In-Silico structural analysis of wild-type human hemoglobin and its mutation resulting in sickle cell anemia

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ABSTRACT

Background: Hemoglobin (HbA) is a metalloprotein having a heme prosthetic group, two α-globin chains, and two β-globin chains. A point mutation in the 6th position of wild-type HBB gene (GAG>GAG) substitutes Glutamic acid (E) to Valine (V) in the β-chains of hemoglobin molecule (HbS) resulting in Sickle Cell Anemia. Structural deformities in the β-globin gene leads to disruptive conformation of red blood cells, disturbs the oxygen transportation and causes functional abnormalities. Aims and Objective: The purpose of the study was to understand the structural, genetic and metabolic effect of the point mutation on HBB gene that causes sickle cell disease. Materials and Methods: The 3D structures of HbA and HbS proteins were retrieved from PDB and the changes in their physical properties were analyzed using Swiss PDB viewer and Molegro molecule viewer. Gene networks were constructed using GeneMANIA server to study genetic and metabolic interactions of the HBB gene. Results: In-silico studies showed that the normal bond length between Glu6-Glu7 in HbA molecule is 1.32Å and that of HbS is 1.33Å. After comparing the two proteins, it was observed that sickle cell hemoglobin suffers a change in bond angle from 122.4º in HbA to 119.35º in HbS. Comparative energy minimization of Glu6 and Val6 in wild-type and mutant hemoglobin respectively yielded 8.78 and -9.083 KJ/mol net energy values, suggesting a more reactive HbA and less reactive HbS. Gene networks were determined on the basis of physical, genetic and co-expressive interactions of HBB gene which revealed a strong connection between HBB and HBA1 genes within the association constituted of various metabolic functions. Conclusion: Sickle cell disease results from a sequence of events which start with a single nucleotide substitution that ultimately leads to severe anemia and other cardiovascular problems. Incorporation of computational exercises correlates to a better probability of discovering precision medicine through target site-specific drug designing.

Key words: HbA; HbS; sickle cell anemia; In-silico

INTRODUCTION

Hemoglobin is a diverse superfamily of proteins detected in many animals and distributed mainly in red blood cells (RBCs).¹ Hemoglobinopathies are inherited genetic disorders resulting from a mutation in globin gene that produces biological, structural and functional abnormalities. A single amino acid substitution is only confined to one aberration in the primary and secondary structure in hemoglobinopathy. The oxygen carrying capacity to all cells and tissues in the body is dependent upon hemoglobin which is the key constituent of Red Blood Cells (RBCs). The RBCs are regular and round in shape when it contains normal hemoglobin. A substitution mutation in the 6th amino acid of β-chain of hemoglobin molecule concurrently changes glutamic acid (E) to valine (V), thereby causing structural deformities. This disruptive conformation of RBCs leads to sickle cell disease or sickle cell anemia. The homozygous and heterozygous states involving one hemoglobin gene encoding HbS protein
is responsible for reduced red blood cell survival and hemolysis in Sickle Cell Disease (SCD). The occurrence of the autosomal recessive affected gene allele (heterozygous) in both parents who are asymptomatic carriers may transmit the defective gene to their child (homozygous, Hb SS) thereby resulting in the inheritance of SCD. HbS forms highly crystallized structure under low oxygen pressure conditions which lead to pathological or physical stress. The formation of intracellular crystals cause polymerization of RBCs resulting in reduced oxygen uptake, as well as blocking of small blood vessels which promote RBC dehydration. The impact of RBC sickling and polymerization in sickle cell trait is considered to be the primary factor responsible for vaso-occlusive crises. The other dysfunction includes severe tissue hemolysis, ischemia, inflammation, hypoxia, endothelial injury, increased viscosity, acidosis and hypothermia. Approximately, 2,00,000 babies in Africa each year are born with severe autosomal sickle cell genotype that constitutes hemoglobinopathies worldwide, but the frequency of occurrence of sickle cell mutation in sub-Saharan Africa, middle-East and scattered populations throughout India is apparently up to 18%. In developed countries, preventive measures have been implemented to bring down the progressive survival loss amongst patients originating from North America, United Kingdom, and other European countries. Targeting the sickle cell mRNA transcripts by antisense RNA technology has been augmented as a holistic approach to reducing the levels of HbS polymerization associated with SCD. An initial challenge to gene therapy methods have been overcome, and efficient transduction of hematopoietic stem cells with lentiviral vectors has become adverse and preclinical laboratory investigations are evolving for patients with SCD. The current definitive clinical approach relies upon hydroxyurea and providing supportive care. Other in-silico studies showed that the genetic variations could alter the expression and function of the hemoglobin gene. Alanazi, Abduljaleel found that 80% of the non-synonymous single nucleotide polymorphisms in the HBB gene were deleterious which may affect protein function.

Our in-silico studies highlight a notable way on changes in bond lengths, bond angles, and mutational analyses of hemoglobin molecule that result in reduced oxygen-carrying capacity of globin protein. We further investigated the impact of sickle cell mutation on various biological interactions of the normal hemoglobin.

**MATERIALS AND METHODS**

**Data Collection and Protein Structure Visualization**

The structures of normal hemoglobin molecules in humans and its mutated form (i.e. sickle cell hemoglobin) were downloaded from www.rcsb.org/pdb having PDB id: 2HBB and 2HBS respectively. Both the normal and the mutated protein structures were visualized using RasWin and Molegro Molecular Viewer tools for analysis of the 3D crystallized structures.

**Computational analysis of protein structure**

Swiss PDBe Viewer v4.1.0 was used to analyze the β-globin chain of the hemoglobin protein in both normal (HHB) and mutated (HbS) structures, particularly at the site of mutation, i.e. the 6th amino acid amino acid position, where Glu6 is replaced by Val6. The structural differences between the two types of protein were calculated for different atomic positions within the amino acids on the basis of bond lengths and bond angles. Molegro Molecular Viewer was further used to corroborate the data generated.

**Energy minimization study**

The two different protein structures HHB and HbS were subjected to energy minimization studies to evaluate the energy of the atoms in the space of the hemoglobin protein. The energy minimization studies were achieved by GROMOS96 implementation of Swiss-PDB Viewer v4.1.0 for comparison between the two protein structures to detect the stability of the proteins. The GROMOS molecular simulation package for energy minimization incorporates a force field of energy interactions amongst all the interacting amino acids based on their 3D representation which enabled the study of individual amino acids, particularly the amino acids in and around the 6th position (Glu6 for HbA and Val6 for HbS).

**In silico prediction of inter-relationships of Hbb gene with other genes for functional assessment**

Prediction of the inter-relationship between HBB gene coding for Hemoglobin β-chain and several other genes present in a large associated functional dataset and assays were computed with the help of GeneMANIA server, a web-interface online tool for analyzing gene function. The physical protein-protein interactions and genetic interactions were studied through BioGRID and PathwayCommons database, which documented similar associations. Co-expression of HBB gene with other gene sets were predicted with the help of expression studies referenced in Gene Expression Omnibus (GEO) (Warde-Farley, D., S. L. Donaldson, et al. 2010).

**RESULTS AND DISCUSSION**

**In silico identification of functional parameters - Bond lengths (Distance) and bond angles using Swiss PDBe-viewer and Molegro molecular viewer**

The mutation in hemoglobin disrupts the structural conformity of red blood cells which affects the flow of...
oxygenated blood to body tissues, leading to a detrimental disease called Sickle Cell Anemia. The bond lengths between Pro5-Glu6 in HbA and Pro5-Val6 in HbS of β-chain are 1.33 Å and 1.33 Å respectively which do not show any alteration. However, a change is observed between Glu6-Glu7 (HbA) and Val6-Glu7 (HbS) with variable bond lengths of 1.32 Å and 1.33 Å respectively (Figure 1).

Further, the bond angles between Pro5-Glu6 (HbA) change from 119.35° to 122.4° in Pro5-Val6 (HbS) and between Glu6-Glu7 (HbA), it changes from 111.93° to 121.88° in Val6-Glu7 (HbS) (Figure 2). All the structural and conformational interpretations measured using Swiss PDB-viewer are shown in (Table 1).

Change in bond lengths and angles result in increasing or decreasing bond enthalpies which in turn affects the stability of the amino acid. Bond angles are less restrained than bond lengths and as such show more variation than bond lengths. Increase in bond angle between three adjacent atoms after mutation from Glu6→Val6 signifies a change in enthalpy and electronegativity wherein electron cloud of the atoms are dispersed further apart spatially. Glutamate is a polar, negatively charged and hydrophilic amino acid which is involved in binding ligands due to its presence in active sites of protein. But valine is a hydrophobic, Cβ branched amino acid with reduced freedom for conformational changes within the backbone chain, thereby restricting their secondary structure to mainly β-sheets. This mutation would affect the binding stability and salt bridge formation ability of hemoglobin. These different structural changes in conformation of the hemoglobin protein provide an in-depth understanding of the impact of deletion of one amino acid on the oxygen transportation and binding affinity of the hemoglobin protein. Functional parameters like bond lengths and bond angles ascertain the relative importance of non-synonymous mutation on protein’s structure and function.

The bond lengths and bond angles of HbA and HbS were studied using Molegro Molecule viewer (Table 2). The bond length between Pro5-Glu6 changed from 1.33 Å (normal HbA) to 1.327 Å in Pro5-Val6 (HbS) and that of Glu6-Glu7 changed from 1.32 Å (normal HbA) to 1.328 Å in Val6-Glu7 (HbS) (Figure 3). Structural alterations provided a more focused interpretation of the bond angles between Pro5-Glu6 which changed from 119.35° (normal HbA) to 122.4° in Pro5-Val6 (HbS). Moreover, in HbA and HbS, the bond angle altered significantly from 111.93° (Glu6-Glu7) to 121.88° (Pro5-Val6) respectively (Figure 4). This investigation characterized the impact of bond length and bond angle changes distort the structural integrity of the HbA protein resulting in abnormal, rigid and distorted RBCs. Evidently, it was reported that bond length provides appropriate information about the mechanism of cooperativity and affinity for oxygen molecule. But the axial distance between the α1 chain of Pro114 and α2 of Glu116 residues were found to be 3.71 Å and 3.91 Å respectively and between β1 of Glu121 and α2 of Pro114 were 3.71 Å and 3.79 Å. The unique contacts were formed by tetramer 1 and 2 of HbA.

The three-dimensional visualization of ribbon structure of hemoglobin protein and its mutated version is displayed using RasMol (figure. 5a and 5b). This relevant analysis observed from three concurrently used bioinformatics tools provided insight into detection of functional
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Table 1: Detailed comparison of normal hemoglobin and sickle cell hemoglobin terms of bond length and bond angle using swiss-PDB viewer

| β-chain of Protein | PDB Code | Wild –type amino acids | Mutated Amino acids | Atom Name | Amino acids position | Bond Length(Å) | Bond Angles |
|-------------------|----------|------------------------|---------------------|-----------|----------------------|----------------|-------------|
| Normal Hemoglobin | 2HHB     | Glu6                   | -                   | PRO5 C6- GLU6 N7 | Pro5-Glu6           | 1.33           | 119.35°     |
|                   |          |                        |                     | GLU6 CA6- GLU7 N7 | Glu6-Glu7           | 1.32           | 111.93°     |
| Sickle Cell Hemoglobin | 2HBS     | Val6                   | -                   | PRO5 C6- VAL6 N7 | Pro5-Val6           | 1.33           | 122.4°      |
|                   |          |                        |                     | VAL6 C6- GLU7 N7 | Glu7-Val6           | 1.33           | 121.88°     |

Table 2: Detailed comparison of normal hemoglobin and sickle cell hemoglobin in terms of bond length and bond angle using molegro viewer

| β-chain of Protein | PDB Code | Wild –type amino acids | Mutated Amino acids | Atom Name | Amino acids position | Bond Length(Å) | Bond Angles |
|-------------------|----------|------------------------|---------------------|-----------|----------------------|----------------|-------------|
| Normal Hemoglobin | 2HHB     | Glu6                   | -                   | PRO5 C6- GLU6 N7 | Pro5-Glu6           | 1.33           | 119.35°     |
|                   |          |                        |                     | GLU6 CA6- GLU7 N7 | Glu6-Glu7           | 1.32           | 111.93°     |
| Sickle Cell Hemoglobin | 2HBS     | Val6                   | -                   | PRO5 C6- VAL6 N7 | Pro5-Val6           | 1.327          | 122.4°      |
|                   |          |                        |                     | VAL6 C6- GLU7 N7 | Glu7-Val6           | 1.328          | 121.88°     |

Figure 5: Visualization of Ribbon structure of the two proteins using Rasmol (a) four chains of Normal Hemoglobin protein (2HHB) (α-chain: red, β-chain: blue, γ-chain: yellow and δ-chain: green) and (b) Mutated hemoglobin Protein (2HBS)

Impact of Energy minimization on β-globin protein

It has been observed that the total energy for native type protein structure (2HHB) was -8311.889 KJ/mol and the mutant type protein (HBS) was -33546.063 KJ/mol. After implementing energy minimization using GROMOS96 force field with an exhaustiveness of 100 steepest descent, the energy of 2HHB was -26309.943 KJ/mol and of 2HBS was -54317.750 KJ/mol respectively (Table 3). Evidently, Alanazi, Abduljaleel11 checked the stability of protein differences at the structural level. Thus, the present observations are in accordance with the fact that mutation in HbA protein leads to Sickle Cell Disease. But in one study, the prediction of biological process and molecular function of normal hemoglobin and sickle hemoglobin was found to be similar.17 The polymerization reaction leading to the sickling effect of HbA to become HbS had been revealed using HbA expression system in bacteria18 and fungi19 by site-directed mutagenesis of β chains of HbA. Alternatively, it has been seen that, by recruiting homologous recombination pathway through providing a non-mutant β globin gene template, a repair mechanism of mutant or diseased causing region could be initiated.20-28

Table 3: Detailed comparison of normal hemoglobin and sickle cell hemoglobin in terms of bond length and bond angle using swiss-PDB viewer

| β-chain of Protein | PDB Code | Wild –type amino acids | Mutated Amino acids | Atom Name | Amino acids position | Bond Length(Å) | Bond Angles |
|-------------------|----------|------------------------|---------------------|-----------|----------------------|----------------|-------------|
| Normal Hemoglobin | 2HHB     | Glu6                   | -                   | PRO5 C6- GLU6 N7 | Pro5-Glu6           | 1.33           | 119.35°     |
|                   |          |                        |                     | GLU6 CA6- GLU7 N7 | Glu6-Glu7           | 1.32           | 111.93°     |
| Sickle Cell Hemoglobin | 2HBS     | Val6                   | -                   | PRO5 C6- VAL6 N7 | Pro5-Val6           | 1.33           | 122.4°      |
|                   |          |                        |                     | VAL6 C6- GLU7 N7 | Glu7-Val6           | 1.33           | 121.88°     |

Figure 3: (a) Bond length between Pro5-Glu6 (1.33Å) of HbA protein (b) Glu6-Glu7 (1.32Å) of HbA protein (c) Bond length between Pro5-Val6 (1.327Å) of HbS protein (d) Val6-Glu7 (1.328Å) of HbS protein using Molegro Molecular Viewer

Figure 4: (a) Bond angle between Pro5-Glu6 (119.35°) of HbA protein (b) Glu6-Glu7 (111.93°) of HbA protein (c) Bond length between Pro5-Val6 (122.40°) of HbS protein (d) Val6-Glu7 (121.88°) of HbS protein visualizations using Molegro Molecular Viewer
Table 3: A comparative energy minimization data of normal hemoglobin and mutated hemoglobin based on three amino acids with the 6th position of β-chain at the centre (Proline, Glutamic acid and Valine). Net energy calculations were formulated for the entire protein molecules before and after minimization with GROMOS96

| β-Globin Protein | Residue | Bonds | Angles | Torsion | Non-Bonded | Electrostatic | Total E= |
|------------------|---------|-------|--------|---------|------------|--------------|----------|
|                  | Individual residues around 6th amino acid position before energy minimization | | | | | | |
| HbA              | Pro B5  | 2.653 | 8.201  | 34.768  | -18.05     | -27.15       | 1.430    |
|                  | Glu B6  | 3.714 | 7.808  | 9.335   | -11.74     | 29.41        | 41.369   |
|                  | Glu B7  | 4.340 | 2.939  | 4.294   | -35.90     | 3.44         | -17.602  |
| HbS              | Pro B5  | 2.809 | 20.952 | 11.953  | -10.46     | -27.18       | 1.427    |
|                  | Val B6  | 0.924 | 1.636  | 1.040   | -0.09      | 6.89         | 10.526   |
|                  | Glu B7  | 0.986 | 2.825  | 2.506   | -34.10     | 2.42         | -25.228  |
|                  | Individual residues around 6th amino acid position after energy minimization | | | | | | |
| HbA              | Pro B5  | 0.457 | 14.522 | 20.232  | -23.67     | -29.24       | -15.879  |
|                  | Glu B6  | 1.037 | 9.941  | 6.680   | -29.50     | 16.59        | 8.78     |
|                  | Glu B7  | 0.422 | 1.197  | 5.463   | -45.56     | -6.23        | -43.921  |
| HbS              | Pro B5  | 0.520 | 18.328 | 15.962  | -20.84     | -27.83       | -11.532  |
|                  | Val B6  | 1.357 | 3.735  | 1.747   | -18.85     | -0.88        | -9.083   |
|                  | Glu B7  | 0.362 | 2.388  | 2.476   | -45.40     | -2.46        | -41.448  |

Table 4: Table shows the description of genes co-expressed with HBB gene network

| Symbol | Description | Co-expression |
|--------|-------------|---------------|
| HBD    | Hemoglobin Subunit Delta | Yes |
| HBZ    | Hemoglobin Subunit Zeta | No |
| HBG1   | Hemoglobin Subunit Gamma 1 | Yes |
| HBE1   | Hemoglobin Subunit Epsilon 1 | Yes |
| NFE2   | Nuclear Factor, Erythroid 2 | Yes |
| GDAP1  | Ganglioside Induced Differentiation Associated Protein 1 | No |
| HBA1A  | Hemoglobin Subunit Alpha 1 (Hsp70) Member 1A | Yes |
| HSPA1A | Heat Shock Protein Family A | No |
| HBG2   | Hemoglobin Subunit Gamma 2 | No |
| HBA2A  | Hemoglobin Subunit Alpha2 | Yes |
| SELT   | Selenoprotein T | No |
| HBG1   | Hemoglobin Subunit Theta 1 | Yes |
| HBM    | Hemoglobin Subunit Mu | Yes |
| ALAS2  | 5-aminolevulinate synthase 2 | Yes |
| PRDX2  | Peroxiredoxin 2 | No |
| ALDOA  | Aldolase, fructose-bisphoshate A | Yes |
| HP     | Haptoglobin | No |
| DACH1  | Dachshund family transcription factor 1 | No |
| PF4    | Platelet Factor 4 | Yes |
| MB     | Myoglobin | Yes |

structure for solvent accessibility of amino acid residue and found that the total energy of native protein structure (4HHB) and mutant type structures were -334380.5kJ/mol. The structural variations could be visualized on the basis of the energy variations of Glu6 and Val6 of HbA and HbS proteins respectively. While Glu6 had a net energy of 41.369 KJ/mol which was minimized to 8.78 KJ/mol following GROMOS96 calculations, the net energy of Val6 was minimized to -9.083 KJ/mol from 10.526 KJ/mol (Table 3). In Sickle Cell Disease, there is a decreasing tendency of metabolic processes to control oxidative stress which results in free radical generation thereby impairing sugar phosphate pathways. This results in energy deficiencies in critical metabolic systems as a consequence of oxygen deprivation to red blood cells, thereby leading to irreversible damage of membrane and ionic channels of sickled erythrocytes. Since the net energy of Glu6 is higher than Val6 even after minimization process, it could be inferred that Glu6 is more reactive in nature than Val6 which ultimately affects the binding of red blood cells with oxygen moieties during oxidative stress.

Functional activity detection of wild-type normal protein (HBB gene) through GeneMANIA Server

The genes that are co-expressed and have physical interactions or properties are shown in Figure 6a and 6b, and the description of the genes co-expressed with HBB network is listed in Table 4. The HBB network gene function and its appearance in network and genome are listed in Table 5. Gene network functional assessment on the basis of Gene Ontology data generated several composite functions of HBB gene which has specific role in blood microparticle association, i.e., its presence in RBCs, its role in gaseous transport, its response to reactive oxygen species, hydrogen peroxide and oxidative stress and its antioxidant activity within the cellular environment. It was reported that there exists functional interactions between VCAM-1 gene and other similarly related genes which plays a major role in cell adhesion binding, membrane docking, microvillus and leukocyte cell-cell adhesion.
various cell types including erythrocytes and elevated levels of blood MPs in Sickle Cell Anemia has been observed in patients which lead to activation of blood clotting through thrombin generation.\textsuperscript{31} The various metabolic and cellular responses to reactive oxygen species and oxidative stress were governed by the HBB, HBA1, PRDX2 and HP networks for providing functional capabilities to hemoglobin beta subunit for oxygen transport. The antioxidant activity, oxidoreductase activity, and peroxidase activity also involve the network of HBB, HBA1 and PRDX2 genes. Involvement of HBB and HBA1 genes of hemoglobin molecule suggests their role in oxidative transportation and metabolic redox reactions within the body. The networks with featured functions depict the networking of HBB and HBA1 in all the demonstrated activities along with their co-expressivity during gene function (Table 4). However, despite sharing network activity in critical functions of oxidative responses and reactive oxygen species, HP and PRDX2 do not co-express with HBB.

As a result of these interactive genes involved in a network performing a common metabolic function, a mutation in any one of them could lead to complete network disruption resulting in loss of function and bioprocess system disintegration as characterized by Sickle Cell Disease (Table 6). $\beta$-haemoglobinopathies of the HBB gene, particularly HbC, HbD, HbE and HbS have been reported to express abnormal molecular interactions within protein chains and also with external ligands which disrupt binding and transport of oxygen across cells.\textsuperscript{11} Moreover, single nucleotide polymorphisms and genome variants in Hb generally do not lead to life-threatening conditions but certain mutations do cause structural and biochemical alterations that cause severe anemia.\textsuperscript{32}

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**Table 5:** Table shows the HBB network genes functions and its appearance in network and genome

| Features                                      | FDR  | Genes in network | Genes in Genomes |
|-----------------------------------------------|------|------------------|------------------|
| Blood microparticle                           | 2.73e-7 | 7                | 108              |
| Hydrogen Peroxide Catabolic Process           | 9.52e-6 | 4                | 14               |
| Cytoplasmic membrane-bounded vesicle lumen    | 5.85e-5 | 5                | 76               |
| Vesicle lumen                                 | 5.85e-5 | 5                | 76               |
| Hydrogen Peroxide Metabolic Process           | 5.85e-5 | 4                | 25               |
| Cellular response to Hydrogen Peroxide        | 1.64e-4 | 4                | 35               |
| Response to Hydrogen Peroxide                | 2.44e-4 | 4                | 40               |
| Gas Transport                                 | 3.11e-4 | 3                | 10               |
| Cellular response to reactive oxygen species  | 8.69e-4 | 4                | 58               |
| Endocytic vesicle lumen                       | 1.16e-3 | 3                | 16               |
| Response to reactive oxygen species           | 1.91e-3 | 4                | 74               |
| Cellular response to oxidative stress         | 4.40e-3 | 4                | 95               |
| Reactive oxygen species metabolic process     | 4.40e-3 | 4                | 95               |
| Oxidoreductase activity, acting on peroxide as receptor | 6.75e-3 | 3                | 32               |
| Peroxidase activity                           | 6.75e-3 | 3                | 32               |
| Response to inorganic substance               | 2.37e-2 | 4                | 153              |
| Response to oxidative stress                  | 2.41e-2 | 4                | 156              |
| Antioxidant activity                          | 2.77e-2 | 3                | 54               |

FDR: False discovery rate (If FDR is greater than or equal to the probability that this is a false positive, then it will lead to incorrect rejection of hypothesis)

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**Figure 6:** (a) Genes that have physical interaction with HBB gene and (b) Genes co-expressed with HBB gene
CONCLUSION

Structural aberrations in wild form of hemoglobin disrupt the normal functioning of erythrocytes resulting in oxygen deprivation and severe anemia. A comparative analysis of the spatial 3D structure of HbA and HbS was done which revealed that there is a significant structural difference in terms of both bond length and bond angles between the two proteins when glutamine is mutated to valine due to point mutation. A significant compromise of the structural integrity of the sickle cell hemoglobin impairs the oxygen binding capacity of hemoglobin due to its transformation into a sickle shape. Dynamic energy calculations and net minimization studies provided a clear picture regarding the reactivity and cooperativity of hemoglobin. The HbA molecule had a much higher net energy value (-26309.943 KJ/mol) than its HbS counterpart (-54317.750 KJ/mol) substantiating the reactive capacity of HbA. Even the mutated amino acids at the 6th position of the beta chain had significantly variable total energy values (E_total = 8.78 KJ/mol for Glu6, E_total = -9.083 KJ/mol for Val6). The protein-protein physical and interactions and co-expression of HBB genes using GeneMania server shed light into the diverse functional modalities of HbA. HBB gene shared a common network function spread across a wide array of genes with HBA1 gene which also encodes for HbA protein, which explains the shared metabolic functions related to their gene ontology. The gene co-expression study further strengthened the functional relatedness of HBB and HBA1 genes in HbA protein. The overall computational study inferred that sickle cell disease is a systemic condition wherein a chain event within the metabolic process are affected due to the single nucleotide (GAG → GTG) resulting in a cascade of events that culminate into severe anemia. More in silico studies are needed on a broader scale to decipher the molecular mechanisms involved in the manifestation of this disease for a better chance of targeted drug designing and precision therapy.

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Table: 6 Summary of the normal and each of the abnormal mutated hemoglobin

| Hemoglobin HB disorder | Position chromosome 11 | Mutational position | Mutation | Biological process |
|------------------------|------------------------|---------------------|----------|--------------------|
| HHB                    | None                   | None                | Glu-Val(E→V) | Oxygen Transportation |
| HB S                   | 6                      | Beta 6              | Glu-Lys(E→K) | Oxygen Transportation |
| HB C                   | 6                      | Beta 6              | Glu-Lys(E→K) | Oxygen Transportation |
| HB E                   | 26                     | Beta 26             | Glu-Lys(E→K) | Oxygen Transportation |
| HB D                   | 121                    | Beta 121            | Glu-Gln(E→Q) | Oxygen Transportation |
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SN- Concept and design of the study; interpreted the results, prepared first draft of manuscript and critical revision of the manuscript; NP- Statistically analyzed and interpreted; reviewed the literature and manuscript preparation; MN- Design of the study, statistically analyzed and interpreted, preparation of manuscript and revision of the manuscript; BD- Concept and coordination of the overall study.

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