SIGNIFICANCE INCREASED OF XANTHINE OXIDASE ACTIVITY IN PLACENTA WITH PREGNANCY COMPLICATIONS OF PREECLAMPSIA AND GESTATIONAL DIABETES COMPARED TO NORMAL

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Abstract

Certain risky complications in pregnancy are due to failure of pseudo-vasculogenesis of arteria spiralis, which leads to inadequate nutrition, hypoxia, and inflammation. These can be caused by eating disorders, stress, or other risk factors. Preeclampsia (PE) and Gestational Diabetes (GD) are pregnancy complications that are currently of concern. In Indonesia, the prevalence of PE reaches 3-10%, with a maternal mortality rate of 50%. Meanwhile, the prevalence of GD ranges from 1.9-3.6%, and half of GD patients are diagnosed with type 2 Diabetes Mellitus (DM) after 5-10 years of giving birth. Oxidative stress arises as a result of those pregnancy complications. Various studies have also been conducted to determine the pathways that produce reactive oxygen species (ROS), the leading causes of oxidative stress. Xanthine oxidase (XO) is an enzyme that contributes to ROS production. Uniquely this enzyme is the converted form of xanthine dehydrogenase (XDH), which plays a minimal role in ROS production. The conversion of XDH to XO significantly increased in PE and GD cases, characterized by increased XO activity in maternal and fetal circulation and placental tissue compared to normal pregnancies. This paper will describe the conversion mechanism of XDH to XO in PE and GD conditions and compare its enzyme activity to normal pregnancy.

Keywords: xanthine dehydrogenase, xanthine oxidase, ROS, gestational diabetes, preeclampsia

Introduction

Pregnancy is a condition with high metabolic activity. It is vital to maintain the homeostasis of the metabolic system in order to avoid pregnancy complications.
Pregnancy complications are health problems involving the mother's health, the baby's health, or both. Many complications arise during pregnancy due to inadequate nutrition, eating disorders, stress, or other risk factors. Preeclampsia (PE) is one of the complications in pregnancy that appears in the second or third trimester, characterized by maternal hypertension and proteinuria. The incidence of preeclampsia depends on geographical conditions, nutrition, and ethnicity. The prevalence ranges from 3-8% globally and 3-10% in Indonesia, of which 3.2% of them developed eclampsia. The maternal mortality rate due to preeclampsia and eclampsia ranges from 50-65%.

The second and third trimesters of pregnancy are also at risk for the emergence of Gestational Diabetes (GD). It is a severe complication characterized by chronic hyperglycemia during pregnancy, even though the mother was not diagnosed with diabetes mellitus (DM) before pregnancy. The International Diabetes Federation (IDF) stated that 14% of cases of GD occur in 18 million births in the world. In Indonesia, the prevalence of GD ranges from 1.9-3.6%, and half of the women with GD were diagnosed with type 2 DM after 5-10 years of giving birth.

Until now, the mechanism of PE and GD is not fully understood. Various studies have tried to examine the pathways and factors that cause these complications with various objectives, mainly to observe how severe oxidative stress is formed in PE and GD. The common oxidative stress marker is Malondialdehyde (MDA) as a lipid peroxidation product. One of the common sources of free radical production is Xanthine oxidase (XO). This enzyme produces ROS and thus also serves as an oxidative stress marker, especially during hypoxia. XO is a molybdoflavine enzyme that catalyzes two terminal reactions in purine degradation, oxidizing hypoxanthine into xanthine and oxidizing xanthine into uric acid. This enzyme is initially transcribed from Xanthine Oxidoreductase (XOR) genes distributed in several tissues such as capillary endothelial cells of the heart, liver, kidneys, intestines, muscle cells, and placenta. It is known that XOR consists of two forms of interconvertible enzymes, Xanthine Dehydrogenase (XDH) and Xanthine Oxidase (XO). The appropriate conditions (e.g., hypoxia, inflammation, or oxidative stress) can convert XDH to XO, which has a high oxygen affinity. It affects higher ROS production that can damage tissues.

Hypoxia is a physiological condition detected during the first trimester of normal pregnancy because of placental development. The presence of PE and GD will cause prolonged hypoxia, leading to increasing severity. Placenta samples from PE and GD conditions showed higher ROS production than normal pregnancies. Therefore, this paper will describe the conversion mechanism of XDH to XO in PE and GD conditions and compare its activity in normal pregnancy.

**Xanthine Oxidase: Structure and Conversion Mechanism**

XO (EC 1.17.3.2) is the interconvertible form of xanthine dehydrogenase (XDH, EC 1.17.1.4). Both enzymes are transcribed from the same gene, located on chromosome 2, called xanthine oxidoreductase (XOR). XOR (especially the XDH form) belongs to a
housekeeping enzyme.\textsuperscript{9,10} XOR can use electron acceptors in the form of oxygen, NAD\textsuperscript{+}, ferricyanide, or methylene blue. Reactions involving one electron can produce superoxide, whereas reactions involving two electrons produce peroxides. Therefore, XOR belongs to the enzyme with low specificity; the substrate being oxidized, and the electron acceptor used depends on the physiological conditions that occur at that time.\textsuperscript{10-13}

Xanthine oxidoreductase in mammals is a 145 kDa homodimer subunit. Each subunit consists of three domains as redox reaction centers of enzymes. The largest domain (85 kDa) was the molybdopterin cofactor (Mo-co) in C-terminal, the intermediate domain (40 kDa) was the flavin adenine dinucleotide (FAD) cofactor, and the smallest domain (20 kDa) was two Fe/S clusters in N-terminal. The two Fe/S clusters are determined as Fe/S I and Fe/S II by potential redox differences. The Fe/S II has a higher redox potential than Fe/S I. Electrons will be transferred from Mo-co to FAD through both centers of Fe/S during the substrate hydroxylation reaction. Then, the electrons will be captured by the acceptor, which can be either NAD\textsuperscript{+} or oxygen molecules.\textsuperscript{14}

The conversion rate of XDH to XO will increase if XOR is released into circulation. This conversion occurs due to physiological conditions such as cell turnover or pathological conditions such as hypoxia, inflammation, ischemia/reperfusion, oxidative stress, or transplantation effects. Circulating XOR can reach tissues or organs and attach to glycosaminoglycans (GAG), especially heparan sulfate groups, on the surface of endothelial cells. Nitric oxide (NO) would be attached to the same sites on the endothelial surface. The increasing XOR would be a competitor for NO and inhibit endothelial NO attachment, which is essential as a vasodilatation regulatory molecule.\textsuperscript{9-10}

XDH can be converted to XO reversibly or irreversibly. XDH is converted to XO irreversibly if there are proteolytic activities in the plasma, which causes a conformational change in the enzyme structure. XDH is reversibly converted if there is sulfhydryl (-SH) oxidation of cysteine. Both decrease the enzyme's affinity against NAD\textsuperscript{+}.\textsuperscript{9-11} (Figure 1).

![Figure 1. Scheme of XDH conversion to XO and conformational changes in its FAD subunit affecting substrate affinity. Adapted from Kelley EE, 2019.\textsuperscript{9}](image)
In irreversible conversion, proteases will break three peptide bonds in XDH, Lys-551, Leu219, and Lys569. In the reversible conversion, oxidation occurs when forming a disulfide bridge (−S−S−) between Cys535 and Cys992 residues. The bridge can be reduced again with reducing agents such as dithiothreitol (DTT) in vitro.10–11

Xanthine dehydrogenase has a greater affinity against NAD\(^+\) than O\(_2\). The \(K_M\) and \(K_{cat}\) of XDH against NAD\(^+\) were 20.8 ± 2.2 M and 12.3 ± 1.2 s\(^{-1}\). Meanwhile, the \(K_M\) and \(K_{cat}\) XDH values against O\(_2\) were 111 ± 5.3 M and 2.5 ± 0.1 s\(^{-1}\), respectively. On the contrary, the XO affinity against NAD\(^+\) could not be detected, and its affinity against O\(_2\) is much greater, with \(K_M\) and \(K_{cat}\) values of XO being 37.7 ± 0.6 M and 15.1 ± 0.2 s\(^{-1}\), respectively. Those kinetic parameters were measured at 0.15 mM xanthine, pH 8.5, temperature 25°C.\(^{16}\) Thus, the use of O\(_2\) as an electron acceptor will result in harmful products such as hydrogen peroxide (H\(_2\)O\(_2\)) and superoxide anion (O\(_2\)\(^{•−}\)).\(^8\)

**XO activity and Oxidative Stress in Preeclampsia (PE)**

Preeclampsia is a pregnancy disorder characterized by hypertension, proteinuria, and edema. Various things that underlie preeclampsia are still not known with certainty, but it is possibly related to an abnormal placental process. Early pregnancy is marked by the invasion of cytotrophoblast cells in the embryo into the decidua and myometrium. It causes cytotrophoblasts to access and infiltrate uterine blood vessels from the mother, which connect the arterial and venous circulation to the intervillous space.\(^{12–17}\)

The success of placenta formation is based on cytotrophoblasts invading the decidua and myometrium and the remodeling of the spiral arteries. After the cytotrophoblast has invaded the uterine wall properly, the blood flow from the veins will stop, and the trophoblast will migrate to the lumen of the spiral arteries, branches of the oxygen-rich uterine arteries, by replacing the endothelial of the vessels and uterine muscle wall. This replacement triggers remodeling of the spiral arteries to increase the diameter of the blood vessels, which is called pseudovasculogenesis. These structures can mediate the passage of nutrients, gases, and wastes between fetal blood circulating in the villi and maternal blood circulating in the intervillous spaces. The remodeling process poses a burden of oxidative stress on the mother.\(^{12,18,19}\)

In pregnancy with PE, trophoblastic cell invasion is not proper, and there is failure to remodel the spiral arteries; this is known as placental maladaptation. Angiogenic factors (Flt-1/VEGFR-1, VEGFR-2) are thought to be essential factors in the regulation of placental vascular system development. Invasive cytotrophoblasts express VEGF, PlGF, and VEGFR-1 (Flt-1), whereas this expression is altered in PE. As a result, the placenta maladaptation causes the spiral arteries not to dilate and decrease the oxygen supply, leading to hypoxia condition.\(^{20}\)

Hypoxia-inducible transcription factors (HIF) control the trophoblast differentiation, which depends on oxygen availability. In addition, HIF regulates erythropoiesis and angiogenesis, which are essential in the placental adaptation process. HIF-1α and HIF-2α are the primary transcription factor of hypoxia signaling in the placenta. They are localized
in the endovascular cytotrophoblast villi and fetoplacental vessels in the first trimester of pregnancy. The amount of HIF-1α and HIF-2α decreases with increasing gestational age. The highest peak of HIF-1α levels occurs at 7-10 weeks of gestation.\(^{21}\)

Expression of HIF-1α and HIF-2α increases in the cytotrophoblast villi, syncytiotrophoblast, and fetoplacental vessels in response to a relatively hypoxic environment during the first trimester of normal pregnancy. Expression of HIF proteins will decrease at the end of the first trimester due to increasing placental oxygenation in intervillous blood flow.\(^{21}\) The hypoxic environment in PE causes overexpression of HIF-1α and HIF-2α proteins. It increases sFlt-1, an antiangiogenic factor which coincides with a decreasing VEGF and PlGF as angiogenic factors. Increased sFlt-1 causes endothelial dysfunction.\(^{20,22}\)

The hypoxic condition triggers the release of inflammatory factors from the placenta into the maternal bloodstream, which causes a systemic inflammatory response in the mother. This response will destroy the endothelial cell in maternal blood vessels, causing vessel leakage. If it occurs in the glomerular capillaries, it causes proteins to leak out of the renal blood vessels, causing proteinuria. In addition, it causes osmotic pressure falls, causing plasma water to come out into the interstitial space, leading to edema or swelling.\(^{12,18,22}\)

The hypoxic condition in PE resulted in increasing XO activity. As explained above, XO has a greater affinity to O\(_2\) than NAD\(^+\).\(^2\) Meanwhile, during hypoxia, hypoxanthine substrate is increased due to the breakdown of ATP. If oxygen is returned, a superoxide radical burst occurs.\(^{17,21}\) In addition, plasma and placental NO levels are decreased in PE. The superoxide anion increment will inactivate NO to form peroxynitrite (ONOO\(^-\)). This species is one of the most potent forms of oxidative stress surrogate marker.\(^2,23-25\) The H\(_2\)O\(_2\) compound produced by the XO can form water if reduced with glutathione peroxidase (GPx). It causes reduced glutathione (GSH) to become oxidized glutathione (GSSG). GSSG can be reduced back using NADPH by the activity of glutathione reductase. In pregnancy with PE, the antioxidants often decrease because NADPH has been heavily oxidized to NAD. The H\(_2\)O\(_2\) molecule can also react with Fe\(^{2+}\) ions resulting in the OH\(^-\) radicals which cause endothelial or tissue damage.\(^2,24,25\) Some literatures show the significance of XO activity in PE cases from some tissue or organ sources; it reaches a 1.5 to 5.8 fold increase from normal (Table 1).

<table>
<thead>
<tr>
<th>Gestational disease</th>
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<th>XO activity</th>
<th>Increasing XO activity (fold)</th>
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</table>
### XO activity and Oxidative Stress in Gestational Diabetes (GD)

Gestational diabetes is defined as glucose intolerance that occurs during pregnancy. Generally, the insulin requirement will be higher during the end of a normal pregnancy. However, in contrast to normal pregnancies, GD consistently shows a decreased insulin response to nutritional intake. GD cases occur in mothers who had no history of diabetes before pregnancy. It is caused by an increase of insulin-antagonist placental hormones released from the fetus, such as human placental lactogen (hPL) and human placental growth hormone (hPGH). The hPL and hPGH are the main hormones that can increase the risk of insulin resistance; they reduce insulin sensitivity by ~50% in the last trimester. It is known that the hPL increases up to 30-fold during pregnancy, whereas hPGH increases 6-8 times and replaces normal pituitary growth hormone (GH) in the maternal circulation at ~20 weeks of gestation.

The placenta is a very metabolically active organ and uses most of the glucose that the mother supplies. Only 40-50% of the glucose taken up will be released to the fetus; the placenta uses approximately 80% of that glucose. It is released into circulation as lactate or stored as glycogen, most of which resides in the endothelial cells of the placenta. The placenta's glucose processing involves and influences a variety of metabolic pathways.

It is known that glucose uptake in tissues requires a glucose transporter called GLUT. GLUT 1 is the main transporter found in almost all cell types during pregnancy in the placenta. In addition, GLUT 3 is also the dominant transporter in the fetoplacental endothelium. Besides those GLUTs, GLUT 4, an insulin-dependent transporter, can also be found in syncytiotrophoblasts in early pregnancy and the placental villi stroma in the last trimester. GLUT 4 is the primary glucose transporter in the mother's heart, skeletal muscle, and adipocytes.

The hPL and hPGH affect the insulin-dependent transporter's uptake (GLUT4) for glucose. In normal pregnancy, insulin binding to its receptor causes a conformational change, activating tyrosine kinase activity. This activation causes autophosphorylation of tyrosine residues in the insulin receptor (IR) beta chain. The presence of this phosphorylation recruits the IR substrate (IRS1/2) to bind IR tyrosine residue. This IRS protein complex acts as a docking protein in downstream signaling. The subunit of PI 3-
kinase (PI3K) called key regulator (p85) binds to the IRS complex and activates the p110 subunit. The increasing PI3K activity will cause downstream signaling and activate Akt. Activated Akt plays an essential role in integrating GLUT4 into the plasma membrane and absorbing glucose.

In pregnancy with GD, serine phosphorylation blocks IR and IRS tyrosine phosphorylation, resulting in decreased PI3K recruitment. Serine kinase activation is caused by proinflammatory cytokines such as TNFα or excess free fatty acids that negatively feed IRS-1. Increased serine phosphorylation promotes IRS1/2 degradation. In both normal pregnancy and GD, pGH increment causes the expression of regulatory protein p85. It leads to enzymatic competition between the binding of p85 to p110 and p85 to IRS1/2, which decreases PI3K activity, prevents the activation of Akt, and decreases glucose uptake by GLUT4 (Figure 2). Minimal glucose absorption will make a hyperglycemic condition in the maternal circulation. The GLUT 1 and GLUT 3 in the placenta will absorb excess glucose, causing the placenta to weigh heavier than average. Hyperglycemic conditions in the placenta inhibit oxygen transport, resulting in hypoxic conditions.

Figure 2. GLUT-4 signaling pathway in pregnancy with gestational diabetes. Adapted from Mccurdy et al., 2010.

The hypoxic condition can trigger XDH conversion to XO. As in PE pregnancy, using O2 as an electron acceptor by XO will produce harmful ROS products. However, ROS
products can be produced from other pathways in DG pregnancy due to the hyperglycemic condition, exacerbating systemic oxidative stress in the mother. The main pathway of the hyperglycemic response is the polyol pathway, in which glucose is reduced by aldose reductase to form sorbitol using NADPH as a coenzyme. Then, sorbitol is converted to fructose by sorbitol dehydrogenase using NAD as a coenzyme. Aldose reductase has a very high $K_M$ value for glucose, so this enzyme is only active in hyperglycemic conditions. The presence of NADPH consumption in the initial conversion of glucose causes the availability of NADPH to be depleted and inadequate to support glutathione reductase to form GSH, which is an important cellular antioxidant. This phenomenon triggers ROS increment, which leads to oxidative stress.\textsuperscript{34,35} Some literatures show the significance of XO activity in GD cases from several tissue or organ sources. It reaches 1.1 until 4.5-fold increase than usual (Table 1).

**Conclusion**

XO and XDH originated from the same gene, XOR. XDH conversion to XO increases either reversibly or irreversibly under hypoxic conditions. XO has a greater affinity for $O_2$ than NAD$^+$; therefore, the use of $O_2$ as an electron acceptor contributes to high ROS production. Gestational abnormalities such as PE or GD increase the risk of prolonged hypoxia than normal pregnancies, increasing XO activity in tissue or plasma. Several studies in this paper show a significant increase in XO activity in maternal plasma, fetal plasma, or placenta of both gestational abnormalities compared to normal pregnancy.

**Competing Interests**

Authors declare no conflicts of interest.

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