Summary

Diabetes mellitus represents a major metabolic disorder affecting millions of people all over the world. The disease is associated with acute and chronic pathophysiological complications, the reasons of which are not yet clear.

In diabetes mellitus, hemoglobin is glycated by glucose in a nonenzymatic glycation reaction. The present work is concerned with the studies on the change in the structural and functional activities of glycated hemoglobin (HbA1c) as compared to nonglycated one (HbA0).

Nonglycated (HbA0) and glycated (HbA1c) hemoglobin were separated, purified and compared of their different functional and structural properties as presented below:

- H2O2-mediated iron (ferrozine detected) release is more from HbA1c than that from HbA0. Free reactive iron is a potent source of dreadly damaging OH radicals, which is produced by Fenton reaction in the presence of H2O2.
- In presence of H2O2, HbA1c degrades lipid (arachidonic acid) and deoxyribose more effectively than HbA0. The reaction is inhibited by desferrioxamine (iron chelator) or catalase indicating that iron and H2O2 are involved in the degradation reactions.
- Hemoglobin possesses peroxidase-like activity. This activity is significantly less in HbA1c than HbA0.
• Rate of auto-oxidation or NBT mediated co-oxidation of HbA₁c is faster than that of HbA₀. These processes produce methemoglobin, which, in turn, produces Heinz body responsible for erythrocyte damage.

• TFZ, a phenothiazine drug, releases oxygen from HbA₀, but not from HbA₁c. Binding affinity constant (K) and number of binding sites (p) for TFZ interacting with HbA₀ and HbA₁c are more or less similar.

• The mode of interaction of HbA₀ and HbA₁c with TFZ are predominantly hydrophobic in nature.

• The interaction between HbA₀ and TFZ is cooperative in nature and co-operativity is reduced due to glycation.

• Differential spectrophotometric titration of HbA₀ with TFZ shows two isosbestic points around 398 nm and 442 nm region. No isosbestic point is found with HbA₁c.

• A small fraction of heme is found to be released from HbA₁c by TFZ interaction, which is detected by gel filtration.

• SDS-PAGE experiment of HbA₀ or HbA₁c and TFZ complex indicates that heme releases from both hemoglobin species. However, the release is greater from HbA₁c.

• The rate of heme transfer from oxidised (met form) HbA₁c to human serum albumin is faster than HbA₀, suggesting weaker heme-globin linkage in glycated protein than HbA₀.

• Thermal denaturation experiment indicates that HbA₁c is more thermolabile than HbA₀.
- Tryptophan quenching titration experiment with acrylamide indicates that the number of surface accessible tryptophan are more in HbA1c than in HbA0, suggesting a conformational change of hemoglobin due to glycation.

- Circular dichroism experiment exhibits a conformational change in hemoglobin due to glycation.

The results on the effect of TFZ on diabetic and normoglycemic erythrocytes are as follows:

- Microscopic examination suggests that erythrocytes from diabetic patients tend to aggregate and form cluster.

- TFZ induces polymerization of hemoglobin within erythrocytes and membranes become susceptible to lysis as detected by microscopic analysis. These effects are more prominent in erythrocytes isolated from diabetic patients than those from healthy normal individuals.

The level of haptoglobin, a hemoglobin binding glycoprotein is elevated significantly with the extent of diabetic condition, which may have some correlation with the defence mechanism.
Conclusion

Glycation induces conformational change of hemoglobin, which, in turn, alters its functional properties. Glycated hemoglobin (HbA1c) is a potential source for the generation of free radicals causing oxidative stress, which may be associated with several complications in diabetes mellitus - a dreadful metabolic disease.