Serum Paraoxonase 1 as a Biomarker in Toxicology

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Contents

Abstract

Biomarkers are used in many areas of health to diagnose the presence of disease, monitor disease progression, track drug distribution in the body, or determine exposure to chemicals. The increase of free radicals damages biochemical macromolecules and increases the formation of new toxic compounds such as enhanced oxidation protein products. Organophosphate (OP) compound exposure, oxidative stress, and lipid peroxide have been associated with DNA damage, various types of cancer, aging, degenerative disease, and inflammatory diseases in humans. For this section, the evaluation of paraoxonase (PON1), a liver enzyme that can hydrolyze toxic OP compounds, is bound to HDL (High-density lipoprotein) in serum and draws attention with its strong antioxidant properties, as a biomarker is discussed. In this chapter, PON1 as a toxicological biomarker was reviewed in three cases: organophosphate exposure, lipid peroxidation, and liver toxicity. PON1 status against toxic damage; both serum PON1 level and PON1 polymorphisms were discussed together in the light of studies. Finding new reliable biomarkers is an area of research that has become increasingly important in recent years.

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Keywords

Paraoxonase 1 · Biomarker · Organophosphate · Liver · Oxidative stress · High-density lipoprotein · Toxic · Disease · DNA damage

Abbreviations

AChE	Acetylcholinesterase
BuChE	Butyrylcholinesterase
CB	N-Methyl-carbamate
CHD	Coronary heart disease
ChE	Cholinesterase
CLD	Chronic liver disease
DZO	Diazoxon
HDL	High-density lipoprotein
His-His	Histidine-histidine
HTL	Homocysteine thiolactone
Km	Michaelis constant
LDL	Low-density lipoprotein
LDLs	Low-density lipoproteins
OP	Organophosphate
PCR	Polymerase chain reaction
PON1	Paraoxonase 1
Vmax	Maximum velocity

Introduction

Biochemical Properties and Gene Structure of Paraoxonase 1

In the past few years, there have been rapid advances in the field of paraoxonase research. Paraoxonase (PON1) is a Ca^{2+}-dependent enzyme and associated with HDL in serum; it is capable of hydrolyzing many substrates, including highly toxic nerve agents and various organophosphorus insecticides, oxidized lipids, and a number of drugs (Fig. 1). The paraoxonase 1 enzyme is classified by the Enzyme Commission of the International Union of Biochemistry and Molecular Biology as an aryldialkylphosphatase (EC 3.1.8.1). Paraoxonase 1 enzyme has organophosphate activity (e.g., substrate, paraoxon, diazoxon), lactonase activity (e.g., dihydrocoumarin, homocysteine thiolactone), and arylesterase activity (e.g., substrate phenyl acetate) (Fig. 2).

The catalytic mechanism of the paraoxonase enzyme has been elucidated. The Ca^{+2} ion, which has catalytic activity, is 2.2A away from the negatively charged oxygen of the phosphate ion in the substrate. The His pair in the active site takes a proton of the water molecule, increasing its nucleophilic strength. The hydroxyl ion formed attacks the carbonyl or phosphate ester. The complex formed at this time is

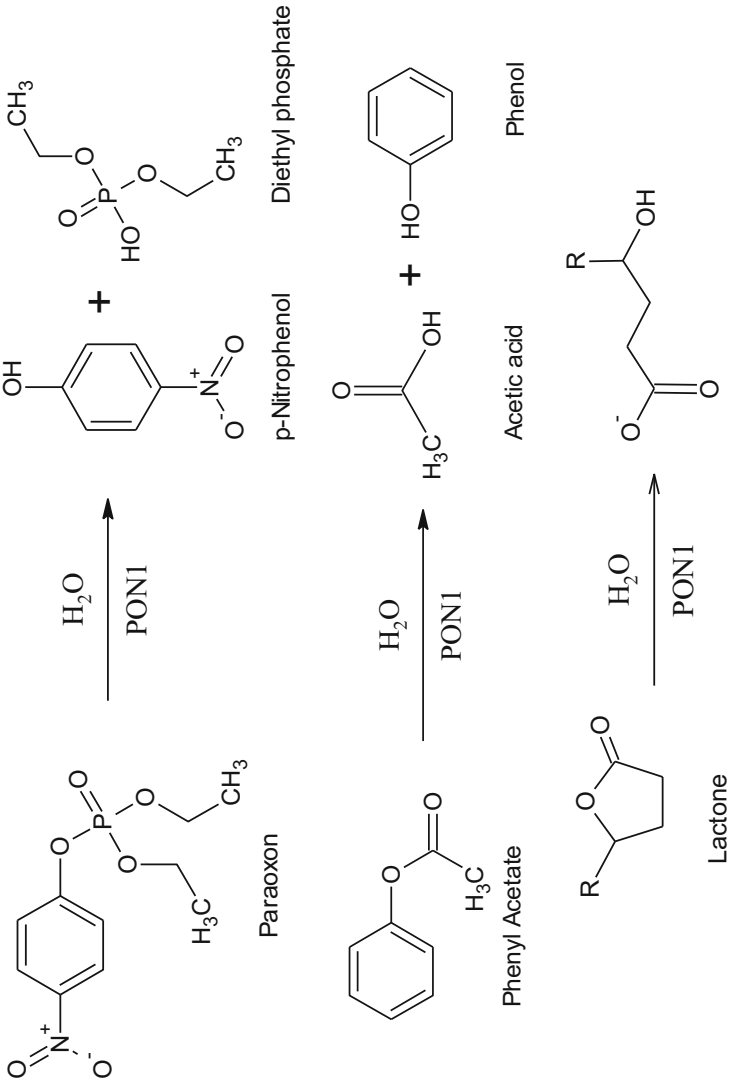


Fig. 1 Activities of paraoxonase 1

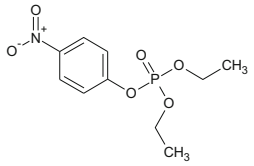
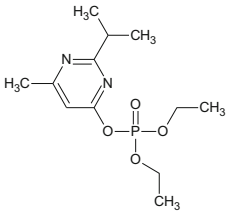
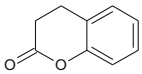
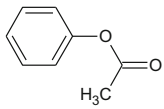
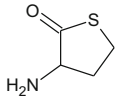
Organophosphates activity		Lactonase activity	Arylesterase activity
 Paraoxon	 Diazoxon	 Dihydroqoumarin	 phenil acetat
		 Homo cysteine thiolactone	

Fig. 2 The structures of paraoxonase 1 substrates

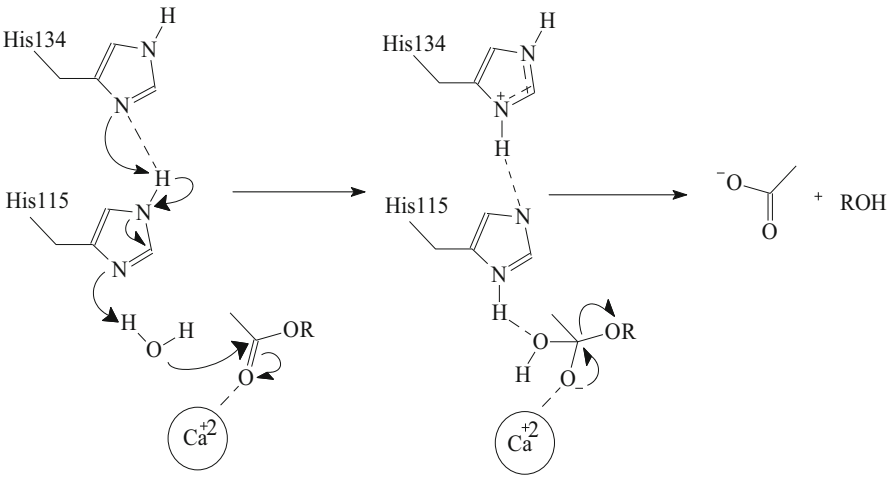


Fig. 3 The catalytic mechanism of paraoxonase 1

stabilized by Ca^{+2} ion as a result of a tetrahedral plane. The Ca^{+2} ion in the formed structure moves away from the negatively charged oxygen, and the bond breaks down on the phosphate or carbonyl again, and the ester bond is broken (Fig. 3).

PON1 is mainly synthesized by the liver, but histochemical experiments have shown the expression of PON1 in many different mammalian tissues, not only in the liver in humans. Since it is dependent on HDL, it is associated with many diseases. PON1 level has been shown to be important in protecting against atherosclerosis, possibly through PON1's physiological role in preventing lipid peroxidation (James 2006). Low serum PON1 levels are associated with many diseases, such as various liver diseases, kidney failure, diabetes, rheumatoid arthritis, and atherosclerosis

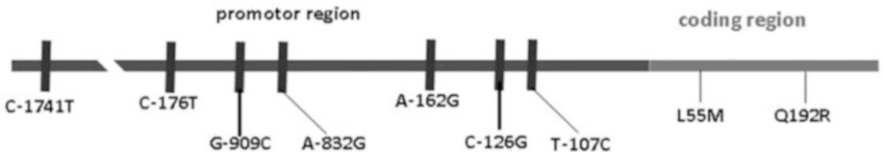


Fig. 4 Polymorphisms in the promoter and coding regions of paraoxonase (PON) 1. (Adapted from Camps et al. (2017))

(Goswami et al. 2009). These metabolic diseases are characterized by low PON1 activity due to dysfunctional HDL (Navab et al. 2007). Now, it is well-known that PON protein plays a vital role in toxicology, inflammation, and infection (Schrader and Rimbach 2011). Although the effects of lifestyle, pharmaceutical modulators, and diet on PON1 activity are known (Costa et al. 2005), the difference in PON1 levels between individuals has the greatest effect due to genetic polymorphisms of PON (Deakin et al. 2002).

The polymorphism of the coding region of the paraoxonase 1 enzyme is characterized by the methionine to leucine polymorphism at position 55 (PON1 Leu55Met or PON1 L55M) and the arginine to glutamine polymorphism at position 192 (PON1 Gln192Arg or PON1 Q192R). PON1 Q192R correlates with the QQ, QR, and RR genotypes, whose polymorphism corresponds to the AA, AB, and BB phenotypes, respectively (Fig. 4). Also, PON1 Q192R polymorphism shows the capacity of PON1 to inhibit LDL oxidation. The Q isoform is more efficient than the R isoform (Mackness et al. 1997; Aviram et al. 1998). The PON1 Q192R polymorphism differs on activity depending on the substrate. For example, paraoxone is hydrolyzed faster by the R isoform, while diazoxon is hydrolyzed faster by the Q isoform. PON1 promoter region T-108C and the PON1 coding region L55M polymorphisms are associated with different serum PON1 concentration levels and different activities too. PON1-55L allele shows higher PON1 serum protein and mRNA levels, so it's higher activity than the PON1-55M allele. PON1-108C allele has larger promoter activity and different serum activity than PON1-108T allele. Many other polymorphisms affect the serum PON1 level less.

Applications to Prognosis, Other Diseases, or Conditions

Organophosphate Exposure

Based on recent studies, biomarkers evaluating human exposure to toxic compounds and their relationship to PON1 will be discussed in this section. Organophosphate (OP) chemicals produced to control insects and pests are unfortunately ubiquitous in the environment. In the last half century, the unconscious use of N-methyl-carbamate (CB) and organophosphate (OP) insecticides has increased considerably around the world. They are especially used in some developing countries of the world to combat a lot of unwanted insects and pests. Paraoxonase (PON1) enzyme metabolizes highly toxic oxon forms of some OPs and pesticides which are commonly used. Some

individuals' health may be more sensitive to harmful compounds. This situation is based on studies with experimental animals; it is thought that paraoxonase (PON1) levels of individuals are an important determinant of susceptibility to insecticides. Studies with transgenic mice have indicated that low serum PON1 activity level is associated with brain acetylcholinesterase inhibition as measured by exposure to diase oxon and chlorpyrifos oxon (Li et al. 2000). Moreover, not only the paraoxonase concentration in the serum but also the Q/R polymorphism at the PON1 192 position affects an individual's OP sensitivity. An individual's PON1 level and polymorphism may be an important determinant of susceptibility to these chemicals. A study of acute OP intoxication observed that cases had significantly higher PON1 Q192 genotype and lower paraoxonase activity than controls (Davies et al. 1996). The PON1 polymorphism differentially impacts the activity of the catalysis of some PON1 substrates. Both PON1 plasma level and PON192 genotype should be evaluated together in determining the risk of exposure of individuals to disease and toxic compounds (Table 1). It was evaluated the association of OP exposure and PON1 Q192R genotype among workers in fruit-growing regions in South Africa. QQ homozygotes or QR heterozygotes were almost three times more likely than RR individuals to show more than one symptom of chronic OP toxicity (Li et al. 2000).

A study suggested that purified serum PON1 protects against OP exposure in mice and using PON1 as a catalytic converter for the treatment of hazardous compound exposures (Li et al. 2000). The difficulties arising from the purification of a quite hydrophobic protein PON1 have also been shown to be an obstacle to the development of the catalytic scavenger (Gan et al. 1991). In general, most PON1 purification attempts have studied human blood for serum as the enzyme material, where the protein is most abundant, and purification has been accomplished by several chromatographic methods (Furlong et al. 1991). In recent developments, the number of steps required to purify human serum PON1 has been decreased (Gencer and Arslan 2009). Evidence has been obtained from different studies that the use of recombinant human PON1 would be appropriate for therapeutic protection against OP exposure. As a result of these observations, it has been shown that human PON1 can be produced with sufficient purity by biotechnological way from bacterial cells and can be injected without any negative effects.

Cholinesterase (ChE) enzymes have two types found in the blood; the first, acetylcholinesterase (AChE), is related with red blood cell membranes, while the

Table 1 Catalytic effect of different OP substrates on purified human serum $PON1_{192}$. (Adapted from Li et al. (2000))

	Paraoxon hydrolysis		Diazoxon hydrolysis		Chlorpyrifos oxon hydrolysis	
	$PON1_{Q192}$	$PON1_{R192}$	$PON1_{Q192}$	$PON1_{R192}$	$PON1_{Q192}$	$PON1_{R192}$
Km (mM)	0.81	0.52	2.98	1.02	0.54	0.25
Vmax (U/mg)	0.57	3.26	222	79	82	64
Vmax/km	0.71	6.27	75	77	152	256

second, butyrylcholinesterase (BuChE), is found in serum. Inhibition of both AChE and BuChE is considered to be biomarker in determining the early biological effects associated with CB/OP exposure. In general, inhibition of AChE is thought to be a better biomarker for determining toxicity, while inhibition of BuChE is a more reliable indicator of most CB/OP exposures. Chlorpyrifos, diazinon, and malathion inhibit BuChE more effectively than AChE. By hydrolyzing the neurotransmitter acetylcholine, AChE has a vital role in the regulation of peripheral and central nervous system transmissions. The major effect of CB/OP toxicity is inhibition of neural acetylcholinesterase (AChE) enzyme activity. In one study, it was determined that PON1 is more ideal as a biomarker for assessing OP exposure, since pesticide handlers are more exposed to hazardous compounds, pesticides, than other agricultural workers (Li et al. 2000). For some OPs, such as chlorpyrifos, PON1-Q192R polymorphism is significant as it determines the catalytic mechanism of hydrolysis. Nonetheless, in the findings obtained, it was determined that PON1 activity showed great differences between individuals who have Q192R genotype. The serum PON1 activity level is meaningful in all wide variety of OPs metabolized by PON1 within the physiological ranges determined. Determination of both serum PON1 activity level and PON1 genotype provides a more meaningful of overall PON1 status rather than Q192R genotype only (Jarvik et al. 2003). Paraoxonase 1 activity level can be affected by nutritional status, smoking, and medications (Mackness and Mackness 2015). But most researches indicate that serum PON1 activity level tends to remain constant over time and is regulated by genetic factors, specifically the PON1 polymorphism promoter region C-108T (Davies et al. 1996). The functional PON1 Q192R genotype of the individual can be identified using the enzymatic assay method with two substrates, which are paraoxon and diazoxon. In this assay, activity is measured using these substrates in plasma samples. The basic principle of this method has been demonstrated in previous studies in the literature. In a 2003 study, the accuracy of the observed Q192R genotype experiment with two substrate analyses was demonstrated by the excellent agreement between the genotype predicted as a result of PCR experiments (Jarvik et al. 2003).

Animal studies related to PON1 polymorphism have shown that PON1's relationship in determining OP toxicity is more sensitive when both phenotype and genotype are considered together, rather than just genotyping. Findings from several epidemiological studies and extensive animal studies have clearly shown that interindividual variability of PON in susceptibility to OP exposure should be considered.

Lipid Peroxidation

Atherosclerosis is a chronic inflammatory disease involving both adaptive and innate immunity. As a result of peroxidation of fatty acid residues in triglycerides, phospholipids, and cholesterol esters, reactive aldehydes are formed, and these initiate inflammatory responses.

Lipid peroxidation is the attack of unsaturated fatty acids by free radicals. Due to the oxidation products formed during the process, it is associated with various diseases. High LDL concentration or oxidized LDLs are directly associated with

the risk of atherosclerosis development. Dysfunction of HDL may be associated with decreased vascular cholesterol flow rate, but the most common consequence is impaired anti-inflammatory/anti-oxidative function. Serum PON1 is found to be associated with high-density lipoprotein (HDL) particle that has a potential positive role in lipid metabolism (Mackness et al. 1991). Paraoxonase 1 has the ability to inhibit lipid peroxidation on low-density lipoproteins (LDLs) both in vitro and in vivo (Watson et al. 1995). HDL can prevent the progression of CHD by delaying the oxidation of LDL. The mechanism for how PON1 delays LDL oxidation has not been fully proven. Several proteins on HDL (Apo AI, apoM, GSPx, PLA2, LCAT), with PON1, contribute to this antioxidant property of HDL (Fig. 5). In studies performed in cell culture, PON1 has been shown to have different atheroprotective effects such as inhibiting cholesterol synthesis, increasing cholesterol efflux, and reducing macrophage oxidative stress. A possible association between PON1 and atherosclerosis showed that patients with myocardial infarction had lower serum PON1 activity than the control group. In patient control studies, low serum PON1 activity levels were seen as a biomarker associated with susceptibility to CHD development. In addition, there are different studies in the literature that PON1 activity is a risk factor for the development of atherosclerosis, regardless of HDL concentration level (Mackness et al. 2003). In some studies, the relationship between risk factors for CHD and PON1 polymorphism has been investigated. Findings, although PON1-Q192R polymorphisms are a risk factor for CHD, no link was found with other PON1 polymorphisms (Ferre et al. 2005).

Low serum PON1 activity levels are associated with many inflammatory diseases as a possible biomarker, including systemic lupus erythematosus, rheumatoid arthritis, and diabetes mellitus (Goswami et al. 2009). Also, these serious health problems are characterized by having dysfunctional HDL, which is hypothesized to be caused by low PON1 activity. Further studies are needed to determine whether low PON1 causes the development of these inflammatory diseases. However, low serum PON1

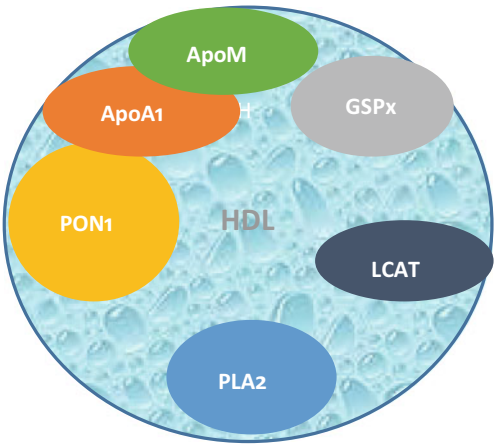


Fig. 5 Schematic representation of HLD with proteins on. Apo AI, apolipoprotein AI; apoM, apolipoprotein M; GSPx, glutathione peroxidase; PLA2, phospholipase A2; PON1, paraoxonase 1; LCAT, lecithin-cholesterol acyltransferase

activity levels, together with other tests, can be seen as a potential marker of inflammatory diseases.

A few studies have shown that there is a decrease in serum PON1 activity with advancing age and consequently a decrease in the protective functions of HDL. In addition, polymorphisms in the PON1 gene are related in healthy aging. Oxidative stress is a situation where the balance between oxidant formation and antioxidant defense is disrupted in the direction of oxidants. It is closely related to the pathogenesis of many pathological conditions and aging. In a study, the results demonstrate that cellular PON1 has a significant role in aging and this multifunctional protein may be associated with aging. Quality aging is an important target for improving living standards, so more studies are needed on the current function of PON1 (reviewed in Mackness et al. 2015).

Considering the studies, it is clear that PON1 contributes to the antioxidant characteristic of HDL and therefore PON1 has atheroprotective role. However, larger epidemiological studies are needed on all possible functions of PON1.

Liver Toxicity

The liver has a wide variety of functions such as synthesis of coagulation factors and other proteins, excretion of bile, and detoxification of harmful products of metabolism. Liver disorders are inflammatory conditions associated with increased plasma cytokine and chemokine concentrations (Visser and van der Heijde 2009). In chronic liver disease (CLD), which is characterized by severe worsening of liver function for at least 6 months, oxidative damage affects the pathological changes that cause liver cirrhosis and hepatocellular carcinoma and cirrhosis (McClain et al. 1999). Since the enzyme paraoxonase 1, which is produced in the liver and subsequently released into the serum with HDL, has a protective effect against oxidative damage, it is reasonable to find a relationship between liver failure and PON1. Studies first performed in the 1970s showed an important reduction in PON1 level among small patient groups with liver cirrhosis (Burlina and Galzigna, 1974). Later, these results were confirmed by examining a larger population of patients with chronic liver injury. When compared to the healthy control group, a significant reduction in serum PON1 activity level was found in patients with chronic hepatitis and a dramatic reduction in patients with liver cirrhosis. In the same study, Ferré et al. concluded that there is a genetic relationship between chronic hepatitis C infection and the PON1-192R allele polymorphism (Ferré et al. 2005). In another study, it was determined that the PON1-192Q allele has stronger effect on hydrolyzing lipid peroxides than the PON1-192R allele (Aviram et al. 2000). In chronic liver diseases, various hypotheses have been proposed as to why serum PON1 activity is reduced (Aviram et al. 1998). Patients suffer from liver damage if they have increased free radicals; this may inactivate PON1 by hydrolyzing lipid peroxides. Changes in HDL structure related to PON1 may effect PON1 activity. When PON1 synthesis is altered by the liver, PON1 activity may decrease accordingly. Changes in serum PON1 levels with alcohol intake depend on the degree of hepatic damage (Deakin et al. 2005).

Studies performed so far have shown that altered serum PON1 levels due to alcohol consumption are not a major factor in being affected by PON1 genetic

polymorphisms (Costa et al. 2005). In the study among chronic alcohol abusers, subjects were grouped according to their level of hepatic disease. The case studies performed have shown that the serum PON1 activity level is reduced in alcoholic individuals and the level of alcohol dependence is correlated with the extent of liver dysfunction. Also, in the same study, an inverse correlation was found between plasma malondialdehyde concentration and serum PON1 activity level in severe chronic alcoholic individuals (Marsillach et al. 2007).

Different nonphysiological substrates can be used to measure PON1 activity, as there is not yet a "natural" substrate for paraoxonase 1. Substrates, especially in the form of OP, are very toxic. The paraoxon substrate, after which it is named, has an LD50 of 0.5 ppm and is not healthy to measure for automated analytical assay systems. Using highly toxic pesticide-derived substrates to measure PON1 activity, which is one of the tests used in the determination of liver damage, is a great dilemma. Fortunately, new healthier method for serum PON1 activity measurement was developed, such as lactonase activity, using nontoxic substrate as homocysteine thiolactone (HTL), which makes it more practical to propose as a biomarker for liver toxicity (Marsillach et al. 2008).

Fatty liver disease in dairy-type cows is a very serious metabolic syndrome that affects most of the herd after calving. It is often associated with low immunity and fertility levels and can even lead to death from liver failure if left untreated (Katoh 2002). For the determination of fatty liver in dairy cows, serum PON1 testing will have an important clinical impact. General biochemical tests to detect liver dysfunction are not sufficient to diagnose fatty liver in dairy cows (Bobe et al. 2004). Therefore, over time, the definitive way to diagnose fatty liver has become the pathophysiological examination of the liver – biopsy. However, this is a costly procedure for dairy farms. Serum PON1 activity measurement to biochemical tests indicates that it will have greater impact in the dairy industry (Mackness et al. 1996).

In a study in dairy cows, serum PON1 activity (arylesterase, paraoxonase, and lactonase) and other hematological and biochemical parameters were measured in Holstein-Friesian cows with 46 fatty liver cases and 46 healthy cows as control group. It was found that serum PON1 activity levels were lower in dairy cows with fatty liver, affecting fertility and immune function, than in healthy cows (Bobe et al. 2004).

Determination of serum PON1 activity as a biomarker has been suggested to be useful in assessing the degree of liver failure. Measurement of serum PON1 level is a powerful diagnostic effect to distinguish patients with liver disease from healthy control subjects. When serum PON1 activity is added to known liver function tests, it will make more reliable contributions in increasing the general sensitivity (Ferre et al. 2005; Camps et al. 2007).

In addition, paraoxonase 1 is closely associated with the improvement of liver function. The value of serum PON1 activity and its effects in patients with severe liver damage and requiring transplant therapy were investigated (Xu et al. 2005). Serum PON1 activity levels were found low when hepatic arteries occluded in liver transplant patients, but it also tended to increase. This finding indicates that the fact

that PON1 activity is included in routine liver function tests will also be useful in determining whether liver transplantation is successful or not.

Key Facts of Cirrhosis

- It is the name given to severe damage to the liver.
- It is caused by many liver diseases such as hepatitis and chronic alcoholism.
- Cirrhosis slows the normal blood flow in the liver and thus increases the pressure in the vessel that brings the blood from the intestines and spleen to the liver.
- The liver damaged by cirrhosis may become unable to clear toxins. Accumulation of these toxins in the brain can cause confusion and difficulty concentrating.

Key Facts of Coronary Heart Disease (CHD)

- It is the name given to diseases caused by problems that block the vessels that feed the heart.
- Some plaques called "hardening of the arteries" can prevent the flow of the blood in the vessels.
- The most common symptom of the disease is chest pain.
- The most important complication of CHD is heart attack.

Mini-dictionary of Terms

Antioxidant: It is a compound that repairs damage at the cellular level caused by reactive oxygen radicals.

Apo-A1: It is one of the apo proteins found on HDL and interacts with cells to mediate cholesterol ester delivery.

Atheroprotective: It means that it protects against the formation of atherosclerosis.

HDL (high-density lipoprotein): It provides the transport of cholesterol from tissues and vessels to the liver.

Km: It is the unit that indicates the affinity of the enzyme for its substrate and equal to the substrate concentration required to reach half the maximum reaction rate.

Malondialdehyde: A specific or quantitative indicator of fatty acid oxidation correlates well with the degree of lipid peroxidation. It can be detected in the blood or urine.

Substrate: A molecule that binds to the active site of an enzyme and chemically converts it to a product.

Vmax: It is the reaction rate reached when the active site of the enzyme is completely saturated by the substrate.

Summary Points

So far, studies with experimental animals and case studies indicate the need for more reliable testing of the diagnosis of liver toxicity, lipid oxidation, and organophosphate exposure. Paraoxonase 1 (PON1) is a high-density lipoprotein (HDL)-associated serum enzyme that can catalyze a wide range of substrates. The reason why PON1 is discussed in this section as a possible toxicological biomarker in the future is because of the superior properties of this enzyme. Paraoxonase protects against exposure to some organophosphorus (OP) pesticides by hydrolyzing toxic metabolites such as oxon and sarin. Also, PON1 is effective in protecting against cardiovascular diseases by metabolizing oxidized lipids. Not only serum PON1 level but also PON1 polymorphism gives preventive and precautionary opinions about toxicity susceptibility of individuals. Some studies suggest that adding serum PON1 activity analyses in standard biochemical tests, like other liver enzymes, may be effective in assessing the degree of liver failure and the degree of fatty liver, not only providing diagnosis. In the future, screening for PON1 status prior to illness may be very useful, particularly to identify individuals with major risk factors or to investigate the effects of OP exposure for agricultural workers.

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