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A review of the factors that influence erythrocyte osmotic fragility

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Abstract

Erythrocyte osmotic fragility is a laboratory test which evaluates the stability of the erythrocyte membrane to osmotic stress. The aim of this review is to highlight research findings on the intrinsic and extrinsic factors that influence erythrocyte osmotic fragility. The extrinsic factors include the type, ionic strength and pH of incubation media, type of anticoagulant and storage time of the blood, ambient temperature, drugs, medicinal plant extracts, xenobiotics, chemical agents; whereas intrinsic factors are age, sex, breed, species, pregnancy, lactation and genetic factors. Membrane composition, ion transports, aquaporin action, lipid peroxidation, and eryptosis of erythrocytes are involved in the variability of osmotic fragility. Increased osmotic fragility and improved osmotic stability of erythrocytes are pathophysiological phenomena that require appropriate interpretation in research or clinical investigation and the understanding of the factors affecting osmotic fragility will aid in the laboratory assessment of conditions related to erythrocytes where erythrocyte osmotic fragility test is used.

Keywords: Erythrocyte, Extrinsic factors, Intrinsic factors, Osmotic fragility, Osmotic stability

Introduction

Osmotic stability and fragility of erythrocytes are related measurable quantities in haematology which estimate haemolysis under hypoosmotic stress. In the interpretation of estimated haemolysis, an increased haemolysis is defined as an increase in osmotic fragility or a decrease in osmotic stability in an inverse corollary (Igbokwe, 2016). Erythrocyte osmotic fragility is determined by conducting a fragility test which is used as aid in the diagnosis of hereditary spherocytosis and haemolytic diseases. It supplements a stained cell examination to detect morphologic erythrocyte abnormalities and alterations leading to destruction of the erythrocytes (Kolanjippan *et al*., 2002). It is used for studies of membrane permeability and as a potential biomarker of oxidative membrane damage in pathologic conditions as well as toxicant or xenobiotic or pesticide-induced oxidative membrane damage to erythrocytes (Sharma *et al*., 2010). It is also a tool for screening for alpha-thalassemia (Sirichotiyakul *et al*., 2004), beta-thalassemia trait (Thool *et al.,* 1998; Bobhate *et al*., 2002; Manju *et al*., 2006), for pigmenturia (Legrange *et al.,* 1995) and for hypertensive patients who will benefit from diuretic therapy (Fansamade, 1999). Transportation stress (Minka & Ayo, 2010) and vitamin E status (Pillai *et al.,* 1992) can be assessed in animals by erythrocyte osmotic fragility test.

Some extrinsic factors which include temperature, pH of isotonic solution (Oyewale *et al.,* 1991;

Oyewale, 1991a; Oyewale, 1991b; Oyewale, 1993; Oyewale, 1994a; Oyewale *et al*., 2011; Islah *et al*., 2016), osmolarlity and type of media (Igbokwe & Igbokwe, 2016a; Igbokwe & Igbokwe, 2016b), oxygenation (Lewis & Ferguson, 1966; Dacie & Lewis, 1995; Mafudvaze & Erlwanger, 2007), season (Oladele *et al*., 2003; Habibu *et al*., 2016), ionic strength of media (Igbokwe & Igbokwe, 2015), medicinal plants (de Freitas *et al*., 2008) and drugs (Boelsterli *et al.,* 1983; Chikezie, 2007) can affect erythrocyte osmotic fragility. Certain intrinsic factors such as age (Perk & Perk, 1964a; Oyewale & Ajibade, 1990; Oyewale 1991b; Igbokwe *et al*., 2016), genes (Krogmeier *et al.,* 1993; Gueorguiev *et al.,* 1999), species (Soliman & Amrousi, 1966; Coldman *et al.,* 1969; Aldrich *et al*., 2006), breed (Schalm *et al.,* 1975; Oyewale & Durotoye, 1988; Olayemi & Oyewle, 2002; Habibu *et al*., 2013), phenotype (Igbokwe *et al*., 2015a), gender (March *et al.,* 1966; Habibu *et al*., 2014; Igbokwe *et al*., 2016), pregnancy and lactation (Habibu *et al*., 2014; Igbokwe *et al*., 2015b), egg laying (Oyewale, 1990), size (Troiano *et al*., 2000) and differences in erythrocyte membrane composition (Fairley *et al.,* 1988; Hagve *et al.,* 1993) can also affect the osmotic fragility of erythrocytes. The resistance of erythrocytes to haemolysis may be increased or decreased in haematological disorders (Jain, 1973), glycemia (Sekar & Selvam, 1994) and disease processes (Kobo *et al*., 2014). Glycosylation of haemoglobin and erythrocyte membrane proteins can also cause changes in osmotic fragility of erythrocytes (Ramana *et al.,* 1997).Chikezie *et al.* (2010) reported that differences in erythrocyte osmotic fragility of humans are under the control of individual genotype of erythrocytes. Variations in some physicochemical properties and oxidant levels of red blood cell genotypes contribute to differences in mechanical stabilities and capacities of erythrocyte to withstand osmotic stress (Srour *et al*., 2000a, Srour *et al*., 2000b; Senturk *et al*., 2001; Richards *et al*., 2007). Extracts of some medicinal plants (Suboh *et al*., 2004; Akinwande *et al*., 2007; Gomes de Sou *et al*., 2007, Chikezie & Uwakwe, 2011) and haemoparasites can also affect erythrocyte fragility (Silva *et al.,* 1989; Santoro *et al.,* 1994).

The aim of the present review was to identify and understand the factors that could influence erythrocyte osmotic fragility and how they affect erythrocyte osmotic stability.

Intrinsic Factors

Genetic factors

Decreased osmotic fragility in C57BL/6J strain of mice, compared to another strain DBA/2J, was directly controlled by their genotype (Dewey *et al.,* 1982) and the osmotic resistance allele was autosomal and recessive to the susceptible one (Schaefer & Dewey, 1989). Gene effect on osmotic fragility seemed to influence erythrocyte membrane ion transport in mice (Norman & Dewey, 1985; Armsby *et al.,* 1996) and correlated with milk fat traits in dairy cattle (Krogmeier *et al.,* 1993). Erythrocytes from sheep with extra α-gene were larger (with increased MCV) and had increased erythrocyte osmotic fragility than those of sheep without the genes (Pieragostini *et al*., 2003). Chikezie *et al. (*2010) reported that osmotic fragility differed in three erythrocyte genotypes (HbAA, HbAS and HbSS) of males in humans. Erythocytes with HbSS genotype exhibited the least stability and HbAA showed the most stability. Phenotypic drift caused physiological variation in erythrocyte osmotic fragility in Sahel goats (Igbokwe *et al*., 2015a).

Species

Elephant erythrocytes are highly resistant to osmotic lysis when compared to erythrocytes from other species of mammals due to the increased surface to volume ratio of their large erythrocytes (Nirmalan *et al.,* 1967; Silva & Kuruwita, 1994). Erythrocyte osmotic fragility was reported to be highest in West African dwarf goat (0.55-0.75%); lowest in White Fulani cattle (0.35%-0.55%) and that of sheep was in between the two (0.45%-0.65%) when the three species were compared (Olusanya & Adepoju, 1979). Buffenstein *et al.* (2001) reported that erythrocytes from kangaroos were more resistant to osmotic stress than erythrocytes of sheep. In a study involving adult animals (Perk *et al.,* 1964b), osmotic fragility of erythrocytes of the following animals was reported in an increasing order; camel, chicken, dog, pig, rabbit, guinea pig, mouse, rat, hamster, horse, donkey, ox, cat, sheep and goat. Birds with elliptical erythrocytes are less osmotically fragile than species with round erythrocytes (Viscor & Palomeque, 1982). Erythrocytes from the guinea fowl are more fragile than those of the domestic fowl (Durotoye & Oyewale, 1988). Erythrocytes from chicken were reported to be more fragile than those of ostrich (Olowookorun & Makinde, 1998).

Breed

Erythrocyte osmotic fragility was lower in Nigerian indigenous dogs than in German shepherd dogs (Olayemi *et al*., 2009; Ogunyemi & Olayemi, 2016). However, Adebiyi *et al.* (2014) reported no difference in erythrocyte osmotic fragility between Rottweiler dogs and Nigerian indigenous dogs. Oyewale & Durotoye (1988) reported that erythrocytes from local Nigerian breeds were more susceptible to osmotic haemolysis than those of exotic breeds of fowl. Among exotic breeds, breed differences exist between the osmotic fragility of erythrocytes from White Leghorn and New Hampshire breeds of fowl, and Marshal, Ross and Hubbard-Anak cross breeds of broiler chickens (March *et al.,* 1966; Habibu *et al*., 2013), with the Marshal breed erythrocyte as the most stable.

Age

Oyewale (1991b) reported that adult guinea fowls (156 weeks of age) had more osmotically fragile erythrocytes than the younger ones (21 weeks of age). Erythrocytes were osmotically more fragile in the young (8-10 weeks old) than adult (52-80 weeks old) local Nigerian ducks (*Anas platyrhynchos*) (Oyewale *et al.,* 1998a) and turkeys (Oyewale & Ajibade, 1990; Azeez *et al*., 2011a) due to variation in nutrition and cholesterol-phospholipid ratio in the membrane. Erythrocyte osmotic fragility decreased as Gezel and Makoei breeds of sheep (Asri-Rezaei *et al*., 2006) and cattle (Basarab *et al.,* 1980) grew older. Erythrocyte membrane stability increased from age 20 to 94 years in women (Penha-Silva *et al*., 2007), while erythrocytes of premature infants were less osmotically fragile than those of full-term infants (*Bautista et al*., 2003). In normal males between the ages of 18 and 78 years, age-related effect was shown to increase both the mean fragility of erythrocytes and the variability of the fragilities of the erythrocytes within individual blood sample (Bowdler *et al.,* 1981). Mosior & Gomulkiewicz (1988) reported decreased osmotic fragility in older human and bovine erythrocytes as their erythrocytes aged in circulation, while Rifkind *et al.* (1983) and Mosior & Gomulkiewicz (1985) reported an increase in osmotic fragility as human erythrocytes aged in circulation.

Sexual dimorphism hormones and other hormones

Erythrocyte osmotic fragility was higher in males than in females in cattle (Olayemi 2004, Olayemi, 2007), birds (March *et al.,* 1966; Oyewale & Durotoye, 1988; Oyewale, 2004) and sheep (Durotoye 1987) probably due to stabilizing effect of estrogen. Erythrocytes from females were osmotically more fragile than those of males in dogs (Ogunyemi & Olayemi, 2016), African giant rats (Oyewale *et al.,* 1998b), goats (Habibu *et al*., 2014), turkeys (Azeez *et al*., 2011b) and humans (Olorunshola *et al*., 2012). However, sexual dimorphism was absent in swine (Makinde, 1986), camels and donkeys (Oyewale *et al*., 2011), cattle (Basarab *et al.,* 1980) and Sahel goats (Igbokwe *et al*., 2016).

Circulating estrogen in high levels protected erythrocyte membrane and decreased erythrocyte fragility in cows (Olayemi, 2004; 2007) and birds (March *et al.,* 1966; Oyewale, 1990). Testosterone had no effect on erythrocyte osmotic fragility in birds (March *et al.,* 1966). Parathyroid hormone (PTH) caused an increase in osmotic fragility of human erythrocytes (Bogin *et al.,* 1982; Malachi *et al*., 1986). PTH caused a significant influx of calcium into erythrocyte, which was not associated with potassium leak. The increased calcium influx affected the spectrin-actin of the cytoskeletal network of the erythrocyte and altered the stability and integrity of the cell membrane. Osmotic fragility of erythrocytes was increased in birds with hypothyroidism when compared with normal birds (Dariyerli *et al*., 2004). Leylek *et al.* (1998) reported significant increase in erythrocyte fragility in groups of women with labour induction or augmentation with oxytocin than in other groups. Progesterone interacted with the soluble protein component of erythrocyte membrane and improved the stability of the membrane (Devenuto *et al.,* 1969) but progesterone reduced erythrocyte osmotic fragility of human erythrocytes (Kaya & Saito, 1985; Yoong *et al*., 2003).

Pregnancy and lactation

Erythrocyte osmotic fragility increased in pregnant women due to destabilizing effect of progesterone on the membrane (Nakamura, 1983; Arora *et al.,* 1994; Emembolu & Mba, 1994) than non-pregnant women mostly in the third trimester of pregnancy (Magid *et al.,* 1982). However, no difference was recorded between mean erythrocyte fragility of pregnant and non-pregnant women (Suhail *et al*., 2010) and Red Sokoto does (Habibu *et al*., 2014).

Krogmeier *et al.* (1993) reported a decrease in erythrocyte osmotic fragility in Holstein cows that were in late lactation as compared to those in early lactation. Habibu *et al.* (2014) reported no significant difference in osmotic fragility between erythrocytes of lactating and non-lactating Red Sokoto does. In Sahel does, erythrocyte osmotic fragility decreased in late pregnancy due to improved membrane stabilization by progesterone and increased during lactation because of decreased concentration of cholesterol and trigycerides in the membrane (Igbokwe *et al*., 2015b).

Membrane composition

The composition of the erythrocyte membrane determines its elasticity and deformability and influences the ability of the erythrocyte to withstand osmotic stress. In humans, low deformability, increased level of surface phosphatidylcholine and low stomatin level in erythrocytes were associated with increased erythrocyte osmotic fragility (Orbach *et al*., 2017). The osmotic stability of erythrocytes in control and heat exposed hamsters was increased with the addition of linolenoyl and stearoyl sorbitol which revealed that unsaturated acyl groups of membrane lipids contribute to the higher osmotic stability of erythrocyte (Livne *et al.,* 1972). Raz & Livne (1973) reported that unsaturated fatty acids (stearic, oleic, linoleic and linolenic acids, the methyl esters of these acids, as well as their hydroxy analogs) are able to protect erythrocytes from hypotonic haemolysis. In human erythrocytes, linoleic acid caused haemolysis and increased erythrocyte osmotic fragility due to its detergent-like action (Thomas-George *et al.,* 1979). Matured human erythrocytes were osmotically more stable than reticulocytes due to increased membrane cholesterol (Karai *et al.,* 1982a) which was suggested to be due to the inactivation of lecithin-cholesterol acyltransferase (Karai *et al.,* 1982b). Hagve *et al.* (1993) observed an increased level of n-3 fatty acids in the erythrocyte membrane of healthy females fed fish oil for twenty-eight days which led to a decrease in osmotic fragility. A similar report was made by Kiron *et al.* (1994) in rainbow trout fish, but Kogawa *et al.* (1998) reported an increase in osmotic fragility of rabbit erythrocytes treated with free fatty acids.

In camels, decreased erythrocyte osmotic fragility was attributed to high concentration of total lipids, cholesterol, proteins, sphingomyelin and phosphatidylcholine in the membranes when compared with the concentrations of these parameters in the erythrocyte membranes of sheep and goats (Al-Qarawi & Mousa, 2004).

The variations observed in the lipid composition and structure of erythrocytes of humans, goats and sheep affect their ability to resist detergent induced lysis (Koumanov *et al*., 2005). Increased erythrocyte osmotic fragility was observed in rabbits fed high fat diets due to distortion of ion fluxes and membrane properties (Abdelhalim & Moussa, 2010). Rats fed dietary oils had decreased erythrocyte osmotic fragility indicative that the fatty acid composition of rat erythrocyte membranes was affected by the fatty acid composition of the dietary fats (Kirchgessner *et al.,* 1994). Mineo & Hara (2005) reported same in rat erythrocytes incubated in saline medium containing short-chain fatty acids.

Esterases are enzymes in erythrocyte membrane that catalyse the hydrolysis of an ester into its alcohol and acid (Fukami and Yokoi, 2012) and some of these esterases could be responsible for the resistance of erythrocytes to osmotic stress. Al-Qarawi &Ali (2003) reported that the activities of three esterases; aspirin esterase, cholinesterase, and nitrophenylacetate esterases were lowest in camels, highest in goats while those of cattle and sheep fell in between.

Extrinsic Factors

Incubation temperature and time

Erythrocyte osmotic fragility increased when incubation temperature was decreased in sheep (Oyewale 1991a), rat, rabbit, cattle, and pig (Oyewale, 1992), domestic fowls and guinea fowls (Oyewale, 1991b), pigeon, lizard and toad (Oyewale, 1994a) and humans (Murphy, 1967; Seeman *et al.,* 1969; Aloni *et al.,* 1977). In camels (Aloni *et al.,*. 1977), ducks (Oyewale *et al.,* 1998a) and peafowls (Oyewale, 1994a), erythrocyte osmotic stability decreased as the incubation temperature increased. The osmotic resistance of erythrocytes exposed to detergents (Triton X-100® and sodium dodecyl sulfate) decreased with increase in incubation temperature (Bielawski, 1990). However, increasing the incubation temperature to 46^0C for 1hr did not affect erythrocyte fragility in humans (Van der Walt & Russell, 1978).

Potential of hydrogen (pH) of media

An inverse relationship between the pH of the media and osmotic fragility of erythrocytes has been reported in camels (Aloni *et al.,* 1977; Islah *et al*., 2016), sheep and goats (Oyewale, 1991a; Oyewale *et al.,* 1991), pigeon and lizard (Oyewale, 1994a), domestic fowl (*Gallus domesticus*) (Oyewale, 1991b), rat, cattle, pig, rat (Oyewale, 1992), and humans (Jacob & Parpart, 1931) with an increase in the pH of the hypotonic saline medium causing a decrease in erythrocyte osmotic fragility attributed to oxidative damage from elevation of endogenous cytotoxic metabolites and release of metals with variable valencies from metalloproteins after acidification.

Anticoagulant and storage of blood sample

When used as an anticoagulant, ethylene diaminetetra-acetic acid (EDTA) increased erythrocyte osmotic fragility when compared with heparin (Kafka & Yermiahu, 1998). Mafudvaze & Erlwanger (2007) reported that blood from ostriches collected in EDTA, had an increased osmotic fragility compared to blood collected in heparin after 12 hours of storage. Erythrocytes stored over a long period of time develop artifacts (Veale *et al*., 2011; van de Watering, 2011) and become very fragile (Epps *et al.,* 1994). Erythrocyte osmotic fragility increased after storage for 40 days when blood samples from dogs (Price *et al.,* 1988) and humans (Ogunro *et al*., 2010) were preserved in citrate-phosphate-dextrose-adenine (CPDA-1) medium. A marked increase in osmotic fragility was observed when erythrocytes were stored in CPD-A2 for 42 hrs (Beutler *et al.,* 1982). Erythrocyte osmotic fragility was not affected after storing non-detergent washed human erythrocytes for 24 hours (Okwusidi, 2004, 2011) and 48 hours (Okwusidi, 2002).

Erythrocytes stored in glass bottles at 0^{0}C showed increased haemolysis and osmotic fragility when compared to erythrocytes stored in polyvinylchloride (PVC) bags plasticized with di (2-ethylhexyl) phthalate (DEHP) at the same temperature. The addition of DEHP to erythrocytes stored in glass bottles at 0^{o}C decreased haemolysis and osmotic fragility to levels equal to those in PVC bags (Yamamura *et al.,* 1991). Kanias *et al.* (2013) reported reduced erythrocyte osmotic fragility in blood stored in bags made with butyryl trihexyl citrate (BTHC) than in bis (2-ethylhexyl) phthalate (DEHP). A comparison of the osmotic fragility of erythrocytes from stored blood samples from various species revealed that after 24hrs at 10^{o}C, osmotic fragility increased in erythrocytes of domestic fowl, lizard and toad (Oyewale, 1994b), goat and pig (Oyewale, 1993); but decreased in erythrocytes of cattle, mouse, rabbit and rat (Oyewale, 1993), pigeon and peafowl (Oyewale, 1994b). After 24hrs of storage at 4^{o}C, the osmotic fragility of camel erythrocytes increased. Osmotic fragility of erythrocytes of camel and donkey also increased after 78hrs of storage at 4^{o}C, (Oyewale *et al*., 2011). Erythrocytes stored in isotonic phosphate-adenine-guanosine-glucose-saline-mannitol (PAGGSM) or erythrosol-4 had improved osmotic stability when compared with erythrocytes stored in saline-adenine-glucose-mannitol (SAG-M) media due to the reduction in size (Veale *et al*., 2011). Oxidative injuries to erythrocytes during storage led to membrane damage and made the erythrocytes more fragile with increased susceptibility to osmotic lysis (Chaudhary & Katharia, 2012).

Incubating media other than saline

The haemolysis of human erythrocytes in 0-10 g/dl glucose solutions was described by a decreasing sigmoid curve with complete or no haemolysis occurring at concentrations of 0-2 g/dl (0-68 mosmol/l) or 4-10 g/dl (137-342 mosmol/l), respectively (Lemos *et al*., 2011). Glucose caused haemolysis in erythrocytes by altering the membrane (Marar, 2011). No haemolysis of human, rabbit, cattle, hamster, guinea pig, pig and sheep erythrocytes occurred in solutions of ≥0.4% glucose (Matsuzawa & Ikarashi, 1979). Haemolysis of sheep erythrocytes was prevented in glucose concentration of 0·6%. As the concentration of glucose increased, the degree of haemolysis decreased from 100% to about 2% haemolysis in 0·4% glucose solution, but then rose in much higher concentrations for dog, mouse and rat erythrocytes (Matsuzawa & Ikarashi, 1979). Igbokwe & Igbokwe (2016a) reported a decrease in erythrocyte osmotic fragility of Sahel goats in hyposmolar concenrtrations of glucose when compared with similar concentrations of saline speculated to be influenced by glucose transporter protein and ion fluxes. Incubation in sucrose media reduced erythrocyte osmotic fragility in Sahel goats media due to decreased permeability of sucrose (Igbokwe and Igbokwe, 2016b) and in humans by inducing shrinkage (Martins *et al*., 2012).

Nutrition

Erythrocytes were osmotically more stable in hypotonic solutions amongst well-nourished humans (Penha-silva *et al*., 2007) and kangaroos (Buffenstein *et al*., 2001) than from the malnourished which later became normal after nutritional therapy (Kaplay, 1978). Hyperglycaemia increased osmotic fragility of human erythrocytes (Jain, 1989).

Decrease in osmotic fragility and change in the shape of erythrocytes were reported in vitamin B6 deficient rats (Kual *et al.,* 1995). Pre-treatment of rats with vitamin C reduced erythrocyte osmotic fragility which was increased due to exposure to chlorpyrifus (Ambali *et al*., 2010; Uchendu *et al*., 2011). Vitamin C (ascorbic acid) administration before transportation protected the erythrocyte membrane in pigs (Adenkola *et al*., 2010a, Adenkola *et al*., 2010b) and goats (Minka & Ayo, 2010) transported by road for several hours. Vitamins C and E supplementation also reduced erythrocyte osmotic fragility and oxidative damage in rats (Etlik *et al.,* 1997; Kraus *et al.,* 1997) and chickens (Azeez *et al*., 2011a). In Gezel and Makoei breeds of sheep, increased vitamin E (α-tocopherol) levels associated with the increase in age caused a decrease in erythrocyte osmotic fragility (Asri-Rezaei *et al*., 2006). Erythrocytes from vitamin E deficient sheep were protected against detergent induced haemolysis after the cells were preincubated with α-tocopherol *in vitro* (Steveson & Jones, 1989).Vitamin E was reported to inhibit membrane peroxidation and protein oxidation and restored activities of superoxide dismutase and catalase on membrane of erythrocytes treated with mefenamic acid (Ahmad & Suhail, 2002) and glucose (Marar, 2011) thereby improving erythrocyte osmotic stability. Marques *et al.* (1986) reported the possibility of a toxic effect of higher doses of vitamin E supplementation rather than a protective role on erythrocyte membrane stability after *in vitro* treatment of erythrocytes with alpha-tocopherol.

In vivo treatment with vitamin E decreased erythrocyte osmotic fragility in patients on haemodialysis and peritoneal dialysis (Uzum *et al*., 2006). Rats treated with acetaminophen had decreased erythrocyte glutathione content and activity of Na^+ K^+-ATPase enzyme with increased osmotic fragility but supplementation with vitamin E restored the glutathione content, Na^+ K^+-ATPase activity and osmotic fragility to normal (Suhail & Ahmad, 1995). Treatment with vitamin C (Alhassan *et al*., 2010), E or combination of vitamins E and C during hot-dry season increased packed cell volume (PCV) and haemoglobin concentration but decreased erythrocyte osmotic fragility in Wistar rats (Wahab *et al*., 2010).

Extracts of leaves, plants and herbs

Leaf extract of *Carica papaya L* stabilized erythrocyte membrane and reduced osmotic fragility in dengue patients (Ranasinghe *et al*., 2012). Aqueous extracts of *Anacardium occidentale, Psidium guajava, and Terminalia catappa* leaves (Chikezie & Uwakwe 2011) and methanol extract of *Solanum aethiopicum* (Anosike *et al*., 2012) reduced the osmotic fragility of human erythrocytes by stabilizing the membrane. Crude extracts of *Artemisia absinthium, Lippia sp., Cymbopogon citratus and Mentha villosa* decreased erythrocyte osmotic fragility while extracts of *Bryophyllum sp.* and *Solidago microglossa* increased erythrocyte osmotic fragility in humans (de Freistas *et al*., 2008). Biltto *et al.* (2012) reported that pre-incubation of erythrocytes with rutin and α-naphtho flavone did not have any effect on osmotic fragility but quercetin and 3, 5, 7-tri- hydroxy-4'-methoxy flavone-7-rutinoside reduced osmotic fragility of human erythrocytes. Oral administration of ethanol extracts of *Jatropha gossypifolia* (Pohl) (Oyedeji *et al*., 2015a) and *Adenopus breviflorus* (Benth) (Oyedeji *et al*., 2015b) did not affect erythrocyte osmotic fragility in male Wistar rats. Ferrali *et al.* (1997) also reported that quercetin reduced osmotic fragility of rat erythrocytes. A mixture of flavonoids decreased erythrocyte osmotic fragility in rats experimentally infected with *Trypanosoma brucei* (Kobo *et al*., 2014). Increased osmotic fragility due to membrane damage was reported in rat erythrocytes treated *in vivo* and *in vitro* with onions (*Allium cepa*) and garlic (*Allium sativa*) (Salami *et al*., 2012). Vanillin, a naturally occurring food-flavoring agent protected erythrocytes against carbon tetrachloride (CCl_4)-induced erythrocyte damage in Wistar albino rats by decreasing osmotic fragility, lipid peroxidation and degradation of membrane proteins (Makni *et al*., 2012). Erythrocytes of rabbits fed diets containing *Hibiscus sabdariffa,* a plant reported to have antioxidant effect improved osmotic stability when compared with erythrocytes from rabbits that were sham treated (Adenkola & Oluremi, 2014). Aqueous extract of *Alstonia congolensis* increased erythrocyte osmotic fragility in guinea pigs (Akinwande *et al*., 2007).

Sharma *et al.* (1981) reported increased erythrocyte osmotic fragility in calves poisoned by lantana. Erythrocyte osmotic fragility increased due to lipid peroxidation and oxidative stress in cattle intoxicated with *Senico sp* (Bondan *et al*., 2005). Gossypol, a yellow polyphenolic compound, from whole cottonseed or cotton seed meal had been reported to increase the osmotic fragility of lactating cows (Mena *et al*., 2004), growing heifers (Colin-Negrete *et al.,* 1996), cattle (Wyse *et al.,* 1991), sheep and goats (Menges 1991; Matondi *et al*., 2007). Herman (1969) reported that extracts of *Plasmodium lophurae* increased erythrocyte osmotic fragility in ducks.

Transportation stress

Erythrocyte osmotic fragility increased in pigs (Adenkola and Ayo, 2009) and goats (Minka & Ayo, 2010) but decreased in Nera black chicken (Azeez *et al*., 2011a) and humans (Olorunshola *et al*., 2012) after transportation stress. Olaifa *et al.* (2012) reported increased erythrocyte osmotic fragility in donkeys due to post packing stress.

Exercise

In horses, aerobic exercise increased the temperature and pH of blood, reduced the size of the erythrocytes due to changes in the lipid composition and protein structure of the cell membrane and then decreased the erythrocyte osmotic fragility (Hanzawa *et al.,* 1996). However, anaerobic exercise decreased the pH of blood, increased the size of the erythrocytes and increased erythrocyte osmotic fragility (Hanzawa *et al.,* 1996; Hanzawa, 2000). Hanzawa *et al.* (1999) observed that regardless of splenic contraction for the release of erythrocytes, osmotic fragility increased with heavy exercise in normal and splenectomized horses.

Increased erythrocyte osmotic fragility caused by high-intensity exercise was recorded in horses (Hanzawa *et al.,* 1998) and humans (Davis & Brewer, 1935; Anuradha *et al.,* 1995). Yusof *et al.* (2007) reported a decrease in erythrocyte osmotic fragility due to *in vivo* alterations in erythrocyte membrane proteins, especially in the α- and β-spectrins in six non-smoking experienced male ultra-marathon runners. Increased erythrocyte osmotic fragility was reported in humans after whole body vibration exercise (Monteiro *et al*., 2013). Erythrocyte osmotic fragility decreased in the rainbow lizard (*Agama agama*) after swimming exercise (Azeez & Oyewale, 2010).

Season

Habibu *et al.* (2016) reported that erythrocytes from Red Sokoto and Sahel goat kids were osmotically more fragile in the hot-dry than in the cold-dry season. Adenkola *et al.* (2011) reported that the osmotic fragilities of goat and cattle erythrocytes were higher during the cold harmattan than in hot dry season. However, osmotic fragility of erythrocytes from rats kept at hypothermic temperature decreased when compared with those from rats kept at room temperature (Peinado *et al.,* 1993). Increased erythrocyte osmotic fragility was seen in heat-exposed hamsters due to changes in membrane properties than those not exposed to heat (Kuiper *et al.,* 1971; Livne *et al.,* 1972).

Water deprivation

Increased erythrocyte osmotic fragility was reported in chickens (Yagil *et al.,* 1976) and Peking ducks (Baloyi *et al*., 2006) deprived of water for more than 24 hours. However, Mafudvaze *et al.* (2008) did not observe any change in erythrocyte osmotic fragility of guinea fowls deprived of water for up to 48 hours when compared with those of guinea fowls that were drinking water *ad libitum*. Erythrocytes of dehydrated camels were osmotically more stable in hypotonic saline solutions than those of hydrated camels (Yagil *et al.,* 1974).

Diseases

A two-fold increase in erythrocyte osmotic fragility was reported in cattle infected with *Anaplasma marginale* which correlated positively with intra-erythrocytic parasitaemia (Silva *et al.,* 1989). Increased erythrocyte osmotic fragility was also observed in cattle affected with trypanosomiasis and theileriosis (Pati *et al*., 2017). In camels, erythrocyte osmotic fragility increased due to direct attack of reactive oxygen species on the erythrocyte's plasma membrane (Saleh *et al*., 2009). Rats infected experimentally by *Trypanosoma evansi* (Mijares *et al*., 2010) and *Trypanosoma brucei* (Ikejiani, 1946; Oyewale, 1987; Kobo *et al*., 2014) had increased erythrocyte osmotic fragility. Lambs experimentally infected with *Dictyocaulus filariae* had increased erythrocyte osmotic fragility (Sharma *et al.,* 1989). Makinde & Bobade (1994) reported an increase in erythrocyte osmotic fragility in dogs infected with *Babesia canis* and *Erlichia canis*. Dogs diagnosed with leptospirosis had decreased erythrocyte osmotic fragility (Santoro *et al.,* 1994).

Erythrocytes of dogs affected with a heritable muscle disorder that clinically resembles a muscular dystrophy had decreased osmotic fragility (Abhold *et al.,* 1983). Erythrocytes from patients with Duchenne muscular dystrophy had increased osmotic fragility (Kim *et al.,* 1980). Cattle with traits of "double muscle" had increased erythrocyte osmotic fragility (Basarab *et al.,* 1980).

There was an increase in osmotic fragility as well as fluctuation in the lipid fraction of erythrocytes in elderly patients with anaemia (Tadano *et al.,* 1981). Human patients diagnosed with hereditary stomatocytosis and haemolytic anaemia had increased autohaemolysis and osmotic fragility (Mutoh *et al.,* 1983). Erythrocyte osmotic fragility decreased in dogs with immune-mediated anaemia (Paes *et al*., 2013) and humans with sickle cell anaemia (Harris *et al.,* 1956; Dash & Kar, 1999) but increased in cats with anaemia (Kohn *et al*., 2000; Tritschler *et al*., 2016). Normal erythrocyte osmotic fragility was reported in a girl diagnosed with hereditary spherocytosis (Korones & Pearson, 1988) and in dogs with malignant hyperthermia (Cribb *et al.,* 1986). Fansamade (1999) reported that osmotic fragility of erythrocytes was higher in hypertensive than in normotensive patients.

In alloxan-induced diabetic rats, erythrocyte osmotic fragility increased due to oxidative stress which was corrected after placing the rats on a diet containing antioxidants (Kowluru *et al.,* 1996). In humans, increased erythrocyte osmotic fragility was reported in diabetic patients (Agte *et al*., 2004; Ibanga *et al*., 2005; Kung *et al*., 2009; Mostafavi *et al*., 2013). Increased erythrocyte osmotic fragility was reported in rats (Endoh *et al.,* 1992) and rabbits (Yuan *et al.,* 1988) with burnt body surfaces. Erythrocyte osmotic fragility increased in hyperlipidemic and dyslipidemic dogs probably due to alteration of cholesterol content in the membrane (Behling-Kelly & Collins-Cronkright, 2014).

In cervical cancer patients, decreased erythrocyte membrane vitamin E concentrations led to reduced erythrocyte glutathione which in turn led to an increase in erythrocyte osmotic fragility (Kolanjiappan *et al*., 2002). The fragility of erythrocytes increased in rats inoculated with a variant of Walker 256 rat tumour cell (Cavalcanti *et al*., 2003) but the fragility decreased in anaemia caused by Walker 256 rat tumour cell (Vido *et al*., 2000). An increase in erythrocyte fragility was reported in buffaloes with dystocia (Prabhakar *et al*., 2013).

Chemicals

Wang *et al.* (2010) reported membrane damage, haemolysis, K^+ leakage, alterations in erythrocyte shape, increased erythrocyte osmotic fragility, and inhibition of enzymatic activity after treatment with the melamine-cyanurate complex in humans. Melamine or cyanuric acid alone had no effect on erythrocyte membrane. Potassium bromate ($KBrO_3$) reduced glutathione content, induced oxidative stress on erythrocyte, impaired the anti-oxidant defense system and increased erythrocyte osmotic fragility in humans (Ahmad & Mahmood, 2012; Ahmad *et al*., 2014). Ethanol created pores on the membrane of human erythrocytes, causing leakage of K^+ from the cell which led to haemolysis and increased osmotic fragility in a dose-dependent fashion (Chi & Wu, 1991; Tyulina *et al*., 2002).

Organophosphorus insecticides (methlybromphenvinphos, diclorvos, malathion and methyl parathion) increased the resistance of pig erythrocytes to lysis and, thus, decreased their osmotic fragility (Blasiak *et al.,* 1991). A significant increase was recorded in the erythrocyte osmotic fragility of Wistar rats after chronic exposure to chlorpyrifos when compared with control rats (Ambali *et al*., 2010). Swiss albino rats exposed to ethanol in drinking water had increased erythrocyte osmotic fragility due to increased cholesterol content and cholesterol to phospholipid ratio in erythrocyte membrane (Sözmen *et al.,* 1994). Exposure of adult male black Nera chickens to iodosteryl (a disinfectant) in drinking water for six weeks increased erythrocyte osmotic fragility (Azeez *et al*., 2012) due to intravascular heamolysis.

Drugs

A reduction in erythrocyte production and an increase in osmotic fragility was reported in rhesus monkeys treated with broad-spectrum antiviral ribavirin (1-beta-D-ribofuranosyl-1, 2, 4-triazole-3-carboxamide) (Canonico *et al.,* 1984). Pharmacological dosages of methylprednisolone produced a decrease in membrane deformability of erythrocytes in cardiac surgical patients leading to decreased fragility (Rand *et al.,* 1997). A gradual and significant decrease in osmotic fragility with an increase in concentration of saline solutions was observed in dogs under pentobarbitone anaesthesia (Olaifa *et al.,* 1999). Lidnocaine protected erythrocytes against oxidative stress by inhibiting potassium efflux and delaying the occurrence of haemolysis (Lenfant *et al*., 2000). Biltto (1990) reported a significant increase in osmotic fragility after *in-vitro* incubation of human erythrocytes for 30 to 60 minutes in 1, 2 and 4mg/mL of aspirin. Chlorpromazine, a tranquilizer has the ability to protect human erythrocytes from osmotic haemolysis by increasing the mean cellular volume and changing the shape of the cell in an isotonic medium (Freeman & Spirtes, 1963; Kwant & Van Steveninck, 1968). Low concentration of phenothiazine reduced osmotic fragility of erythrocytes in hypotonic sodium chloride solutions (Seeman *et al.,* 1969). Ebselen was reported to reduce erythrocyte osmotic fragility in humans by acting as an inhibitor of haemoglobin glycalation after glucose-induced haemolysis (Soares *et al*., 2014).

Detergents

Triton® X-100 (non ionic detergent) and sodium dodecyl sulfate (anionic detergent) increased erythrocyte osmotic fragility by the formation of pores in various membrane lipid regions, releasing vesicles from erythrocytes (Bielawski, 1990; Chernitsky & Senkovich, 1997) and time dependence of the opening probability of these pores (Chernitsky & Senkovich, 1998). Parameters of detergent-induced haemolysis are sensitive to the changes of the charge and structural state of erythrocyte membrane (Chernitsky *et al*., 2001). Triton® X-100 caused swelling and pores in the membrane followed by the haemolysis of erythrocytes while sodium dodecyl sulfate fragmented and destroyed erythrocytes. The rate of haemolysis increased with an increase in detergent concentration (Bielawski, 1990). Gaehtgens & Benner (1974) reported that three detergents (Pluronic® F 38, F 68, and F 108) exhibited anti-haemolytic effect and decreased osmotic fragility in human erythrocytes.

Metals

Elevated erythrocyte calcium caused a progressive increase in erythrocyte osmotic fragility (Cueff *et al*., 2010; Costa *et al*., 2010). Calcium levels of erythrocytes from goats were reduced by blocking calcium channels using diltiazem which decreased the level of oxidative damage (Das & Bhattacharyya, 2010). Erythrocyte deformability, membrane fluidity, and osmotic fragility improved significantly with nifedipine therapy in patients with systemic scleroderma. Impairment of ATP-Ca^{2+} pump occurred in the patients and there was the accumulation of Ca^{2+} in the erythrocytes which increased osmotic fragility (Spengler *et al*., 2007). Mostafavi *et al.* (2013) reported that intra-erythrocytic calcium reduced erythrocyte osmotic fragility in patients with type 2 diabetes.

Humans diagnosed with iron deficiency anaemia (Kirchgessner *et al.,* 1994) and iron deficient pregnant rats (Al-Hashimi *et al*., 2015) had decreased erythrocyte osmotic fragility.

Osmotic fragility of erythrocytes obtained from zinc-deficient rats was increased when compared with that from normal rats (O'Dell *et al.,* 1987; Kraus *et al.,* 1997). Roozbeh *et al.* (2009) reported a significant decrease in osmotic fragility of erythrocytes from humans on haemodialysis administered zinc supplements for 6 weeks.

Karuppasamy *et al.* (2005) reported an increase in erythrocyte fragility of *Channa punctatus* fish exposed to sub lethal dose of cadmium. However, the ingestion of cadmium in food and drinking water by Swiss male rats did not cause any significant change in erythrocyte osmotic fragility (Demir & Öner, 1995).

Erythrocytes from copper-deficient rats had increased membrane lipid peroxidation and lower osmotic fragility than erythrocytes from rats fed diets containing various concentrations of copper (Jain & Williams, 1988). Toxic concentrations of copper in the blood resulted in increased membrane permeability and erythrocyte osmotic fragility in rats (Kirchgessner *et al.,* 1994). Copper-dextran complex (C-79) in doses of 0.4-0.8mg/kg stabilized the erythrocyte membrane for several hours but lower doses produced a weak stabilizing effect while a dose of 1.6mg/kg increased erythrocyte osmotic fragility in rabbits (Debowy *et al.,* 1985). Hong-Wei *et al.* (2008) reported an increase in erythrocyte osmotic fragility, the rate of haemolysis and damage to erythrocytes when high concentrations of copper sulphate were added to media in which erythrocytes were incubated.

Chronic exposure of people working in lead refining factories to lead (Pb) caused alterations in membrane proteins which decreased the permeability of erythrocyte membrane and erythrocyte osmotic fragility (Karai *et al*., 1981, 1982a, Fukumoto *et al*., 1983). *In vitro* treatment of erythrocytes of rats (Levander *et al.,* 1977) and humans (Qazi *et al.,* 1972; Lessler & Walters, 1973; Mrugesh *et al*., 2011) with Pb decreased osmotic fragility. Decreased mean corpuscular volume (MCV) and erythrocyte osmotic fragility were reported in lead poisoning squeal to intracellular water leakage accompanied with K^+ loss due to erythrocyte membrane damage caused by the increased cholesterol level recorded in the erythrocyte membrane (Karai *et al.,* 1979; Karai *et al.,* Karao *et al*., 1982a; Karai *et al.,* 1982b). However, Oyedeji & Alabi (2015) reported no change in erythrocyte osmotic fragility of male Wistar rats after oral administration of lead acetate for five weeks.

Mercury increased erythrocyte osmotic fragility in humans (Lessler & Walters, 1973). Okuda & Tsuzuki (1977) reported decreased erythrocyte osmotic fragility in low doses of methylmercury and no change in osmotic fragility in higher doses in male Wistar rats. Erythrocyte osmotic fragility decreased in female mice fed methyl mercury (10nmoles/g feed) (Yamamoto & Suzuki, 1982). Erythrocytes treated with mercuric ions showed resistance to osmotic shock after 5 minutes of incubation but they began to haemolyse when the incubation time was increased (Zolla *et al*., 1994). *In vitro* treatment of human erythrocytes with mercuric chloride resulted in shrinking of the erythrocytes and conferred protection against osmotic haemolysis (Mel & Reed, 1981). Igbokwe *et al.* (2018) reported an increase in osmotic stability of Sahel goat erythrocytes incubated in saline, glucose or sucrose media after *in vitro* treatment with mercuric chloride due to inhibition of osmotic permeability by blockage of aquaporins or by steric mechanism.

Various concentrations of aqueous solutions of tris acetylacetonate aluminium (III) (Al $(acac)_3$, tris maltolate aluminium (III) $(Al(malt)_3$ and tris aluminium lactate $(Al(lac)_3)$ caused erythrocytes to be osmotically fragile (Zatta *et al.,* 1989). Treatment of rat erythrocytes with aluminium chloride caused increased osmotic fragility due to increased lipid peroxidation (Gutteridge *et al.,* 1995; Hernández *et al*., 2008; Al-Qayim *et al*., 2014; Oztürk & Ozdemir, 2015; Zhang *et al*., 2016). However, Bazzoni *et al.* (2005) reported improved stability of erythrocyte membrane and reduced erythrocyte size and aggregation in adult male rats after parenteral treatment with aluminium hydroxide.

In conclusion, many intrinsic and extrinsic factors as seen in this review can affect erythrocyte osmotic fragility or stability. A good understanding of these factors will guide in blood sample collection, preservation and analysis such that inferences can be drawn from erythrocyte osmotic fragility tests with little or no interference from the factors.

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