Hemoglobin N Seattle in a Bengali Child: Diagnostic Challenges and Insights into Genetic Diversity – A Case

Munim Ahmed[1*], Bikash Chandra Chanda[2], Tufael[3], Sharmine Zaman Urmee[2]

Abstract

Background: Hemoglobin N Seattle is an exceedingly rare hemoglobin variant characterized by a lysine-to-glutamic acid substitution at position 61 of the beta-globin chain. While primarily reported in individuals of African and mixed ethnic origins, its occurrence in the Bengali population remains undocumented. Case Presentation: A 1-year, 6-month, and 24-day-old male child presented with poor appetite, frequent respiratory infections, and anemia at Anower Khan Modern Medical College and Hospital, Bangladesh. Initial complete blood count revealed Hb 9.4 g/dL, MCV 64.2 fL, and MCH 18.1 pg. Capillary zone electrophoresis (CZE) demonstrated reduced levels of Hb A (69.0%), Hb A2 (1.6%), and an abnormal peak (29.2%) in Zone 14. High-performance liquid chromatography (HPLC) identified a significant peak (27.4%) in the labile A1c window, indicative of Hb N Seattle. Genetic analysis confirmed the presence of the Hb N Seattle variant in the child's mother, while the father exhibited normal findings. Both mother and child were asymptomatic for diabetes, distinguishing this case from previous reports. Conclusions: This case represents the first documented occurrence of Hb N Seattle in the Bengali population, expanding our understanding of its geographic distribution. The findings underscore the value of advanced diagnostics, including CZE, HPLC, and genetic analysis, in identifying rare hemoglobin variants. Comprehensive family screening and genetic counseling are recommended for accurate diagnosis and management.

Keywords: Hb N Seattle, Bengali population, hemoglobin variant, genetic counseling, capillary electrophoresis

Significance | This case highlights Hb N Seattle's first detection in a Bengali child, emphasizing diagnostic challenges and genetic diversity insights.

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Introduction

Hemoglobin disorders are a significant public health concern worldwide, affecting at least 5.2% of the global population and accounting for approximately 3.4% of childhood mortality before the age of five (Modell & Darlison, 2008). These genetic disorders predominantly include thalassemias and sickle-cell diseases, which arise from mutations in genes encoding hemoglobin, resulting in structural and functional abnormalities (Cao & Kan, 2013). The prevalence of hemoglobin disorders is particularly high in regions historically plagued by malaria, including the Mediterranean area, the Middle East, Transcaucasus, Central Asia, the Indian subcontinent, and the Far East (Modell & Darlison, 2008). These inherited conditions manifest through various genetic mutations, most commonly as single amino acid substitutions in the globin proteins, although they can also result from complex variations such as multiple amino acid substitutions, deletions, anti-termination mutations, and changes in post-translational processing (Thom et al., 2013).

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The increasing awareness of hemoglobin disorders has led to the establishment of extensive genetic databases, such as the HbVar database (Patrinos et al., 2004) and ITHANET (Kountouris et al., 2014), which collectively report over 1,500 different genetic variants associated with these disorders. These platforms serve as crucial resources for researchers and clinicians in understanding the epidemiology and genetics of hemoglobinopathies. Diagnostic techniques have evolved considerably, with capillary zone electrophoresis (CZE) and cation exchange high-performance liquid chromatography (CE-HPLC) being the most commonly utilized non-molecular approaches for identifying hemoglobin disorders due to their proven efficacy and reliability (Higgins et al., 2009; Keren et al., 2008; Stephens et al., 2015).

In this context, the discovery of rare hemoglobin variants is particularly noteworthy. A striking example is the hemoglobin variant Hb N Seattle, first identified in a Seattle man of African ancestry in 1968, characterized by a mutation that replaces the lysine residue at position 61 of the beta globin chain with glutamic acid (Jones et al., 1968). Instances of this variant have generally been associated with benign or mild clinical presentations, making its detection crucial for genetic counseling and further understanding of its implications on health (Kimura et al., 2002). In a recent case study reported by Pradhan et al. (2017), Hb N Seattle was identified in a diabetic female patient using both CZE and CE-HPLC methodologies, emphasizing the necessity for thorough screening practices to unveil such rare variants.

Although Hb N Seattle has been documented in a small number of cases primarily within African and Indian populations, its relationship with the Bangladeshi population remains largely unexplored. Notably, previous investigations have primarily focused on more prevalent hemoglobinopathies like alpha and beta thalassemias within this community (Khan et al., 2017). This gap in research underscores the importance of expanding our understanding of hemoglobin disorders in Bangladesh, where unique ethnic backgrounds and environmental factors may shape the characteristics and prevalence of these conditions. This case report aims to highlight the discovery of Hb N Seattle in a male patient from Bangladesh, illustrating the potential for further investigations into the occurrence and clinical implications of rare hemoglobin variants in this population while promoting genetic counseling and awareness of hemoglobin disorders more broadly.

Recognizing and correctly diagnosing hemoglobin disorders, especially rare variants like Hb N Seattle, is essential for effective medical management and patient outcomes. As such, further research into the genetic diversity of hemoglobinopathies in different populations is warranted, particularly in regions where the carrier rates may be higher than previously recognized. Moreover, understanding the implications of these conditions will contribute significantly to public health initiatives aimed at prevention, early diagnosis, and appropriate management of hemoglobin disorders globally.

2. Case Report

A 1-year, 6-month, and 24-day-old male child presented to Anower Khan Modern Medical College and Hospital with complaints of poor appetite, frequent episodes of the common cold, and anemia identified during routine complete blood count (CBC) analysis. The initial CBC revealed the following red blood cell (RBC) indices: hemoglobin (Hb) 9.4 g/dL, mean corpuscular volume (MCV) 64.2 fL, and mean corpuscular hemoglobin (MCH) 18.1 pg (Table 1).

These findings prompted further investigation with capillary zone electrophoresis (CZE), conducted at the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). Results showed decreased levels of adult hemoglobin (Hb A, 69.0%), Hb A2 (1.6%), and the presence of an abnormal peak (29.2%) in Zone 14 (Figure 1). Based on these findings, the patient was suspected to have Hb N Seattle, a rare hemoglobin variant.

High-performance liquid chromatography (HPLC) was subsequently performed using the D-10 BioRad system. The analysis identified a prominent peak (27.4%) in the labile A1c window with a retention time of 0.67 seconds, further supporting the diagnosis of Hb N Seattle (Figure 2). To confirm this diagnosis, genetic counseling and family screening were advised.

DNA sequencing of the parents' blood samples revealed that the mother carried the Hb N Seattle variant, whereas the father's results were normal. Interestingly, the mother's RBC indices were within normal limits, suggesting an asymptomatic carrier status. Genetic counseling was provided to the family, and the clinical implications of this finding were discussed.

3.Discussion

3.1 Overview of Hb N Seattle

Hb N Seattle is an exceptionally rare hemoglobin variant that has not been widely reported in the Bengali ethnic population bordering the Bay of Bengal, India, and Myanmar. The variant involves a substitution at position 61 of the beta-globin chain, replacing lysine with glutamic acid (HBB:c.184A→G, p.Lys61Glu). A similar variant, Hb Pocos de Caldas, replaces the same lysine residue with glutamine (HBB:c.184A→C, p.Lys61Gln).

The Hb N Seattle variant was first identified in a man of African ancestry (Black American blood donor) in Seattle in 1968 (Jones et al., 1968). Another case was reported in 2002 involving a 30-year-old woman of mixed Indian and Italian descent from Pocos de Caldas, a city in Southeastern Brazil (Kimura et al., 2002). In most instances, Hb N Seattle is clinically silent in the heterozygous state and is more commonly observed in males of African ancestry (Kimura et al., 2002; Jones et al., 1968).

3.2 Previously Reported Cases

Table 1. Hematological parameters of the patient

Parameters	Results	Unit
WBC	6.30	X 10^3/µL
RBC	5.19	X 10^6/µL
Hb	9.4	X g/dL
Hct	33.3	%
MCV	64.2	fL
MCH	18.1	pg
MCHC	28.2	g/dL
Plt	332	X 10^3/µL

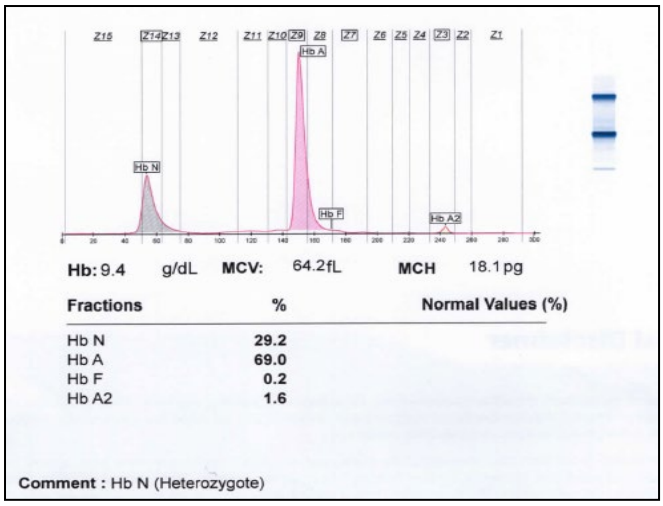

Figure 1. Capillary zone electrophoresis showing abnormal peak in zone 14 corresponding to hemoglobin N Seattle (Patient).

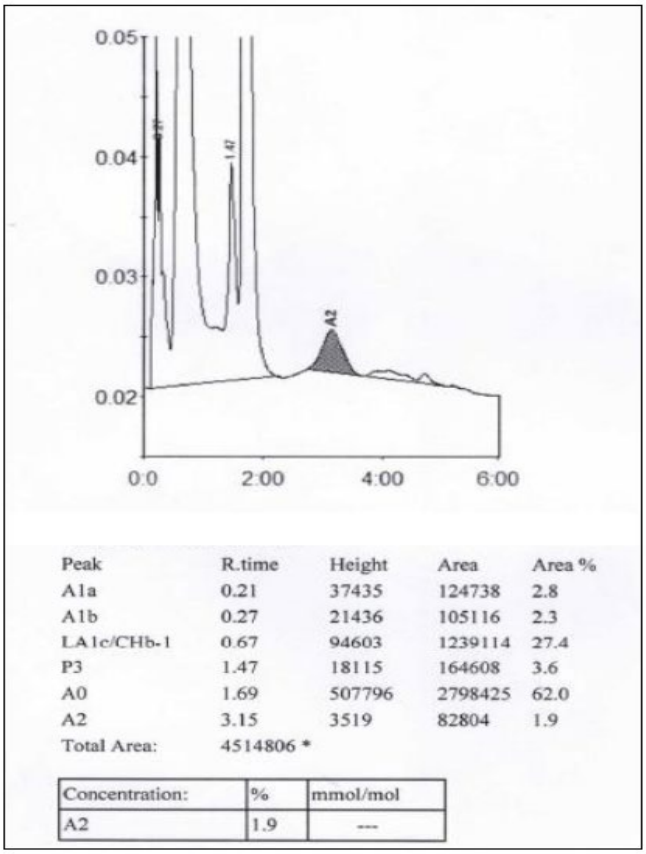

Figure 2. High performance liquid chromatography (HPLC) corresponding to hemoglobin N Seattle (Patient).

In 2017, Pradhan et al. documented a case of Hb N Seattle in a 55-year-old diabetic female from Bhubaneswar, India. The patient presented with increased urination, weight loss, and high random blood sugar levels. Although family screening and molecular confirmation were recommended, the patient was lost to follow-up (Pradhan et al., 2017). In contrast to the current case, our patient and his carrier mother showed no evidence of diabetes, underscoring the heterogeneity of clinical presentations associated with Hb N Seattle.

3.3 Diagnostic Challenges and Implications

The detection of Hb N Seattle in the current case relied on the use of both capillary electrophoresis and HPLC. The abnormal peaks identified in both diagnostic modalities aligned with previously reported patterns for this variant, including the distinctive peak in the labile A1c window observed in HPLC (Pradhan et al., 2017). Such findings highlight the importance of incorporating advanced diagnostic techniques for the detection of rare hemoglobin variants. Spuriously low HbA1c levels can serve as an indicator of rare hemoglobin variants, as observed in the present case. This phenomenon is particularly relevant in regions where hemoglobinopathies are underreported or underdiagnosed. Family screening and molecular confirmation are essential to elucidate the genetic basis of these findings and to provide appropriate genetic counselling.

3.4 Hb N Seattle in the Bengali Population

To date, there have been no prior reports of Hb N Seattle in the Bengali population. This case underscores the need for further research into the genetic diversity of hemoglobin variants within this population, particularly given the unique ethnic and environmental factors that may influence their prevalence. The discovery of Hb N Seattle in a male patient and his mother in Bangladesh provides an opportunity to expand our understanding of hemoglobinopathies in this region and to promote awareness and accurate diagnosis of such conditions.

3.5 Public Health Implications

Recognizing and diagnosing rare hemoglobin variants such as Hb N Seattle is critical for effective medical management and patient outcomes. This case highlights the importance of integrating advanced diagnostic techniques, such as CZE and HPLC, into routine clinical practice in regions with high rates of hemoglobinopathies. Additionally, public health initiatives should prioritize genetic counseling, family screening, and community awareness to mitigate the burden of these disorders globally.

4. Conclusion

This case report provides a novel instance of Hb N Seattle in the Bengali population, diagnosed in a young male child through capillary electrophoresis and HPLC. Family screening revealed the mother as an asymptomatic carrier, emphasizing the importance of genetic counseling. Hb N Seattle, though rare and clinically silent in heterozygotes, can spuriously lower HbA1c levels, complicating diabetes diagnostics. This underscores the need for advanced diagnostic tools and awareness of rare hemoglobin variants in regions with high genetic diversity. Further research is warranted to understand the prevalence and clinical implications of Hb N Seattle in Southeast Asia.

Author contributions

M.A. designed the study. Bikash Chandra Chanda (BCC) and T drafted the manuscript. S.Z.U. contributed to the discussion and reviewed the manuscript. M.A supervised the study. All authors contributed to the article and approved the submitted version.

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Competing financial interests

The authors have no conflict of interest.

References

Cao, A., & Kan, Y. W. (2013). The prevention of thalassemia. Cold Spring Harb Perspect Med, 3(2), a011775. https://doi.org/10.1101/cshperspect.a011775

Higgins, T., Mack, M., & Khajuria, A. (2009). Comparison of two methods for the quantification and identification of hemoglobin variants. Clin Biochem, 42(7-8), 701-705. https://doi.org/10.1016/j.clinbiochem.2009.01.004

Jones, R. T., Brimhall, B., Huehns, E. R., & Motulsky, A. G. (1968). Structural characterization of hemoglobin-N-Seattle. Biochim Biophys Acta, 154(2), 278-283. https://doi.org/10.1016/0005-2795(68)90041-x

Keren, D. F., Hedstrom, D., Gulbranson, R., Ou, C. N., & Bak, R. (2008). Comparison of Sebia Capillarys capillary electrophoresis with the Primus high-pressure liquid chromatography in the evaluation of hemoglobinopathies. Am J Clin Pathol, 130(5), 824-831. https://doi.org/10.1309/AJCPQY80HZWHHGZF

Khan, W. A., Banu, B., Sadiya, S., & Sarwardi, G. (2017). Spectrum of Types of Thalassemias and Hemoglobinopathies: Study in a Tertiary Level Children Hospital in Bangladesh. . Thalassemia Reports(7(1)). https://doi.org/10.4081/thal.2017.6354

Kimura, E. M., Jorge, S. B., Ogo, S. H., Cesquini, M., Albuquerque, D. M., Fattori, A., Saad, S. T., Costa, F. F., & Sonati, M. F. (2002). A novel beta-globin variant: Hb Pocos de Caldas [beta 61(E5)Lys-->Gln]. Hemoglobin, 26(4), 385-388. https://doi.org/10.1081/hem-120016375

Kountouris, P., Lederer, C. W., Fanis, P., Feleki, X., Old, J., & Kleanthous, M. (2014). IthaGenes: an interactive database for haemoglobin variations and epidemiology. PLoS One, 9(7), e103020. https://doi.org/10.1371/journal.pone.0103020

Modell, B., & Darlison, M. (2008). Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ, 86(6), 480-487. https://doi.org/10.2471/blt.06.036673

Patrinos, G. P., Giardine, B., Riemer, C., Miller, W., Chui, D. H., Anagnou, N. P., Wajcman, H., & Hardison, R. C. (2004). Improvements in the HbVar database of human

hemoglobin variants and thalassemia mutations for population and sequence variation studies. Nucleic Acids Res, 32(Database issue), D537-541. https://doi.org/10.1093/nar/gkh006

Pradhan, S., Chauhan, S., & Samal, P. . (2017). Incidental Detection of a Rare Hemoglobin Variant (Hemoglobin N Seattle) Leading to Undetectable Levels of HbA1c in a Diabetic Female: A Case Report. . Thalassemia Reports(7(1)). https://doi.org/10.4081/thal.2017.5860

Stephens, A. D., Colah, R., Fucharoen, S., Hoyer, J., Keren, D., McFarlane, A., Perrett, D., Wild, B. J., & International Council for Standardization in, H. (2015). ICSH recommendations for assessing automated high-performance liquid chromatography and capillary electrophoresis equipment for the quantitation of HbA2. Int J Lab Hematol, 37(5), 577-582. https://doi.org/10.1111/ijlh.12413

Thom, C. S., Dickson, C. F., Gell, D. A., & Weiss, M. J. (2013). Hemoglobin variants: biochemical properties and clinical correlates. Cold Spring Harb Perspect Med, 3(3), a011858. https://doi.org/10.1101/cshperspect.a011858