LETTER TO THE EDITOR

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

Orak Hücreli Anemide Leucin-Rich Alpha-2 Glucoprotein 1 (LRG1) ve Proanjiogenik Aracılar

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To the Editor,

The recent article titled "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] was interesting. However, several limitations may impair the validity and reliability of the presented results. First, the sample size of patients with sickle cell disease (SCD) was relatively small (50 patients, including 25 in the steady-state [SS] phase and 25 in the critical vaso-occlusive crisis [VOC] phase), which may not be sufficient to detect statistically significant differences or represent the SCD patient population as a whole. Furthermore, the classification of patients as being in the VOC or SS phase was based on the time period during which the samples were obtained with no longitudinal follow-up, which may not accurately 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 hypoxia-inducible factor 1-alpha (HIF1A) and C-reactive protein (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 other variables, such as LRG1 and markers of inflammation or cell damage like lactate dehydrogenase, which could provide more information about LRG1's role.

Some interesting points for further debate include the following: 1) Why did LRG1 and vascular endothelial growth factor A (VEGFA) levels not differ between the SCD-SS and SCD-VOC groups, despite the VOC phase being more clinically severe? 2) Does HIF1A, a hypoxia marker, have a link with CRP, which measures inflammation? Would that imply that hypoxia in the VOC phase 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 the VOC phase 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 the VOC phase, suggesting that it is a structural biomarker of the disease. While HIF1A and CRP levels were considerably elevated in the VOC phase, they may have been markers of acute events rather than the current illness status. In terms of the receiver operating characteristic curve for HIF1A, the area under the curve value of 0.694 demonstrated 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 larger sample sizes, long-term follow-up, and better control of confounding variables in research.

Keywords: Leucine, Proangiogenic, Mediation, Sickle cell

Anahtar Sözcükler: Lösin, Proanjiogenik, Aracı, Orak hücre

Ethics

Informed Consent: Not applicable.

Authorship Contributions

Concept: A.K., V.W.; Data Collection or Processing: A.K., V.W.; Analysis or Interpretation: A.K., V.W.; Literature Search: A.K., V.W.; Writing: A.K., V.W.

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Reference

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Reply from the Authors:

To the 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" [1]. We address the main issues below, based on both our own research and the current scientific literature.

First, we performed a priori power analysis with an effect size of Cohen's f=0.44, alpha of 0.05, and 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 that level, which gave us 92.4% statistical power for comparing LRG1 levels. While this indicates statistical adequacy, we agree that larger multicenter studies would enhance the generalizability, and we acknowledged that limitation in our article [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 the 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 the groups (SS: 52%; VOC: 60%), minimizing intergroup bias. Furthermore, we applied strict exclusion criteria and excluded patients with sickle cell anemia-related complications. We also adjusted for age and sex in multinomial logistic regression models. Details on drug usage, statistical adjustments, and exclusion criteria were clearly presented in the relevant sections of the article.

We observed a weak correlation between hypoxia-inducible factor 1-alpha (HIF1A) and C-reactive protein (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 vascular endothelial growth factor A (VEGFA) or HIF1A, and no explicit correlation with lactate dehydrogenase (LDH) was noted, 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 the SS and VOC phases 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 [4], as we explained in the discussion and limitation sections of our article.

Although the area under the curve of the receiver operating characteristic curve for HIF1A 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 such as CRP, LDH, or LRG1 may enhance diagnostic accuracy.

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

Sincerely,

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