Study of the levels of advanced Glycation End Products in Sickle Cell Patients

Thesis

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List of Abbreviations

Abb.	Full term

ACS .....Acute chest syndrome"

AGEs .....Advanced glycation end products

ASSC .....Acute splenic sequestration crisis

AUC .....Area under curve

BNP .....Brain natriuretic peptide

CBC .....Complete Blood Count

CML .....Carboxymethyl lysine

d-ROMs .....Derivatives of reactive oxygen metabolites

EDTA .....Ethylene diamine tetra acetic acid

GH .....Growth hormone

H2O2.....Hydrogen peroxide

HbS .....Haemoglobin S

HbS .....Sickle hemoglobin

HPLC .....High-performance liquid chromatography

HRP .....Horse radish peroxidase

IHC .....Immunohistochemistry

IQR .....Inter-quartile range

MRI .....Magnetic Resonant imaging

MS .....Mass spectrometry

NO .....Nitric oxide

OD.....Optical density

PAH .....Pulmonary hypertension

List of Abbreviations (Cont...)

Abb.	Full term
PS	*Phosphatidylserine*
RAGE	*Receptor for advanced glycation end products*
RBC	*Red blood cell*
ROC	*Receiver operating characteristic curve*
ROS	*Reactive oxygen species*
SCA	*Sickle cell anemia*
SCD	*Sickle cell disease*
SOD	*Superoxide dismutase*
sRAGE	*Soluble receptor for advanced glycation end product*
TCD	*Trans cranial Doppler*
TLC	*Total leukocyte count*
WBCs	*White blood cells*

INTRODUCTION

Sickle cell disease (SCD) is a hemoglobinopathy characterized by hemolytic anemia, increased susceptibility to infections and vaso-occlusion leading to reduced quality of life and life expectancy. SCD is caused by a point mutation in a single gene, which results in a mutant β-globin protein (HbS), in which the sixth amino acid is changed from glutamic acid to valine. In the homozygous and some compound heterozygous states, deoxygenated HbS molecules form polymers, which damage the red cell membrane and increase its rigidity ***(Rees et al., 2010)***.

SCD is characterized by a lifelong continuous oxidative stress, which is the result of an imbalance between oxidants and antioxidants in favor of the former. Such imbalance triggers a cascade of oxidative reactions damaging lipids, proteins, and DNA, ultimately leading to premature cell death ***(Nur et al., 2010)***. Oxidative stress plays a significant role in pathophysiology of hemolysis, vaso-occlusion and ensuing organ damage in sickle cell patients. Several mechanisms contribute to the high oxidative burden in sickle cell patients, including: (1) The excessive levels of cell-free hemoglobin with its catalytic action on oxidative reactions, (2) the characteristic recurrent ischemia-reperfusion injury, (3) a chronic proinflammatory state, and (4) higher autoxidation of sickle hemoglobin (HbS) ***(Nur et al., 2011)***.

Advanced glycation end products (AGEs) are well-established markers of oxidative stress, and were associated with disease severity in diabetes and inflammatory diseases ***(Nur et al., 2010; Loomis et al., 2017)***. AGEs are a heterogeneous group of compounds formed by the nonenzymatic glycation of proteins, lipids or nucleic acids within the so-called "Maillard reaction" ***(Vlassara and Palace, 2003)***. This reaction consists of several steps, beginning with the formation of "Schiff bases", which are transformed by structural irreversible rearrangements into Amadori products (e.g. the HbA1c). The Amadori products undergo further structural changes through oxidation, dehydration and degradation to finally yield highly stable AGEs compounds ***(Mendez et al., 2010)***. Usually the reaction leading to the formation of AGEs may take weeks to years. Under certain conditions, such as increased substrate availability (e.g. hyperglycemia), increased temperature and increased oxidative stress, the formation of AGEs can be reduced to several hours ***(Stirban et al., 2014)***. Three main groups of AGEs have been described: (1) fluorescent crosslinking AGEs (e.g. pentosidine and crossline); (2) non-fluorescent crosslinking AGEs such as imidazolium dilysine cross-links resulting from reactions between glyoxal derivatives and lysine residues; (3) non-crosslinking AGEs (e.g N-ε- carboxymethyl lysine (CML)) ***(Hartog et al., 2007; Hegab et al., 2012)***.

AGEs act either by modifying substrates, or by interacting with specific receptors ***(Loomis et al., 2017)***. Some of the mechanisms mediating the detrimental effects of AGEs on the vasculature are related to inflammation and oxidative stress, increased glycation of low-density and high-density lipoproteins, activation of the pro-inflammatory inducible nitric oxide (NO)-synthase (iNOS) and decreased NO availability ***(Stirban et al., 2014)***.

Several studies have revealed increased plasma AGE levels in sickle cell patients and proved their association with haemolysis and haemolysis-related complications. Thus, AGEs were suggested to be implicated in the pathophysiology of the haemolytic phenotype of SCD ***(Somjee et al., 2005; Nur et al., 2010)***.

Aim of the Work

The aim of the present work is to study the AGE levels in sickle cell patients and determine its relationship to the presence of SCD-related organ complications, in addition to exploring the association of AGE levels to other clinical and laboratory data.

Chapter 1

SICKLE CELL ANEMIA

Sickle cell anemia (SCA) is one of the most common inherited blood anemias. It is a blood disorder caused by inherited abnormal hemoglobin. The abnormal hemoglobin causes sickled red blood cells that are fragile and prone to rupture. When the number of red blood cells decreases from hemolysis, anemia is the result. The irregular sickled cells can also block blood vessels causing tissue and organ damage ***(Stuart et al., 2014; Alnajjar, 2018)***.

SCA is inherited as an autosomal recessive condition. In order for sickle cell anemia to occur, a sickle cell gene must be inherited from both parents. The inheritance of just one sickle gene is called sickle cell trait ***(Steinberg, 2019)***.

I. Incidence and Epidemiology

Sickle cell anemia is considered the most common type of sickle cell disease. The global incidence of SCD is 300,000 to 400,000 per year, with high childhood mortality in regions with developing economies, such as Africa and India. Sickle hemoglobin emerged independently in four or more sites worldwide. Both sickle cell trait (HbAS) and hemoglobin C trait (HbAC) protect against severe forms of cerebral malaria, without causing a significant hematologic syndrome ***(Piel et al., 2013)***. Between the beginning of the sixteenth century and the

end of the twentieth century, millions of Africans, mostly from West and Central Africa, were forced to move to the Caribbean and the Eastern coast of the Americas through the slave trade. This human traffic from areas of high prevalence of the sickle mutation to regions in which hemoglobinopathies were absent left a profound impact on populations of the Americans. The frequency of the HbS mutation in African Americans is often similar to those observed in the African subcontinent, resulting in sickle cell disease being the most common inherited blood disorder in Africa ***(Brousseau et al., 2010)***.

II. Genetics

SCD is inherited in an autosomal recessive manner. If one parent is a carrier of the HBB HbS pathogenic variant and the other is a carrier of any of the HBB pathogenic variants (e.g., HbS, HbC, β-thalassemia), each child has a 25% chance of being affected, a 50% chance of being unaffected and a carrier, and a 25% chance of being unaffected and not a carrier. Carrier detection for common forms of SCD is most commonly accomplished by isoelectric focusing or HPLC. Prenatal diagnosis for pregnancies at increased risk for SCD is possible by molecular genetic testing if the HBB pathogenic variants have been identified in the parents ***(Adam et al., 2017)***.