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Value of Red Blood Cell derived Extracellular Vesicles as Novel Biomarker for Thalassemia: Review article

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ABSTRACT

Background: Hereditary hemolytic anaemia is a broad range of anemias characterised by a reduced ability of red blood cells to survive as a result of genetic haemoglobin, enzyme, or membrane abnormalities. Red blood cells that have been affected are more fragile, less deformable, exhibit greater vesiculation, and are more vulnerable to oxidative stress and shear stress. Extracellular vesicles contain a variety of bioactive substances that have been associated with cellular activation, intercellular communication, and a number of biological and pathological processes.

Objective: The aim of the study was to evaluate value of red blood cell derived extracellular vesicles as novel biomarker for thalassemia.

Methods: The study was conducted in Assiut University Hospital, Assiut, Egypt.

Conclusion: Bioactive chemicals are selectively sorted into microvesicles during the carefully regulated process of red blood cell microvesiculation. Hereditary hemolytic anaemia has a variety of molecular abnormalities that may have an impact on red blood cell vesiculation. Regarding hereditary hemoglobinopathies such as thalassemias, unstable haemoglobin causes membrane proteins and lipids to oxidise, haemoglobin to deposit on the membrane, and alterations to the intracellular viscosity, all of which lead to RBCs that are difficult to deform.

Keywords: Bioactive chemicals, Thalassemia and Haemolytic anaemia.

INTRODUCTION

Hereditary hemolytic anaemia is a broad range of anemias characterised by a reduced ability of red blood cells to survive as a result of genetic haemoglobin, enzyme, or membrane abnormalities. Red blood cells that have been affected are more fragile, less deformable, exhibit greater vesiculation, and are more vulnerable to oxidative stress and shear stress.

Similar to other cells, red blood cells vesiculate, or form phospholipid extracellular vesicles, both in vivo and in vitro. This class of extracellular vesicles includes a wide range of vesicles with internal sources and various sizes. If they come from multi-vesicular bodies, they are referred to as exosomes. When they emerge from the plasma membrane during the budding process in a single step, they are known as microvesicles.

Apoptotic bodies when they develop as blebs from the plasma membrane of cells going through programmed cell death. Extracellular vesicles contain a variety of bioactive substances that have been associated with cellular activation, intercellular communication, and a number of biological and pathological processes. Theoretically, only microvesicles are released by adult red blood cells. With an emphasis on red blood cell vesiculation, we address recent developments in our understanding of extracellular vesicles biology in this review.

The molecular abnormalities of hereditary hemolytic anemias, particularly thalassemia, and their relationship to red blood cell deformability and vesiculation are also reviewed, along with recent scientific findings on these topics. Understanding the pathogenesis of thalassemia and other hereditary hemolytic anemias would need integrating bio-analytical results on abnormalities of red blood cells and their microvesicles.

EXTRACELLULAR VESICLES AND THEIR PATHOPHYSIOLOGICAL SIGNIFICANCE

A heterogeneous class of cell-determined structures having a lipid bilayer film is known as extracellular vesicles. They originate from endosomes or are expelled from the plasma film in physiological and neurotic conditions [(1)].

Exosomes, ectosomes, shedding microvesicles, apoptotic blebs, and diverse extracellular vesicle subsets are just a few examples of the many and varied vesicles that are classified under the name extracellular vesicles based on their biogenesis and delivery method [(2)]. Extracellular vesicles should be arranged into three distinct main groups, according to the International Society for Extracellular Vesicles (ISEV): exosomes, microvesicles, and apoptotic bodies or apoptotic blebs [(3)].

Extracellular vesicles (Exo) are described in contemporary logical writing as having endocytic origins that escape from multivesicular endosomes (MVEs), whereas microvesicles are created by blebbing the plasma layer and subsequent membrane bleb separation [(4)].

Microvesicles, which are bigger vesicles with a diameter of 100 to 500 nm, are created during the growth or exocytosis cycle of the plasma layer. Endosomal multivesicular bodies, which have a diameter of 50 to 150 nm, release exosomes [(5)].

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Extracellular vesicles may also initiate phenotypic changes in a target cell by migrating to one that has dynamic receptors like CCR5, EGFRvIII, or MET [6]. The parent cell source and the availability of various target cell types to occlude the extracellular vesicles in motion are likely to have a role in extracellular vesicle biodistribution. To describe the organ uptake and clearance of different extracellular vesicle populations, more thorough research comparing different injector cells, donor cells, and healthy and sick circumstances is required [7].

Extracellular vesicles play a key part to play in significant biological cycles. The intercellular correspondence can happen between cells by moving extracellular vesicles that is considered as an exchange mediator of proteins, lipids, and RNAs. For example, surface membrane mediator exchange and also RNA transport among adjoining and distant cells. This perspective is broadly researched in tumors, neurodegenerative diseases, and immune system problems including autoimmune diseases and ageing [8].

RED BLOOD CELL-DERIVED VESICLES

One of the fundamental vesicle-emitting cells in the flowing blood, erythrocytes represents 40% of the complete blood volume [9].

In healthy people, platelets make up most of the coursing microvesicles, yet when certain abnormal circumstances like jungle fever and sickle cell illness are available, levels of RBC-determined microvesicles are higher [10].

Regardless of how reticulocytes, or juvenile RBCs, act in this manner during the remodelling system that occurs nearby their maturation, adult RBCs are probably the only cells that only secrete microvesicles instead of exosomes [11].

Erythrocyte-determined microvesicles have a lipid bilayer rich in different phospholipids and proteins and are submicron-sized membranous structures. They develop from the parent red blood cells. Their size typically ranges from 100 to 200 nm. Typical RBCs vesiculate during the course of their 120-day lifespan, losing 20% of their haemoglobin and membrane layer [12].

Conditions for storage, length of storage, and the expansion of blood items all affect these lesions [13]. Erythrocytic membrane components assume a vital part in the development and arrival of microvesicles, and understanding their capabilities might help how we might interpret RBC vesiculation. Proteins 4.1R, 4.2, and ankyrin interface the phospholipid bilayer that makes up the RBC film to the cytoskeleton organization of actin and spectrin and are penetrable to basic proteins (like band 3, glycophorins An and C, and Rhesus-related antigen) (Figure 1).

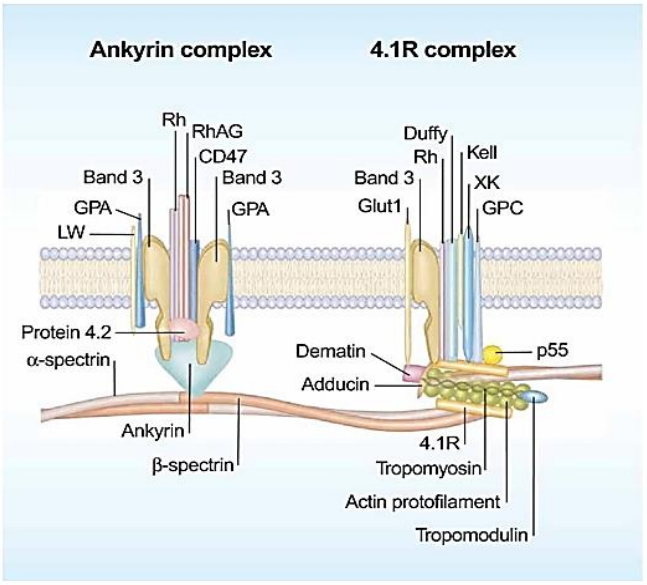

FIGURE 1 | Schematic representation of the red blood cell membrane. The red blood cell membrane consists of a phospholipd bilayer that is anchored to the 2-dimensional elastic network of skeletal proteins (mainly spectrin) through transmembrane proteins (reproduced with permission from Mohandas and Gallagher, 2008).

The plasma layer is kept stable by the RBC film and cytoskeleton proteins, which additionally give RBCs their deformability and mechanical strength [14]. A few RBC layer proteins likewise can move particles. For example, the transportation of glucose and water is constrained by aquaporin-1 and glucose carrier 1 (GLUT1), individually. Particle channels are one more part of the erythrocytic membrane that manage particle gradient and the level of hydration in RBCs [15]. Scramblase is initiated and flippase is hindered by high intracellular calcium levels, which prompts the breakdown of the lipid unevenness and externalization of phosphatidylserine.

It is vital to take note of that the calcium-incited phospholipid scrambling and microvesicle delivery may both happen through various pathways. As indicated by **Bucki *et al.*** [16] spermine's ability to balance out the phospholipid deviation didn't forestall calcium-actuated RBC vesiculation [16]. The specific synthetic systems behind RBC vesiculation, nonetheless, remain ineffectively comprehended. It is indistinct how various boosts, including calcium inundation, amphiphiles, shear pressure, and ATP exhaustion, may prompt the shedding of microvesicles with various pieces. Despite the fact that it is accepted that the rebuilding of blood storage looks like the maturing of red blood cells, there might be varieties in the elements of cytoskeleton proteins in vesicle development between in vitro and in vivo [17].

Moreover, various RBC morphologies have fluctuated approaches to oozing microvesicles. RBCs in

Scott syndrome, an uncommon genetic draining issue, oppose vesiculation when enacted with the calcium ionophore A23187 in spite of having a typical shape and showing no irregularities of the cytoskeleton [18].

Taking everything into account, not all erythrocyte-determined microvesicles are made equivalent, and the qualities of the microvesicles that are delivered might be affected by the aggregate of the red blood cells and the setting off occasions. RBC-inferred microvesicles have many physiological and clinical applications. Two physiological impacts result from the externalization of the adversely charged phosphatidylserine, which is the primary quality of microvesicles shaped from red blood cells: advancing coagulation; and sending the "eat me" message to macrophages. The procoagulant activity of phosphatidylserine relies upon its synergist action. Phosphatidylserine fills in as a limiting surface for prothrombinase and other coagulation factors, speeding up the rate at which prothrombin, an urgent coagulation particle, changes into thrombin [19].

The comparative purifying cycle is workable for microvesicles that uncover phosphatidylserine. RBC vesiculation has been speculated to work as a guarded system to forestall the untimely clearing of RBCs by eliminating the harmed RBC film parts. Erythrocyte-determined microvesicles "penance" themselves in this design since their parental RBCs, which probably had their lipid deviation re-established after vesiculation, are dispensed with by the reticuloendothelial framework while the phosphatidylserine-uncovered microvesicles remain [20]. Such immunosuppressive ways of behaving may influence post-bonding safe concealment and contaminations. Nonetheless, **Shelf *et al.*** [21] tracked down that P. falciparum- contaminated RBCs had the option to make microvesicles that were full loaded with host and parasite proteins and that these microvesicles significantly affected inborn invulnerable framework cells**.** During storage of blood, RBCs foster storage injuries, which compromise the respectability of the RBC film and cause the arrival of the oxy-hemoglobin content of the RBCs as free cell oxy-hemoglobin and vesicular oxy-hemoglobin. These two varieties may be better at rummaging NO, an intense vasodilator. It was found that, conceivably because of NO rummaging, how much storage- related hemolysis expanded with how much put away blood transfused [21]**.**

Hereditary Hemolytic Anemia: Understanding The Molecular Biology

Inherent RBC abnormalities are the main causes of hereditary hemolytic anemias. In this study, we concentrate on this subset of illnesses, which can result from changes in the genes encoding for transmembrane/cytoskeleton proteins, enzymes required for RBC metabolism, or haemoglobin. Hereditary hemolytic anemias can have phenotypes that are mildly asymptomatic or severely life-threatening. These illnesses' molecular causes are discussed in this section, along with any possible connections to RBC vesiculation.

Hereditary Hemolytic Anemias Due To Hemoglobinopathies

In humans, haemoglobin serves as the primary oxygen carrier. This protein is a tetramer made up of two pairs of various globin chains. Hemoglobin A (Hb A), which is made up of two -globin and two -globin chains, makes up the majority of normal adult haemoglobin. The integrity, solubility, and oxygen affinity of these haemoglobin molecules, as well as their concentration (32–35 g per 100 mL of packed RBCs), are all essential for maintaining the RBC's primary function, oxygen transport. It has been discovered that over 1000 mutations in the globin-encoding genes impact the synthesis, solubility, stability, or oxygen affinities of haemoglobin. Such hereditary haemoglobin abnormalities may affect the RBCs' internal viscosity and deformability, leading to an increase in the sequestration of poorly deformable cells in the spleen. Additionally, these disturbances may set off proteolytic processes linked to the generation of reactive oxygen species, which may ultimately lead to hemolytic anemias [22]. Haemoglobin deficiencies may be related to quantitative or qualitative problems.

Qualitative aberrations result from altered structural features of haemoglobin that have perturbed chemical and/or physical properties. Quantitative hemoglobinopathies are caused by incorrect synthesis of otherwise healthy globin chains. Some pathological conditions connected to the first group include sickle cell anaemia, hereditary met-hemoglobinemia, high oxygen affinity polycythemia, and low oxygen affinity cyanosis. Examples of pathological disorders linked to the quantitative anomalies include thalassemias. The two most prevalent and serious disorders are thalassemia and sickle cell anaemia [23]**.**

Thalassemias

Reduced production of one of the two globin chains required to create the adult haemoglobin tetramer Hb A, causes a wide range of inherited anemias known as thalassemias. The name of each thalassemia is determined by the damaged globin chain. For example, individuals with -thalassemia have no or very few globin chains [22]**.**

In thalassemias, the globin chains frequently have a characteristic structure. Underproduction of haemoglobin and the intracellular accumulation of extra globin subunits are two pathophysiological effects of thalassemias.

The capacity to carry oxygen is decreased in the remaining RBCs as a result of insufficient haemoglobin synthesis. Additionally, if excess globin subunits precipitate on the inner leaflet of the membrane, the cytoskeleton may be injured and the RBC's capacity to deform is diminished. Rapid decomposition of free globin chains may potentially result in the removal of RBC precursors from the bone marrow, which would impair erythropoiesis [(22)].

The value of erythrocyte derived extracellular vesicles analysis as novel biomarkers in thalassemia patients

Extracellular vesicles varied times additional within the plasma of thalassemic patients, when compared to healthy controls. it's thought that this is often because of the acute oxidative stress practised by thalassemic red blood cells similarly because the platelets [(24)]. The link between these animate vesicles and clinically vital procoagulant activity has been established. This is often explained by the very fact that thrombin age is made by the interaction with charged phosphatidylserine openness and activated tissue factor on the outer layer of vesicles that starts the coagulation process. Vesicles derived from platelets may activate pro-inflammatory cytokines and chemokines.

Natesirinilkul and colleagues [(25)] additionally rumoured higher substance counts in HbE/b-thalassemic patients, notably in those that had splenectomies [(25)]. Compared to healthy people, the next amount of proteins associated with the oxidative pressure reaction, like peroxiredoxin two (PRDX2), catalase and heat shock proteins seventy (HSP70), were found in vesicles within the plasma discharged from the platelets and erythrocytes of b-thalassemic patients in earlier investigations [(26)]. One more example of proof of expanded red blood cell age in b-thalassemia was discovered, is that the discovery of m-hemoglobin, that is translated from the a high quality (HBM) and sometimes delivered in rope blood reticulocytes [(27)]. Given the robust correlation between vesicular age and also the pathophysiology of HbE/b-thalassemia, it is attainable that the structure of vesicles might replicate the severity of the patients' clinical symptoms. The target of this study was to look at the protein profiles of vesicles exploitation sensitive nano-fluid chromatography mass spectrographic analysis (nano-LC MS/MS) and quantitative random mass tag (TMT) so as to identify variations between the vesicular contents of HbE/b-thalassemic patients and controls. One amongst the most objectives of such study is to search out potential biomarkers that will be wont to gauge the severity of the infection and, more ideal, to work out whether or not blood transfusion is important [(26)].

CONCLUSION

Previously considered to be cell trash, microvesicles generated from red blood cells now appear to have crucial functions in controlling blood homeostasis, regulating immunological response, and other pathophysiological processes.

Bioactive chemicals are selectively sorted into microvesicles during the carefully regulated process of red blood cell microvesiculation. Hereditary hemolytic anaemia has a variety of molecular abnormalities that may have an impact on red blood cell vesiculation.

There may be differences in the biological impact of the microvesicles secreted in the various hereditary hemolytic anemias. This knowledge might potentially lead to the discovery of brand-new medicinal compounds and diagnostic markers. Regarding hereditary hemoglobinopathies such as thalassemias, unstable haemoglobin causes membrane proteins and lipids to oxidise, haemoglobin to deposit on the membrane, and alterations to the intracellular viscosity, all of which lead to RBCs that are difficult to deform.

DECLARATIONS

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