leucine rich alpha-2-glycoprotein 1; HIF1A, hypoxia inducible factor 1 subunit alpha; VEGFA, vascular endothelial growth factor A.

Table 2. Multinomial logistic regression for the association between study groups and ELISA parameters

		LRG1	HIF1A	VEGFA
Control		**1 (Reference)**	**1 (Reference)**	**1 (Reference)**
SCD-SS	OR (95% CI)*	1.15 (1.053-1.256)	1.025 (1.010-1.042)	1.014 (1.005-1.022)
	p-Value	0.002	0.002	0.002
SCD-VOC	OR (95% CI)*	1.2 (1.076-1.339)	1.029 (1.013-1.046)	1.015 (1.007-1.024)
	p-Value	0.001	<0.001	<0.001

* Adjusted for age and sex. The dependent variable included Control, SCD-SS, and SCD-VOC as categorical groups, with the Control group as the reference. The presence of multicollinearity among independent variables was assessed using variance inflation factors (VIF), and no variable with VIF > 5 was detected. Odds Ratios (OR) and their corresponding 95% confidence intervals (CI) are presented. LRG1, leucine-rich alpha-2-glycoprotein 1; HIF1A, hypoxia-inducible factor 1 subunit alpha; VEGFA, vascular endothelial growth factor A.