Background: Glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49) is a ubiquitous enzyme that has a housekeeping role in all cells. This enzyme catalyzes the first step in the pentose phosphate pathway (PPP) converting glucose 6-phosphate (G6P) to produce 6-phosphogluconolactone with concomitant reduction of oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$) to reduced nicotinamide adenine dinucleotide phosphate (NADPH). NADPH plays a key role in protecting cells against oxidative stress by regulating the level of reduced glutathione (GSH) (Hwang *et al*., 2018). In red blood cells (RBCs), G6PD is the only source for NADPH that is important for defense against oxidative stress If the NADPH concentration in the RBCs cannot be maintained, as in G6PD deficiency (G6PDD), the GSH levels fall and oxidative damage occurs which eventually resulting in hemolytic anemia (Reading *et al*., 2016). The activity of human G6PD is reduced either due to a decrease in catalytic activity or a reduction in the number of active G6PD molecules (Boonyuen *et al*., 2016).
Objectives: The main objective of this study was to understand the molecular basis of G6PDD, the structural and conformational changes of both variants were studied at atomic level using 100ns molecular dynamic simulation.
Method: A systematic search was performed using four databases (PubMed, Embase, ScienceDirect, and Web of Science) to select G6PD variants that led to different forms of G6PDD. The studies were then selected based on published as a primary research paper in a peer-reviewed journal. Combinations of search terms were restricted to "G6PD deficiency" OR "Glucose-6-phosphate dehydrogenase deficiency" or "G6PDD'.
Results: $G6PD_{Mahidol}$ variant has been reported to affect the same codon as $G6PD_{Plymouth}$ variant. $G6DP_{Mahidol}$ variant has an amino acid alteration at position 163 where glycine is replaced with serine (G163S) which is an uncharged polar amino acid. This, resulting in a moderate form of enzyme deficiency (10–60% enzyme activity). As for $G6PD_{Plymouth}$ variant, glycine at position 163 is substituted with aspartic acid (G163D). The substitution of non-polar amino acid with a negative charged amino acid exhibits a severe form of enzyme deficiency with less than 10% enzyme activity (Mason *et al*. 1995). This mutation is located at the tight turn between an α-helix and a β-sheet on the surface of the enzyme molecule distant from the substrate and both $NADP^+$ binding sites (> 28Ă). Computer modelling analysis of $G6PD_{Plymouth}$ demonstrated steric hindrance occurring between aspartic acid (Asp) and amino acids Gln133, Ala134, Asn135 and Ser160 causing repulsion of the adjacent loop which in turn changes the conformation of the connected β-sheet and overall stability of the structural $NADP^+$ binding site (Huang *et al.*, 2008). $G6PD_{Mahidol}$ with serine substitution at the same position shows similar effects but less severe clashes due to shorter serine side chain. Therefore, this suggests that the charged amino acid at position 163 compromises the structural conformation of G6PD protein and the resultant distortion in protein structure affects enzyme functionality (Huang *et al.*, 2008).

Conclusion: The main goal of this research was to determine the difference in stability and flexibility of G6PD WT and mutant enzyme structures using MD simulation approach. The studied mutations, $G6PD_{Plymouth}$ and $G6PD_{Mahidol}$ present in G6PD structure affect the binding of the substrate as well as the structural $NADP^+$, and this interferes with the stability of the enzyme structure. However, findings from this study suggest that dimerization site could also contribute to overall changes of enzyme activity resulting from structural changes associated with the mutation. Further analysis at the dimerization site could provide valuable insights in understanding the behavior of this protein in disease state.

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