LETTER TO THE EDITOR
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Leucine-Rich Alpha-2 Glycoprotein 1 (LRG1) and Proangiogenic Mediators in Sickle Cell Disease

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Dear Editor, the publication on "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 [1]" is interesting. Several constraints may impair the validity and reliability of the presented results. First, the sample size of sickle cell anemia (SCD) patients and controls was relatively small (50 patients, 25 in the steady-state phase and 25 in the critical VOC phase), which may not be sufficient to detect statistically significant differences or be representative of the SCD population as a whole. Furthermore, the classification of patients by VOC and steady-state was based on the time period during which the samples were obtained, with no longitudinal follow-up, which may not correctly reflect biochemical changes over time.
In terms of data analysis, the use of multinomial logistic regression analysis, while appropriate for comparing more than two groups, did not imply that other confounding variables such as medication use or disease complications were controlled, which could have influenced the measured protein levels. Furthermore, the correlation value between HIF1A and CRP was minimal ($r = 0.351$). Although statistically significant, it may not be biologically relevant. There was also no mention of a correlation test between all of the variables, such as LRG1 and markers of inflammation or cell damage like LDH, which could provide more information about LRG1's role.
Some interesting points for further debate include: 1) Why did LRG1 and VEGFA levels not differ between SCD-SS and SCD-VOC groups, despite VOC being more clinically severe? 2) Does HIF1A, a hypoxia marker, have a link with CRP, which measures inflammation? Does this imply that hypoxia in VOC may be caused by inflammation rather than vascular factors? 3) Does the lack of a connection between LRG1, an angiogenesis promoter, and VEGFA or HIF1A during VOC imply that LRG1 may have alternative pathogenetic activities unrelated to hypoxia response?
A new interpretation of the findings could imply that LRG1 plays a stable role in SCD patients, with no further activation during VOC crisis, implying that it is a structural biomarker of the disease. While HIF1A and CRP levels were considerably elevated in VOC, they may have been markers of acute events rather than current illness status. In terms of the HIF1A ROC curve, the AUC = 0.694 demonstrated a decent capacity to predict VOC, however it is insufficient for clinical use. The use of numerous biomarkers or more complicated models may result in more accurate predictions. All of this highlights the importance of bigger sample sizes, long-term follow-up, and better control of confounding variables in research.

Data Availability statement: There is no new data generated.

References

1. Özcan O, Kaçmaz M, Erdoğan FH, Balyen LSD, Oğuzman H, Kaya H, Arpacı A. 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. Turk J Haematol. 2025 May 22;42(2):100-107.

Dear Editor,

We would like to express our sincere appreciation for the valuable comments and constructive criticisms regarding our article entitled, *"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."*

We respectfully address the main issues below, based on both our research and the current scientific literature.

First, we did a priori power analysis with an effect size (Cohen's f = 0.44), an alpha of 0.05, and a power of 0.80. This showed that 18 people in each group would be enough. Our sample of 25 patients in each group was above this level, which gave us 92.4% statistical power for comparing LRG1. While this supports statistical adequacy, we agree that larger multi-center studies would enhance generalizability, and we have acknowledged this limitation in our manuscript [1].

Second, while longitudinal data can provide insights into disease dynamics, our study employed a prospective, cross-sectional design specifically aimed at distinguishing biomarker profiles between steady-state (SS) and vaso-occlusive crisis (VOC) phases. We believe this model is appropriate for detecting phase-specific biochemical differences, laying groundwork for future longitudinal studies.

Third, we acknowledge the importance of controlling for potential confounders such as hydroxyurea usage and disease complications. Treatment rates were similar between groups (SS: 52%, VOC: 60%), minimizing intergroup bias. Furthermore, we applied strict exclusion criteria and excluded the patients with SCA-related complications. We also adjusted for age and sex in multinomial logistic regression models. Details of drug usage, statistical adjustments and exclusion criteria were clearly presented in the relevant sections of the manuscript.

We observed weak correlation between HIF1A and CRP ($r = 0.351$, $p = 0.024$). However, the specificity of this correlation only in the VOC group may reflect the acute interplay between hypoxia and inflammation in this phase of the disease [2]. While LRG1 did not correlate significantly with VEGFA or HIF1A, and no explicit correlation with LDH was presented, its consistent elevation across both clinical states may indicate a role as a chronic marker of vascular remodeling rather than an acute-phase reactant [3].

The lack of significant differences in VEGFA and LRG1 between SS and VOC may also stem from chronic endothelial activation, a recognized hallmark of sickle cell disease. Hydroxyurea's known effects on angiogenesis may further contribute to this pattern as we explained in the discussion and limitation sections [4].

Although HIF1A's ROC AUC of 0.694 suggests limited standalone clinical utility, the establishment of a predictive cut-off (494.5 pg/mL) is a novel contribution. We suggest that future biomarker panels combining HIF1A with other parameters (e.g., CRP, LDH, LRG1) may enhance diagnostic accuracy.

Finally, we concur that LRG1 may represent a structural biomarker of baseline vasculopathy in sickle cell disease rather than a dynamic indicator of VOC. Its role in the angiogenesis in various disease conditions and chronic inflammation supports this hypothesis [5, 6].

We thank the reviewers for their valuable insights, which have improved the clarity and scientific depth of our work.

Sincerely,

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1. Özcan O, Kaçmaz M, Erdoğan FH, Balyen LSD, Oğuzman H, Kaya H, Arpacı A. 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. Turk J Haematol. 2025 May 22;42(2):100-107
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