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Erythrocyte oxidative stress in clinical management of diabetes and its cardiovascular complication: A review

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Key words: oxidative stress, erythrocyte, prediabetes, diabetes mellitus, cardiovascular disease
Abstract

Diabetes mellitus is a chronic disease in its own capacity, but it is also regarded as both a cardiovascular risk factor as well as a cardiovascular disease due to its propensity to progress to a stage of cardiovascular co-morbidity. The pathophysiology of cardiovascular complication in diabetes has been consistently reported to involve hyperglycaemia-induced oxidative stress. The erythrocyte has an array of endogenous antioxidants involved in quenching both oxidant production and the
exponential chain reactions in diabetes. When the erythrocyte is oxidatively stressed, evidenced by depleted level of reduced glutathione and/or increased malondialdehyde in the erythrocyte membrane, the risk of diabetes progression and its cardiovascular sequelae including atherosclerosis and coronary artery disease become exacerbated. Virtually all studies that determined erythrocyte malondialdehyde and glutathione in diabetes have shown consistent increased and reduced levels respectively. Furthermore, cardiovascular complication of diabetes has been reported to commence at the prediabetes stage. Current coronary artery disease screening programs based on the presence of two or more risk factors being present are failing to identify optimal numbers with increased risk of diabetes and cardiovascular complications, thereby limiting early interventions. Screening that includes erythrocyte oxidative stress determination may provide an additional marker for both preclinical and advanced disease. In this review, a concise description of erythrocyte oxidative stress involvement in diabetes mellitus and its cardiovascular sequelae is presented. Antioxidant action and interaction within the erythrocyte is also described with emphasis on why current coronary artery disease screening markers do not extrapolate to the erythrocyte oxidative stress markers.

1. Oxidative stress

Oxidative stress (OS) is a disturbance in the ‘oxidant/antioxidant’ balance, in favour of oxidants. It is a state in which a cell is experiencing alteration of cellular components, due to its being under high exposure to free radicals and ROS beyond its antioxidant capacity. Though, the reaction of ROS are essential for cellular functions such as the utilization of the chemical energy of nutrients for the production of adenosine triphosphate (ATP), excess oxidant exposes the cells involved to OS.
1.1 Erythrocyte oxidative stress: Erythrocyte oxidant stress (EOS) is a type of cellular oxidative stress involving functional impairment of the red blood cells, which arises from over exposure of the cellular components to various ROS. The erythrocyte’s functional mechanisms are overwhelmed by alteration in the normal metabolic and/or physiological activities that generate ROS due to the oxidant challenge exceeding the red cells’ antioxidant producing capacity.

There are three possible sources of ROS. First, the erythrocyte can become oxidatively stressed from normal physiological processes. Due to the role of erythrocytes in oxygen transport and the presence of redox-active haemoglobin molecules, they generate relatively high levels of ROS with its attendant deleterious effects of oxidative stress. Furthermore, there do occur incomplete reduction of oxygen in the mitochondrial electron transport system resulting in \( \text{O}_2^- \) generation. Though there are no mitochondria in erythrocytes, there is risk of the \( \text{O}_2^- \) entering the erythrocyte membrane through superoxide channels, especially during hyperglycaemia and/or dyslipidaemia.

Second, there is hyperglycaemia-induced generation of ROS, which causes oxidative damage of the erythrocyte. The erythrocyte functions in utilizing glucose for the generation of ATP via glycolysis and the pentose phosphate pathway (PPP). The increased rate of glycolysis, to meet the cellular need of ATP, as in DM is associated with increased generation of free radicals, which depletes reduced glutathione (GSH) content. The PPP produces reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is a potent electron acceptor during GSH regeneration, but overproduction of the NADPH could drive the production of \( \text{O}_2^- \) radicals thereby exacerbating oxidative stress.

Thirdly, in the process of methaemoglobin (metHb) reduction to haemoglobin, the GSH level is depleted as it converts to the oxidized glutathione (GSSG), thereby leading to reduced
erythrocyte antioxidant capacity and consequently, oxidative stress. Methaemoglobin reductase is also involved in the reduction of Fe$^{3+}$, but this pathway uses up reduced nicotinamide adenine dinucleotide (NADH), which is a requirement for GSH reductase (GR) activity for the regeneration of GSH from GSSG. Thus, either pathway of metHb reduction results in a decrease of erythrocyte GSH level, which translates to antioxidant imbalance of the cell.

1.2 Erythrocyte oxidant stress and CVD: Accumulating research reports have consistently demonstrated changes in erythrocyte antioxidant and haem components in diabetes complications such as CVD. The generation of ROS in the erythrocytes coupled with depletion of its defensive natural antioxidants enhance activation of the nuclear redox-sensitive transcription factor. This results in up-regulation of events at the gene level such as pro-coagulant tissue factors and pro-inflammatory mediators that lead to endothelial dysfunction and CVD. Hence, EOS may affect certain cardiovascular events (fig 1) including hypercoagulation and endothelial dysfunction.

EOS decreases erythrocyte membrane deformability, which is one of the factors influencing blood flow/shear rate that can lead to thrombotic events. Nevertheless, while there is consensus that atherosclerosis represents increased oxidative stress contributing to important clinical manifestations of coronary artery disease, it has yet to be proven that oxidative modification causes atherosclerosis and CAD.
1.2.1 Blood viscosity: This is an intrinsic resistance of blood to flow in the vascular system. During normal blood flow, erythrocyte deformability property (physiologic changing of shape per se) enables the cells to adapt physically and squeeze past an otherwise rough vascular wall or viscous space.26 27 When EOS occurs through lipid peroxidation of the low density lipoprotein (LDL) content, the cell membrane becomes rigid, less deformable and less adaptable.28 This makes the whole blood more viscous and causes development of the vascular abnormalities including increased red cell aggregation as in atherosclerosis and CAD.23 The associated blood flow abnormalities can be detected primarily at low shear rate as increased viscosity. However, there is school-of-thought that increase in blood viscosity in individuals affected by ischaemia is due to the latter decreasing erythrocyte deformability.29 Thus, whether decreased deformability leads to ischaemia and/or vise versa has yet to be addressed. Moreover, some studies on viscosity were based on plasma,30 31 while some were based on whole blood that includes the erythrocyte factor.21 32 Therefore, the clinical utility of blood viscosity and choice of specimen has yet to be unanimously viewed with regard to the associated EOS.

1.2.2 Endothelial dysfunction: Of emerging interest is hyperglycaemia-induced endothelial dysfunction.33 34 The oxidation of LDL as in diabetic dyslipidaemia, initiates a chronic inflammatory reaction that results in endothelial damage, which in turn culminates in atherosclerosis and CAD.35 There is speculation that oxidant/antioxidant imbalance results in endothelial dysfunction,36 which would be marked by increased plasma homocysteine levels and other biomarkers.22 However, homocysteine is known to competitively inhibit GSH synthesis at the cysteine-dependent de novo synthesis pathway,9 22 37 and/or enhance generation of O₂⁻ and H₂O₂ radicals,38 which results in OS. Therefore, how endothelial damage and OS interact with each other remains unclear.

1.2.3 Hypercoagulation: There are quite a number of studies that reported an association between D-dimer and cardiovascular disease as well as conflicting findings with respect to hypercoagulation in diabetes mellitus.39 For instance, Sommeijer et al. 2004 have reported40 that some coagulation
markers are elevated in DM. A preliminary report from our laboratory has also shown increased D-dimer levels in DM. However, Yano et al. 2003 suggested that in type 2 diabetes, there is hypo-fibrinolysis as thrombomodulin-thrombin complex, which is formed on intact vascular endothelium, may activate thrombin-activatable fibrinolysis inhibitor (TAFI). Indeed, this suggestion is supported by the observation that hyperglycaemia and insulin enhance the synthesis and secretion of PAI-1. These proposals surely mean that fibrinolysis and therefore the generation of D-dimer would be reduced in DM. Therefore, considering that the generation of EOS lead to up-regulation of events at the gene level such as pro-coagulant tissue factors, it implies that D-dimer changes in diabetes have not been adequately explained.

1.2.4 Haemolytic anaemia: Deficiencies of GSH peroxidase (Gpx-1), GSH reductase (GR) and GSH synthetase enzymes, which respectively utilize, regenerate and synthesize GSH, have long been known as rare hereditary erythrocyte defects that cause non-spherocytic haemolytic anaemia. Thus, the involvement of the erythrocyte in cardiovascular complication of DM can also be viewed in association with haemolytic anaemia and/or reduced oxygen supply associated with changes in red blood cell morphology. That is, hyperglycaemia resulting in reduced GSH via the polyl pathway, coupled with the inadequacy of the GR/GST to regenerate/synthesize GSH, exacerbates haemolysis. The effect is a sequence of anaemia, reduced blood/nutrient/O\textsubscript{2} supply, ischaemia and subsequent angina, chronic ischaemic heart disease, myocardial infarct or sudden death.

1.3 Oxidative stress is a target in the management of diabetes and its cardiovascular complications: It is known that development or progression of diabetes can be delayed, and that the key to successful prevention and/or treatment is early diagnosis.

1.3.1 Diagnosis: The diagnosis of DM is based on (i) symptoms including polyuria, polydipsia and unexplained weight loss, plus random blood glucose level \(\geq 11.0 \text{ mmol/L} \); (ii) fasting blood glucose level \(\geq 7.0 \text{ mmol/L} \); or (iii) OGTT \(\geq 11.0 \text{ mmol/L} \) at two hour post glucose load of 75mg at fasting.
For the diagnosis of cardiovascular complication of DM, there are difficulties especially in the asymptomatic or early stages due to either absence or non-specificity of the symptoms. Diagnostic laboratory tests include the traditional risk evaluation markers cholesterol profile and glucose, Emerging markers for CAD in diabetes are ceruloplasmin, C-reactive protein (CRP), D-dimer, factor VIII, fibrinogen and homocysteine. CRP is a marker of low-grade inflammation that may capture the inflammatory aspect of atherosclerosis, particularly because levels of CRP increase dramatically during acute inflammatory episodes. However, based on the very low incidence of high CRP levels in the absence of borderline or abnormal levels of established risk factors, the extent of CRP involvement in CVD events is currently controversial.

Although a moderately elevated plasma level of homocysteine is associated with increased risk for CVD, association of homocysteine with CVD complications in DM requires further studies especially in type 2 DM. In type 1 DM, higher plasma homocysteine levels have been reported compared with non-diabetes control, while lower and similar levels compared with controls have also been reported.

Several studies have been carried out on erythrocyte GSH and malondialdehyde (MDA) status in diabetes as oxidative stress indices and reports have been consistent. For instance, Memisogullari et al. 2003, studied the levels of serum antioxidant proteins and erythrocyte antioxidant and oxidant biomarkers in patients with type II diabetes, with and without complications. They found amongst others, that the levels of erythrocyte MDA was significantly increased and positively associated with glucose levels in diabetes groups; whereas erythrocyte GSH was significantly decreased. Dominguez et al. (1998), determined the presence of oxidative stress at onset and early type I diabetes. Their results showed, that MDA was increased while erythrocyte GSH levels decreased at onset of type I diabetes compared with controls. In another study, lipid peroxidation in erythrocyte cell membrane
was determined in prediabetes with erythrocyte GSH and MDA as indices. The results indicated significant reduction in GSH and elevation of MDA.

Our preliminary results also demonstrated that in both diabetes and prediabetes, erythrocyte MDA is higher, while erythrocyte GSH is lower compared to control. In summary, there is evidence of observable changes in EOS indices in DM and its cardiovascular sequelae. However, there is still controversy about which biomarker is most reliable for clinical diagnostic use. It may be more appropriate to use a panel of tests that include antioxidant and OS markers.

1.3.2 Management: Most management measures are devoted to modification of risk factors such as diet, obesity, physical inactivity, and smoking. Among the medication options, the preservation of normal oxidant-antioxidant balance is considered important for preventing CVD progression. Hence, there are (i) drugs such as anti-inflammatory and hypoglycaemic medication as well as statins that are not used primarily as antioxidants, but there is growing interest in their antioxidant activities; and (ii) drugs such as vitamins A, C and E as well as coenzyme-Q supplements that are primarily used as antioxidants.

2. Antioxidants

Antioxidants are substances that prevent oxidation of cell components by donating an electron to the free radicals that initiate or take part in oxidative reactions. Oxidant stability is achieved by the removal of electrons from (i.e. oxidation of) surrounding molecules to produce an electron pair. However, the surrounding molecule that lost the electron then possesses an unpaired electron and has itself become another free radical (oxidant). If the reactivity is high, further target molecules are attacked. Thus, a single radical may initiate a sequence of electron transfer (redox) reactions. Where the antioxidant becomes the target, the resultant radical will possess a low reactivity and the chain reaction is broken.
2.1 Types of antioxidants: Based on the source, there are three types of antioxidants. First, the endogenous antioxidants constitute a group that is naturally found in cells. In the erythrocytes, this group includes catalase, GSH, glutathione peroxidase (GPx-1), metHb reductase, NADPH, and superoxide dismutase (SOD). Catalase, GPx-1 and SOD are antioxidant enzymes central in erythrocyte antioxidation function.\(^7\)

Second, the nutritional group of antioxidants, obtained from the diet as micronutrients include vitamin C (ascorbic acid) and vitamin E (tocopherol).\(^6\) Others are micronutrients such as [beta]-carotene, cysteine, flavonoids, polyphenols, quercetin; as well as trace metals zinc, selenium, manganese and copper.\(^7\) Sources of such molecules are citrus fruits, strawberries, papaya, red pepper, and broccoli for vitamin C; vegetable oils, whole grain cereals and eggs for vitamin E; carrots, potatoes, pumpkin, spinach, apricots, broccoli and green vegetables for carotenoids; apples and tea for flavonoids.\(^72\)

Third, the antioxidant supplements. This constitutes pharmaceutical products including assorted brands of over-the-counter coenzyme Q, selenium, vitamins and additive to foods and creams.\(^73\)

2.2 Mode of antioxidant action: Maxwell and Lip (1997)\(^7\) as well as Young and Woodside (2003)\(^74\) classified antioxidants into three groups based on their mode of action (Table 1).

3.2.1 Mode of action of antioxidant enzymes: SOD first catalyses the conversion of $O_2^-$ to $H_2O_2$ (EQ 1). The $H_2O_2$ is then reduced to water by either catalase or GPx-1 (EQ 2-3).\(^7\)\(^74\)

\[
2O_2^- + 2H^+ \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2 \tag{EQ 1}
\]

\[
2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \tag{EQ 2}
\]
When the above enzymes fail to neutralize $O_2^-$ and $H_2O_2$ adequately, Fenton reaction and membrane lipid peroxidation occurs resulting in formation of $OH^-$ and lipid peroxyl radicals.$^{12,75}$ Specific to the erythrocytes, Fenton reaction results in methaemoglobinaemia.$^{12,76}$ The limitation to GPx-1 activity is the propensity of GSH regeneration (EQ 4) by GR to maintain a normal GSH/GSSH ratio. This reaction is an important antioxidation mechanism, in preventing oxidative stress that arises from the depleting GSH [EQ 3].$^8$

$^{2GSH + H_2O_2 \overset{GpX-1}{\rightarrow} GSSG + 2H_2O}$  

EQ 3

2.2.2 Mode of action of chain-breaking antioxidants: The principle$^{74}$ of chain-breaking antioxidation is that when an oxidant reacts with a molecule, a new radical is formed. The new radical further reacts with another molecule to produce yet another radical, and this continues exponentially until the radical reacts with a molecule that forms a stable product.$^1$

Beside GSH acting as substrate in antioxidant enzyme catalysis, there are three other pathways by which it acts as a chain-breaking antioxidant. One pathway is the reduction of metHb to haemoglobin (Fig 2 ¶$^3$). This is important in prevention of the Fenton reaction (Fig 2 ¶$^7$).$^{12}$ The second pathway involves the lipid peroxidation process. Upon erythrocyte membrane lipid peroxidation, vitamin E ($\alpha$-tocopherol) performs antioxidation by donating one electron (via an H radical) to the resultant lipid peroxyl radical to form lipid peroxide and tocopheroxyl radicals (EQ 5). The lipid peroxide is further acted upon by GSH to give a stable lipid-OH (EQ 6).$^{74}$

$Lipid-O_2^- + Tocopherol-OH \overset{\text{Glutathione reductase}}{\rightarrow} Lipid-O_2H + Tocopherol-O^-$

EQ 5
The regeneration of tocopherol vis-a-vis ‘vitamin E regeneration system’ (VERS) by coenzyme-Q, reduced glutathione and/or vitamin C, from its oxidant form (tocopheroxyl radical) is quite complex. Nevertheless, in the absence of any of the VERS components, tocopheroxyl exhibits a pro-oxidant property in a process called tocopherol-mediated peroxidation. Thus, in the absence of adequate dietary micronutrient vitamins C and CoQ-H₂, GSH becomes important in preventing vitamin E from exhibiting its pro-oxidant effect.

The antioxidation function of vitamin C is the regeneration of vitamin E [EQ 8]. It occurs at the aqueous phase and involves two single-electron reductions, initially to yield semi-dehydro-ascorbyl radical and later dehydroascorbate. The dehydroascorbate is converted back to ascorbic acid by GSH in the reaction (Eq 10).
Ubiquinol (CoQ-H₂) has direct and indirect antioxidation pathways. Indirectly, it acts by regenerating α-tocopherol [EQ 9]. Directly, it could break the lipid peroxidation chain by donation of electrons and in the process becomes ubiquinone (Eq 11).

The ubiquinone (CoQ) that formed in [EQ 9] and [EQ 10] is converted back to ubiquinol in the respiratory chain. Paradoxically, this ubiquinol/ubiquinone metabolism in the respiratory phase is associated with superoxide production, thereby making ubiquinol both an antioxidant and a pro-oxidant.

2.2.3 Mode of action of transition metal-binding protein antioxidants: The transition metal-binding proteins act by preventing copper and iron from binding with the hydroxyl radicals. Albumin and ceruloplasmin binds to copper while ferritin, lactoferrin and transferritin bind to iron, thereby stabilizing them. One important property that has been hypothesized is that the presence of some antioxidants might paradoxically lead to increased oxidative stress, particularly if copper and iron are present.

2.2.4 Antioxidant interactions and supplement: Overall, there exist complex interactions between antioxidants (fig 2). The nature of oxidant injury or the free radical responsible for the OS determines a prevailing antioxidant that could break the exponential chain reaction. In diabetes, the initial oxidant injury on erythrocyte is O²⁻ followed by its product of dismutation, H₂O₂. The prevailing antioxidant is GSH and its associated enzymes. Fig 2 illustrates some of the main antioxidant interactions that occur in the erythrocyte. The summary is that there are at least five reaction processes (Fig 2: ¶¹ – ¶⁵) that influence GSH concentration in the erythrocyte. This involves
four metabolites including homocysteine and dismuted ROS, which are oxidants directly affecting erythrocyte GSH status. Others are NADPH and methaemoglobin. Virtually all antioxidants and oxidants are involved in competitive, simultaneous interactions. Therefore the importance of a particular antioxidant depends on the micro and macro environment at a specific time, and on the nature of the oxidant injury taking place.

Insert figure 2 here.

For instance, a review of changes in oxidative stress biomarkers in diabetes, their consequences and effect of conventional and alternative drugs, showed that effective antioxidant activity depends on the type of oxidant and diabetic complication. In another study, whether high glucose level leads to disruption in glutathione-dependent antioxidant defences and capacity to handle oxidative stress was tested. The results indicated that glucose/hyperglycaemia toxicity reduces erythrocyte GSH and that at a certain low level of GSH, the maximum GR activity to regenerate GSH is insufficient to restore normalcy. The implication was that GSH is a factor in the occurrence of diabetes complications. Thus, low level of erythrocyte GSH in diabetes requires appropriate supplementation with a GSH precursor such as cysteine to improve erythrocyte GSH synthesis and maintenance. The next sections integrate oxidative stress and antioxidant activity into diabetes and cardiovascular disease progression.

3 DM and CVD complications: Overview of oxidative stress link

Diabetes mellitus (DM), which includes insulin dependent (type I), non-insulin dependent (type II) and gestational diabetes, is a common chronic disease associated with devastating complications including cardiovascular disease (CVD).

3.1 DM as causal to CVD and the prediabetes factor: Hyperglycaemia has been proposed to be the basis of the increased occurrence of CVD complications such as atherosclerosis and coronary artery
disease (CAD) in DM, as found in the Diabetes Control and Complications Trial and reported by others.\(^8\)\(^2\)\(^3\) Thus as DM is increasing in the population, so are its cardiovascular complications, which are the leading cause of morbidity and mortality in persons with DM.\(^5\)\(^8\)\(^1\) Hence DM is a factor associated with the early phase of CVD and diabetes patients need to be screened regularly for early identification of CVD including CAD.\(^5\)\(^1\)

There are individuals that do not have established diagnosis of DM, yet show higher than normal blood glucose levels.\(^8\)\(^4\) These individuals have diagnosed or undiagnosed prediabetes, which is either impaired fasting blood glucose level between 5.6-6.9 mmol/L,\(^8\)\(^5\) or impaired glucose tolerance blood glucose level between 7.0-10.9mmol/L at two hour postprandial following a standard glucose load.\(^8\)\(^6\)

It is now well established that persons with prediabetes are at great risk of developing CVD,\(^8\)\(^7\)\(^8\)\(^8\)\(^9\)\(^0\) in addition to developing diabetes. Hence improving early detection of prediabetes could reduce its human and economic cost.\(^9\)\(^1\)\(^9\)\(^2\)

The criteria of using two or more risk factors (RF) is not helping to identify asymptomatic patients with a higher prevalence of CAD, though the criteria are good enough for a more severe CAD.\(^9\)\(^3\) Therefore, an aggressive diagnostic approach among people with preclinical DM associated with one or less RF for CAD is imperative. Thus, the monitoring of oxidative stress status in prediabetes may become an important adjunct strategy.\(^6\)\(^1\)

3.2 Mechanisms of cardiovascular complication of diabetes: The mechanisms for the propensity to develop CVD among persons with diabetes and prediabetes are varied and includes hyperglycaemia.\(^9\)\(^4\) The identification of risk factors associated with preclinical hyperglycaemia is important in reducing mortality and morbidity associated with diabetes complication.\(^9\)\(^5\) Hyperglycaemia can mediate its adverse effects through multiple pathways including polyol, hexosamine, Protein kinase C, and glycation pathways.\(^3\)\(^4\) The unifying occurrence in these pathways is the overproduction of the oxidant, superoxide ion \((O_2^-)\) that increases susceptibility to intracellular oxidative stress.\(^5\)\(^8\) Oxidative stress is enhanced by
diabetic dyslipidaemia characterized by increased oxidation of membrane lipids, which exacerbates atherosclerosis. Hence it is recommended to screen persons with established diabetes annually and treat them for hyperlipidaemia, if LDL-cholesterol level exceeds 3.38 mmol/L. Metabolic syndrome is another factor. It is related to insulin resistance and characterized by albuminuria, dyslipidaemia, inflammation, hyper-coagulability and hypertension as well as obesity. It has been viewed as a distinct disease condition, but it is now appraised as a state of co-existing cardiovascular risk factors. Nevertheless, metabolic syndrome includes pathophysiological factors associated with oxidative stress and requires early identification in order to reduce the disease burden.

The development of atherosclerosis and CAD involves blood clot formation. Glucose as well as insulin have the capacity to enhance the synthesis and secretion of plasminogen-activator inhibitor type 1 (PAI-1), which promotes the stability and extension of clot formation. Thus, diabetes enhances the development of deep vein thrombosis. Furthermore, insulin resistance causes increased free fatty acid (FFA) entrance from adipocytes into arterial endothelial cells leading to increased FFA oxidation and overproduction of reactive oxygen species (ROS) including O$_2^-$, hydrogen peroxide (H$_2$O$_2$) and hydroxyl (OH$^-$) radicals. Thus, there is evidence that oxidative stress is a unifying mechanism in the development of cardiovascular complications associated with diabetes.

**Conclusion**

Current health care and preventative medicine does not identify a large proportion of people with prediabetes, yet they have a significant risk of cardiovascular disease that requires early intervention. To improve on identification of people with prediabetes and risk of cardiovascular disease, this review emphasized that oxidative stress and/or antioxidant deficiencies are involved in the onset of diabetes and its cardiovascular sequelae. As
erythrocyte levels of MDA and GSH are determinants of oxidative stress & antioxidant status in diabetes, antioxidants can be an effective therapy against oxidative stress. Biomarkers of oxidative stress status and related cardiovascular events are not used as a tool in routine clinical practice as antioxidant interactions in the erythrocyte have not been given proper consideration in the interpretation and use of oxidative stress evaluations in research. This review draws attention to the suggestion that a panel of tests will be important in order to determine whether oxidant activities are high and/or what antioxidant is actually deficient enough to require supplementation.\(^{(90)}\)

It is therefore important to determine how (i) the levels of antioxidant and oxidative stress biomarkers plus the related cardiovascular events differ between normal and pathological conditions as well as with different stages in the progression of diabetes including prediabetes; and (ii) how the identifiable changes in biomarkers relate as a panel of tests, in non-diabetes and diabetes. The findings could be useful for screening and management of OS in prediabetes, which will help in early identification and/or intervention, improve patient care and reduce morbidity and mortality.

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Figures
Diabetes mellitus

- Hyperglycaemia toxicity
  - Polyol pathway
  - Hexosamine pathway
  - Protein kinase c pathway
  - Glycation

\[ \Downarrow \text{Erythrocyte GSH} \]

Erythrocyte oxidant stress
- \[ \uparrow \text{H}_2\text{O}_2 \text{ concentration} \]
- \[ \uparrow \text{Fenton reaction} \]
- \[ \uparrow \text{metHb concentration} \]

Erythrocyte membrane (EM)
- \[ \uparrow \text{Lipid peroxidation} \]

\[ \downarrow \text{EM deformability} \]
\[ \uparrow \text{Cell aggregation} \]

\[ \downarrow \text{blood flow} \]
\[ \uparrow \text{Shear stress} \]

*Malondialdehyde

\[ \downarrow \text{Erythrocyte haemolysis} \]

Hereditary erythrocyte defects
- Glutathione peroxidase (Gpx-1)
- Glutathione reductase (GR)
- Glutathione synthetase (GST)

\[ \downarrow \text{Gpx-1: GSH not utilizable} \]
\[ \downarrow \text{GR: GSH not regenerated from GSSG} \]
\[ \downarrow \text{GST: GSH not synthesized} \]

Defective GSH antioxidant activity

\[ \downarrow \text{Reduced O}_2 \text{ supply} \]

Up-regulation of pro-coagulant tissue factors

\[ \uparrow \text{Tissue hypoxia} \]

Hypercoagulation

Endothelial dysfunction

\[ \uparrow \text{Thrombosis} \]

Atherosclerosis

\[ \downarrow \text{Coronary artery disease (CAD)} \]

Angina
Chronic ischaemic heart disease
Myocardial infarct
Sudden death

Key: * = identifiable biomarkers
Fig 1: Illustration of links between EOS and related cardiovascular biomarkers/events

![Diagram of links between EOS and related cardiovascular biomarkers/events](image)

**Keys:**  
¶1 = Cysteine dependent de novo metabolism; ¶2 = glutathione peroxidase catalysed reaction; ¶3 = metHb reduction by GSH; ¶4 = glutathione reductase catalysed reaction; ¶5 = metHb reductase catalysed reaction; ¶6 = dismutation by SOD; ¶7 = Fenton reaction; * = identifiable biomarkers of interest

Fig 2: Schematic illustration of glutathione interactions with other antioxidants in the erythrocyte

![Diagram of glutathione interactions with other antioxidants in the erythrocyte](image)

**Tables**

Table 1: Classes of antioxidant based on mode of action

<table>
<thead>
<tr>
<th>Antioxidant enzymes</th>
<th>Chain breakers</th>
<th>Metal-binding proteins</th>
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<tbody>
<tr>
<td></td>
<td>Aqueous phase chain breakers</td>
<td>Lipid phase chain breakers</td>
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<tr>
<td>Catalase</td>
<td>GSH/GSSG</td>
<td>Carotenoids</td>
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**(20/41)** (116)
<table>
<thead>
<tr>
<th>GPx-1</th>
<th>Vitamin C</th>
<th>Flavonoids</th>
<th>Ceruloplasmin</th>
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<tr>
<td>GR</td>
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<td>Ubiquinol-10</td>
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<td>SOD</td>
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<td></td>
<td></td>
<td></td>
<td>Transferrin</td>
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Key: Gpx-1 = glutathione peroxidase; GR = glutathione reductase; SOD = superoxide dismutase