

# The interrelation of glutathione reductase, catalase, glutathione peroxidase, superoxide dismutase, and glucose-6-phosphate in the pathogenesis of rheumatoid arthritis

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**Abstract** Rheumatoid arthritis (RA) is the most common form of inflammatory arthritis, a systemic autoimmune disease characterized by chronic inflammation of the synovial joints, ultimately leading to joint destruction and permanent disability, affecting 1% of the world population. Oxidative stress in rheumatoid inflammation, due to the fact that antioxidant systems are impaired in RA and caused by free radicals, might have an essential role in etiology of RA. This review includes the interrelation of antioxidants against free radicals in RA patients. There is much evidence that antioxidant team that covers glutathione reductase, catalase, glutathione peroxidase, superoxide dismutase, and glucose-6-phosphate destroy reactive oxygen species and other free radicals through enzymatic as well as nonenzymatic means. The change in relative levels of antioxidants vis-à-vis free radical formation and level could be used as indicators for effective and earlier diagnosis of RA.

**Keywords** Free radicals · Glucose-6-phosphate · Glutathione peroxidase · Glutathione reductase · Rheumatoid arthritis · Superoxide dismutase

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the synovial joints, ultimately leading to joint destruction and permanent disability [1]. It is the most common form of inflammatory

arthritis and has a substantial social effect in terms of cost, disability, and lost productivity. The course of RA varies ranging from a mild to an aggressive form. The long-term outcome of this disease is characterized by significant morbidity, loss of functional capacity, and increased mortality. It is one of the most prevalent autoimmune diseases, affecting about 1% of the adult population in Western countries [2]. Early diagnosis and treatment reduce joint destruction, preserve function, and improve survival [3].

RA is a debilitating autoimmune disease where diagnosis is based on clinical and radiological features and the presence of rheumatoid factor in the serum. Although the pathogenesis of RA remains incompletely understood, much insight into the cellular and molecular mechanisms involved has been gained in the past decade. Its precise etiology remains to be elucidated, although both environmental and genetic components are believed to influence the development of disease. Of the former, cigarette smoking appears to be of importance in both disease susceptibility and severity [1].

Some investigators have focused on oxidative stress in rheumatoid inflammation. Free radicals might have an essential role when possible alterations in the matrix, and the enzymes that degrade oxygen consumption are taken into consideration [4]. A free radical is any atom, molecule, or ion that contains one or more unpaired electrons that might be formed during a number of physiologic or pathologic reactions. Since pairing of electrons in orbitals depict a lower energy state than the same electrons unpaired, free radicals are usually more reactive than their parent species [4]. These unmapped electrons can initiate destructive reactions against proteins or lipid and nucleic acids in the body, reactivating the free radical molecules [5, 6], which increases the essence of these radicals in physiopathology of many diseases including RA

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[7, 8]. In healthy cells and tissues, any free radicals generated are likely to encounter and react with nonradicals since most cellular constituents are nonradicals, and the chances of most radicals meeting are low. Nevertheless, chemical reactivity of radicals varies enormously. Free radicals and byproducts of free radicals are among essential mediators of inflammation-related diseases [9]. The effects of free radicals on RA have been evaluated since 1970s [8, 10, 11].

Free radicals from oxygen metabolism destroy antioxidant systems [12]. Researchers such as Heliövaara et al. [13] have suggested that enzymatic and/or nonenzymatic antioxidant systems are impaired in RA. RA patients are therefore exposed to oxidant stress [12]. Consequently, a number of different activities of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase, and glutathione reductase (GR) have been reported to be effective in treating RA [14–16]. Some other researchers found that RA patients were more inclined to lipid peroxidation because of the reduced antioxidant defense system [17, 18].

The synovial fluids taken from patients with RA were found to be exposed to high levels of antioxidant damages [19, 20]. According to Babior [21], reactive oxidants are essential tools for the pathogenesis of RA. Through evolution, mammalian cells have developed antioxidant defense systems for survival in an aerobic environment [22]. Cells carry varying antioxidant systems, including low-molecular-weight antioxidant molecules such as glutathione [23] and other antioxidant enzymes to protect themselves against free radicals, most frequently observed of which are defined in Table 1. SOD, which is an essential zinc and copper-containing metalloenzyme, is a primary defense element against oxygen-derived free radicals, which catalyzes the dismutation of the superoxide anion ( $O_2^{\cdot-}$ ) into hydrogen peroxide ( $H_2O_2$ ).  $H_2O_2$  can be transformed to  $H_2O$  and  $O_2$  through catalase [24]. In many studies, the level of SOD has shown varying concentration levels. While researchers such as Kalavacherla et al. [25] and Bae et al. [26] found lower levels of SOD, Olivieri et al. [27] found unchanged SOD and thiobarbituric acid levels.

According to Jira et al. [28], there is a possibility that the lowered levels of SOD activity may be due to the inhibition of the enzyme by hydrogen peroxide, which might be an indicator of high degree of superoxide anion production resulting in increased hydrogen peroxide liberation through dismutation reaction. Rheumatoid patients undergoing therapy with nonsteroidal anti-inflammatory drug (NSAID) are usually good examples of significant correlation between SOD activity and intracellular lysate thiol (LSH) concentrations [29, 30]. However, the correlation vanishes when sulphasalazine is added, which is an indicator of interference with free radical defense mechanisms, similar

**Table 1** Antioxidant defenses [4]

Enzymes	Reactions
Enzymes (mainly intracellular)	removing $O_2^{\cdot-}$ and $H_2O_2$
Superoxide dismutase	$2O_2^{\cdot-} + 2H^{\pm} \rightarrow H_2O_2 + O_2$
Glutathione peroxidase	$2GSH^a + H_2O_2 \rightarrow GSSG + 2H_2O$
Glutathione reductase	$GSSG + NADPH + H^{\pm} \rightarrow 2GSH + NADP^{\pm}$
Catalase	$2H_2O_2 \rightarrow 2H_2O + O_2$
Low molecular mass antioxidants (intracellular and extracellular)	
Glutathione	Scavenges $\cdot OH$ , singlet oxygen, $HOCl$ , $ONOO^-$ , $RO\cdot$ , $RO_2\cdot$
Ascorbic acid	Reduces agent that reacts with $O_2^{\cdot-}$ , $\cdot OH$ , $HO_2\cdot$ , $HOCl$ , $ONOO^-$ , $RO\cdot$ , $RO_2\cdot$ , and singlet oxygen. It may detoxify other radicals by reducing them
A-Tocopherol	Terminates chain reaction of lipid peroxidation, thought to be recycled by ascorbic acid or ubiquinol
Uric acid	Scavenges singlet oxygen, $\cdot OH$ , $HOCl$ , $O_3$ , $ONOO^-$ , and peroxy radicals. Urate radical may be recycled by ascorbate

<sup>a</sup> Glutathione

to the actions of sodium aurothiomalate and auranofin [30]. Similarly, Nivsarkar [31] found that in RA patients, SOD activity was lower than controls, but levels of circulating SOD were up after NSAID therapy. Rister and Bauermeister [32] found that SOD content in polymorphonuclear leukocytes of children with RA was diminished compared to healthy controls. On the other hand, Ozkan et al. [33], Gambhir et al. [18], or Scudder et al. [34] were not able to observe a significant change in SOD levels.

Along with the SOD activity, Rasool and Varalakshmi [35] found that the activity of GSH-Px is decreased in arthritic animals that could be a consequence of enormous production of free radicals. Glutathione is essential in the stability of lysosomal and other cell membranes. Moreover, tripeptide is thought to protect cell membranes against damage from superoxides and free radicals [36]. The tissue damage resulting from the inflammatory effects of RA is partly due to lipid peroxidation and prostaglandin synthesis. Thus the protective effect of glutathione may be due to the inhibition of these reactions as suggested by Munthe et al. [37]. According to this study, the depressed level of erythrocyte glutathione observed in patients with active RA goes up in good responders to D-penicillamine, while no similar increases are observed in nonresponders to the drug unless supplemented by L-cysteine. The concentration of glutathione is regulated through glutathione synthetase, GSH-Px, and GR [36].

GSH-Px is localized in the cytoplasm and mitochondria of cells. It catalyzes the degradation of various peroxides by

oxidizing glutathione with the formation of its conjugates [38]. Munthe et al. [37] found depressed values of erythrocyte GSH-Px activity during active phases of RA, similar to the findings by Tarp [39]. Mèzes et al. [40] observed an upwards movement in GSH-Px activity in (female) rheumatoid patients, while male patients were neutral. Abella et al. [41] did not observe any differences in GSH-Px activity in patients with RA, in contradiction to Vanella et al. [42] who found that both erythrocyte GSH-Px and GR levels were reduced in the observed patients.

Vijayalakshmi et al. [43] found supporting evidences of low GSH-Px activity in liver, kidney, and heart in arthritis, the findings of which were supported by Shah and Vohora [16]. Braven et al. [36] found that the level of erythrocyte GSH-Px activity in patients with RA was high comparatively. This view was opposed to by Tarp [44] for Braven et al. [36] might not have taken into account the potential differences in selenium status between RA patients and controls as also noted by Vijayalakshmi [43]. On the other hand, Cimen et al. [45] found that GSH-Px might not be playing an essential role in rheumatic events. The affinity of GSH-Px for  $H_2O_2$  is stronger than the affinity of catalase, which makes GSH-Px more efficient at low levels of  $H_2O_2$  concentrations.

Catalase is another effective enzyme that functions together with SOD and GSH-Px through enzymic antioxidant defense system and catalyses the decomposition of hydroperoxide to water and oxygen to protect cells against  $O_2$  toxicity and lipid peroxidation [46].

Reactive oxygen species (ROS) play a role in the pathogenesis of a number of inflammatory diseases such as RA. Endogenous antioxidant enzymes such as catalase protect cells against damage caused by ROS. Catalase plays an essential role in the reduction of lipid peroxides and hydrogen peroxides in a reaction involving  $H_2O_2$  as an absolute substrate. SOD, catalase, and GSH-Px are thought to be the fundamental antioxidant enzymes, for they are closely related to the direct elimination of ROS [16]. Despite many reports that do not find catalase activity in serum of patients with RA, Kerimova et al. [47] reported decreased erythrocyte catalase activity in RA patients. These findings are also in line with Taysi et al. [24]. Nevertheless, Gambhir et al. [18] and Cimen et al. [45] found unchanged catalase activity in erythrocytes of RA patients.

Catalase is expressed predominantly within peroxisomes, where it dismutates the ROS and  $H_2O_2$  formed as a result of oxidation of long-chain fatty acids [48]. Expression of catalase in vitro and in vivo affects the expression of genes influencing inflammation [49]. Therefore, increased catalase activity can be protective against RA through limiting the production of ROS or through regulation of the expression of genes involved in inflammation [50]. El Sohemy et al. [50] also found that

differences in catalase activity did not play a significant role in the onset of RA.

Similar to the enzymes such as GSH-Px and SOD, RA is associated also with low antioxidant status related to low activities of GR [51] and linked also to decreased levels of reduced glutathione that is an intracellular antioxidant in the synovial fluid T cells of patients with RA [52].

Mulherin et al. [51] reported that higher basal and stimulated GR might be expected in patients with RA in response to chronic oxidative stress due to synovial inflammation. The association of riboflavin deficiency with increased disease activity suggests that impaired GR activity could facilitate continuing inflammation in these patients. Another study reported impaired GR activity in synovial fluid in RA [53].

The glucose-6-phosphate (G6P) provides the reducing equivalents required for GR conversion of glutathione sulfide back to serum reduced glutathione levels [54]. Hassan et al. [54] also suggest that a state of oxidative stress exists in RA as well as the depletion of serum reduced glutathione levels and that the decrease of detoxifying enzymes such as GR and GSH-Px are thought to be contributing factors. Hassan et al. [54] found similar results in which the RA patients they observed had significantly lower levels of GR, similar to the propositions by Chow and Tappel [55], who thought that G6P dehydrogenase, GR, and GSH-Px work in harmony against oxidative cellular damage. Herve et al. [56] found in a specific study on G6P isomerase (GPI) that GPI was not a specific auto-antigen in RA.

## Conclusions

Although there remains much to be gone through regarding the etiology of RA, there is much evidence that antioxidant team that covers GR, CAT, GSH-Px, SOD, and GPI destroy ROS and other free radicals through enzymatic as well as nonenzymatic means. The change in relative levels of antioxidants vis-à-vis free radical formation and level could be used as indicators for effective and earlier diagnosis of RA.

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