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Review Article

Alkaline phosphatase activity as a biochemical biomarker in aqua-toxicological studies

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Abstract: Alkaline phosphatase (ALP) is a glycoprotein with a metallophosphatase structure that catalyzes the hydrolysis of monophosphate esters of biomolecule esters at alkaline pH. ALP activity is a useful bioindicator to assess the physiological health of cellular membranes, cell growth, apoptosis and cell migration, cellular metabolic status, hepatocyte function, and detoxification activity in hepatocytes. ALP activity is detected in a colorimetric method using the para-nitrophenyl phosphate substrate (p-NPP) at a wavelength of 405 nm in biological samples. Cell hemolysis, especially erythrocytes; increased levels of sex hormones and corticosteroids, biological infections, and poor nutrition can adversely affect ALP activity.

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Introduction

Alkaline phosphatase (ALP) is a metalloenzyme that is anchored to the cell membrane by a glycosylphosphatidylinositol (GPI) (Atkins et al., 2011). ALP is a glycoprotein with an external domain and a catalytic domain with a serine base. The activity of ALP depends on the binding of two Zn^{+2} ions and one Mg^{+2} ion to the active site of the enzyme. The serine base can inhibit the activity of the ALP enzyme (Banaee and Taheri, 2019). Therefore, ALP activity depends on the concentration of these ions in cells and intercellular fluids (Banaee et al., 2011; Nafisi Bahabadi et al., 2014; Soleimany et al., 2016). Therefore, gender, health status, ontogeny (Kim et al., 2001), oral supplements administration (Taheri et al., 2017; Gholizadeh Zare Tavana et al., 2018), or exposure to pollutants, and environmental factors can have a significant effect on ALP activity (Woo et al., 2018).

Alkaline phosphatase plays a vital role in phosphate hydrolysis and membrane transfer (Derikvandy et al., 2020), and is also involved in transphosphorylation of many biomolecules, such as nucleotides, proteins, and alkaloids in alkaline pH (Nematdoost Haghi and Banaee, 2017). The activity of this enzyme is essential for cell growth, planned cell death (apoptosis), and cell migration (Sharma et al., 2014; Banaee et al., 2019c). ALP activity often increases during differentiation of B lymphocytes to plasmocytes (Akcakaya et al., 2007).

Alkaline phosphatase is often synthesized in the liver, bones, and to a lesser extent, in the intestine and kidneys (Telega, 2018). Additionally, the ALP gene is expressed in the endothelial blood vessels and the brain tissue of fish (Goishi and Klagsbrun, 2004). ALP activity is essential for bone mineralization (Atkins et al., 2011). So far, 12 isoenzymes from ALP have been identified that may have different origins and synthesis sites.

Alkaline phosphatase plays a vital role in glycogen metabolism (Kim et al., 2001). The activity of this enzyme in hepatocytes can inactivate phosphorylase and increase the rate of glycogen breakdown in the liver (Raisi et al., 2018; Rezaei Shadegan and Banaee, 2018). Increased levels of synthesis and ALP activity can play an essential role in providing energy for stressed cells (Agrahari et al., 2007; Saha and Kaviraj, 2009).

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Purpose of measuring ALP activity: Alkaline phosphatase is a non-specific indicator for the diagnosis of liver and bone damage. Furthermore, ALP is an excellent biological indicator for assessing cellular stress (Banday et al., 2019). It plays a vital role in the xenobiotic detoxification process in hepatocytes (Vaziryan et al., 2017; Hatami et al., 2019).

ALP activity measurement method: Alkaline phosphatase activity is measured by a quantitative colorimetric method using the para-nitrophenyl phosphate substrate (p-NPP) at a wavelength of 405 nm (Moss and Henderson, 1999).

Reasons for increased ALP activity: Tumor formation in the liver and bone tissue, hyperthyroidism, bile duct obstruction, liver nodules, liver cysts, liver failure, and increased ALP biosynthesis rates in hepatocytes, and intestinal dysfunction can lead to increased ALP activity in the serum or plasma. For example, ALP activity in rainbow trout, *Oncorhynchus mykiss*, and *Cyprinus carpio* fish plasma increased significantly after exposure to diazinon (Banaee et al. 2011, 2012). Increased ALP activity in the body tissues of freshwater fish of *Alburnus sellal* exposed to fenpropathrin indicates damage to cell membranes (Banaee et al., 2014). Additionally, damage to the cell membrane led to the release of ALP into the fish plasma exposed to titanium dioxide nanoparticles (Banaee et al., 2019b). Increased ALP activity in fish hepatocytes exposed to dimethoate and bacillar fertilizer may also be a physiological response to increased glycogen breakdown in the liver (Rezaei Shadegan and Banaee, 2018).

Nematdoost Haghi and Banaee (2017) and Wan Do et al. (2019) stated that damage to the hepatocyte membrane, gallbladder ducts, intestinal epithelial cells, and renal ducts increased ALP in fish plasma exposed to microplastics and paraquat. Banaee et al. (2019b) reported an increase in ALP activity in plasma of fish exposed to paraquat and nano-TiO2. Research shows that sometimes pollutants may increase ALP activity in cells by affecting the process of transphosphorylation in cells (Banaee et al., 2019a). Increased ALP activity in cells of *C. carpio* following oral exposure to zinc oxide nanoparticles may be related to the dysfunction of cells (Taheri et al., 2017). Furthermore, an increase in the production rate of reactive oxygen species (ROS) may elevate ALP activity (Banday et al., 2020; Banaee et al., 2017). Woo et al. (2018) observed elevated activity of ALP in plasma of *C. carpio* exposed to trichlorfon (Woo et al., 2018). An increase in ALP activity was observed after exposure of *Clarias gariepinus* to deltamethrin (Amin and Hashem, 2012; Eni et al., 2019), cypermethrin (Eni et al., 2019), heavy metals (Banday et al., 2019). Similar results have been reported after exposing *Oreochromis niloticus* to deltamethrin (Abdel-Daim et al., 2015; Abdelkhalek et al., 2015), diazinon (Abdelkhalek et al., 2017). An increase in ALP activity was observed in the blood of *Channa punctatus* exposed to monocrotophos (Agrahari et al., 2007).

Reasons for decreased ALP activity: ALP activity may be reduced by malnutrition, especially protein, zinc, magnesium and vitamin C deficiency, and increased vitamin D levels in the diet. Anemia and hypothyroidism can lead to decreased ALP activity. Severe cellular necrosis can be another reason for decreased ALP activity in fish plasma (Banaee et al., 2016). Moreover, disturbance in the transport of zinc, magnesium, and phosphorus ions from cell membranes can lead to decreased ALP activity (Banaee et al., 2019c; Banaee et al., 2020). Decreased absorption of essential ions for ALP biosynthesis can lead to decreased enzyme activity (Veerappan et al., 2012). Besides, the interaction of environmental pollutants with ALP can reduce its activity (Banaee et al., 2019a; b). For example, decreased ALP activity in gill cells of the rainbow trout exposed to cadmium chloride may be due to the effect of cadmium on cell membranes (Evaz-Zadeh Samani et al., 2017).

Exposure of *C. carpio* to Cd reduced ALP activity in muscle tissue (Banaee et al., 2015). Decreased plasma ALP activity may be due to damage to erythrocyte membranes and their hemolysis (Farah et al., 2012). Vaziryan et al. (2017) suggested that reduced ALP activity may be associated with impaired ALP biosynthesis, hepatic cell necrosis, and damage to the intestinal epithelial cells of fish exposed to oral aflatoxins. Decreased ALP activity may affect bone marrow cell activity (Prins et al., 2014). Decrease in ALP activity was reported in serum of *Rhamdia quelen* fed with a feed artificially contaminated with aflatoxin B1 (Anater et al., 2020).

Interventional factors in measuring ALP activity: Sex hormones (Ahmad et al., 2002), viral and bacterial infections (Banaee et al., 2017; Xia et al., 2017), parasites infection, administration of antibiotics (Gora et al., 2018), dietary supplements, hemolysis of blood cells (Farah et al., 2012) and feeding fish with oxidized and fatty foods can interfere with ALP activity in blood or tissue samples.

Conclusion

Measurement of the ALP activity in the intercellular and extracellular fluids can be indirectly useful for evaluating the membrane function of cells exposed to xenobiotics. Interaction of environmental pollutants with the biochemical structure of ALP or lipid peroxidation of cell membranes can cause fluctuations in ALP activity in cells or extracellular fluids. Therefore, each change in ALP activity outside the normal range can indicate stress on the cells.

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