Cellular and Molecular Biology

Journal homepage: www.cellmolbiol.org

The relationship of hs-CRP, vitronectin and NT-proBNP serum levels with the extent and severity of cardiac complications in patients with organophosphate pesticide poisoning

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ARTICLE INFO

Original paper

Article history:
Received: August 18, 2021
Accepted: November 16, 2021
Published: December 01, 2021

Keywords:
Hs-CRP, Vitronectin, NT-proBNP, Organophosphate, Myocardial Injury

ABSTRACT

Acute organophosphate poisoning kills tens of thousands of people annually around the world. These substances are widely used as insecticides in homes, industry, and agricultural environments. Due to the ease of access, they can cause accidental or intentional risks of exposure through the skin or respiratory contact. This study aimed to evaluate the serum levels of hs-CRP, Vitronectin, and NT-proBNP and their relationship with the extent and severity of cardiac complications in patients with organophosphate pesticide poisoning. In this descriptive-comparative study, 160 patients were studied with acute organophosphate poisoning. Also, for better comparison, 40 healthy individuals participated in this study. Diagnosis of organophosphate poisoning was based on clinical findings of serum butyrylcholinesterase levels. The hs-CRP measurement was performed by an autoanalyzer (Abbott, model Alcyon 300, USA) with the ELISA hs-CRP kit (The apDia Company, Belgium). Vitronectin (VN) measurements were performed by ELISA method and Glory science human VN kit with Catalog No: 11668. NT-ProBNP serum levels were analyzed by ProBNP assay kit (Roche, Germany) by ECLIA method using Elecsys 2010 Analyzer. The most important variables studied in this study were the electrical activity and conduction system of the heart, PR distance, QTC interval, and T-wave changes. In this study, most of the patients were women and girls (60.78%). The highest percentage of organophosphate poisoning was in the age group of 15-24 years (37.25%). In most cases (78.43%), poisoning was intentional or suicidal. Evaluation of electrophysiological abnormalities of the heart showed that 89 patients (55.62%) had long QTC interval (>450 msec), 43 cases (26.87%) had possible long QTC (431-450 msec), and 28 cases (17.5%) had normal QTC (<430 msec). Only 9.37% of cases (n = 15) showed an increase in P-R distance, which is characteristic of the first-degree ventricular atrial block. Sinus bradycardia occurred in 57 cases (35.62%) and sinus tachycardia in 43 cases (26.87%); in 60 cases (37.5%), the pulse rate was normal. Smooth T-wave changes were observed in 9.8% of patients and reverse T-wave was observed in 17.6%. A long T-wave was not reported in any case. In only two cases (1.25%) was grade 1 ventricular atrial block and grade 2 and 3 blocks were not observed. In general, there was a significant difference in the hs-CRP, vitronectin, and NT-proBNP serum levels between the patient and control groups in all studied variables. These parameters were also related to the extent and severity of the disease.

DOI: http://dx.doi.org/10.14715/cmb/2021.67.4.26

Introduction

There are more than 100 organophosphate-containing compounds on a large commercial scale in the world with different formulations used as insecticides in agriculture, animal husbandry, and home use (1). These compounds usually consist of phosphate esters, including a central phosphate atom and three sub-organic chains, two of which are ethyl or methyl, and the other, which is more specific, is used to kill insects. In most organophosphate insecticides, groups R1 and R2, occupied by methyl or ethyl groups, are often used as "thio" (P=S) (2). In this place, oxidative metabolism is carried out, and thus the combination of organophosphate or anticholinesterase (in the form of P=0) is activated (3). The side groups are variable and occur through the bond with phosphorus or directly (Phosphinates), through oxygen (phosphates), or nitrogen (phosphoramidites). The X substituent or leaving group is separated and hydrolyzed to release the enzyme after the organophosphorus phosphorylates acetylcholinesterase (4).

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Organophosphate toxin inhibits cholinesterase by phosphorylation of hydroxyl serine in the enzyme (1). Inhibition of acetylcholinesterase activity in blood, brain, and tissues is time-dependent (4). The extent of acetylcholinesterase inhibition depends on the constant amount of enzyme and the binding time of the toxin. This inhibition leads to acetylcholine accumulation in the autonomic ganglia and post-ganglionic nerve endings, central synapses, and the muscle nerve junction (5).

Organophosphate poisoning often causes heart problems, which can be severe and often fatal. Organophosphate toxins affect the electrical activity and conduction system of the heart (6). Cardiac manifestations of organophosphate poisoning include sinus bradycardia, sinus tachycardia, torsade de pointe polymorphic ventricular tachycardia, hypertension or hypotension, and pulmonary edema of non-cardiac origin (1).

Insecticide with cholinesterase inhibitors, such as organophosphates, can cause cardiac dysrhythmias by increasing acetylcholine, causing tachycardia or bradycardia, depending on whether the muscarinic or nicotine effects are superior (7). Increased arterial pressure activates baroreceptors in the aortic arch and carotid sinus and affects the initial heart rate (8). Stimulation of the vagus nerve in the center of the vagus and motor also increases the release of acetylcholine, which in turn causes a potential change in the atrial sinus node, followed by a decrease in heart rate and cardiac output (9). Atrial fibrillation, premature ventricular contractions, ventricular tachycardia, and prolonged QT_C interval are also seen, which can cause fatal cardiac arrhythmias and cardiac arrest (10).

In recent years, elevated serum levels of hs-CRP, vitronectin, and NT-proBNP are independent and potent predictors of heart disease and cardiac complications (11-13). Vitronectin (VN) is a vital plasma protein present in platelets and the extracellular matrix of many tissues. VN binds to several ligands, including integrin, plasminogen activator inhibitor-1 (1-PAI), urokinase receptor, collagen, complement 7-C5b, and heparin (14). These interactions suggest that VN plays an essential role in cell adhesion, migration, homeostasis, and immune defense in various biological stages (15). Thus, VN creates our unique regulatory link between cell adhesion and physiological proteolysis. VN may also play a role in clearing and controlling clotted blood vessels. In this way, the 1-PAI load is bonded and stabilized (16). 1-PAI is a crucial regulator of fibrinolysis. It should be noted that WN also binds to platelet glycoproteins (lla/lib) and VPS, which may act as an intermediate in platelet adhesion and accumulation at sites of vascular injury (17).

CAD-related risk factors are also closely related to endothelial dysfunction (18). Lipid peroxidation and inflammation both work together to play an essential role in the development of atherosclerosis (19). Studies show that in addition to vitronectin, hs-CRP, like other risk factors, plays a role in this process (11-13). Also, proBNP is a cardiac natriuretic peptide synthesized and secreted in response to increased ventricular wall stress in the ventricular myocardium (20).

In the present study, we investigated the serum levels of hs-CRP, Vitronectin, and NT-proBNP and their association with the extent and severity of cardiac complications in patients with organophosphate pesticide poisoning.

Materials and methods

This descriptive-comparative study was performed on 200 patients for three years in the hospital poisoning center. The 160 patients were considered as the patient group and 40 patients as the control group. The PASS software was used to determine the sample size. In this study, the diagnosis of organophosphate poisoning was based on obtaining a history from the patient, those around the patient and measuring serum butyrylcholinesterase.

Frequency distribution in terms of sex, age frequency distribution, frequency distribution according to forms of poisoning, evaluation of number and percentage of patients with respiratory problems during and after admission, pulse changes at the entrance, effects of organophosphate poisoning on cardiac electrical activity, distance changes P-R, QTc interval changes, ST-segment changes, T wave changes and ventricular atrial blocks were measured by electrocardiography using standard methods (21).

To measure hs-CRP in this experiment, which was performed by the immuno-turbidometric method for two-point measurement with a photometer, the CRP in the patient sample with polyclonal antibody against

human CRP attached to latex particles, forms a data complex and creates turbidity. The amount of generated turbidity is directly related to the amount of CRP in the patient sample (22). Hs-CRP measurement of the studied samples was performed using an autoanalyzer (Abbott, model Alcyon 300 USA) with the ELISA hs-CRP kit (The apDia Company, Belgium).

Vitronectin (VN) measurements were performed by ELISA method and Glory science human VN kit with Catalog No: 11668. Quantitative measurements of human VN can be performed on serum, plasma, tissue, and cultured cell samples. The VN kit is based on standard sandwich ELISA technology (23). VN-specific monoclonal antibodies are coated on the surface of the wells. Human VN-specific antibodies have been biotinylated. Diagnostic biotinylated samples and antibodies are added to the wells. Strepto-avidin HRP complex is also added to form the immune complex, and unconnected conjugates are washed by rinsing with PBS or TBS. The substrate used (TMB) is chromogen A, B to detect HRP enzyme activity. HRP catalyzes TMB to produce a blue color that eventually turns yellow by adding an acidic stop solution. The yellow concentration or OD indicates the amount of VN.

NT-ProBNP serum levels were analyzed by ProBNP assay kit (Roche, Germany) by ECLIA method using Elecsys 2010 Analyzer (24). The ProBNP Cut off point curve was used to determine the ROC value.

Data were analyzed using SPSS software version 16. Findings were shown as mean ± standard error. The student's t-test was used to compare the mean between patients and the control group, and a one-way analysis of variance (ANOVA) was used inside the patient group. $P < 0.05$ was considered statistically significant. Tukey's mean comparison test was performed for intra-group comparison.

Results and discussion

In this study, most of the patients with acute poisoning were women and girls (60.78%), indicating the prevalence of suicide in women. The highest percentage of organophosphate poisoning was in the age group of 15-24 years (37.25%) and then the age group of 25-45 years (33.33%), which can be due to puberty and psychological crises or due to their lack of understanding by parents or teachers. In most cases (78.43%), the poisoning was intentional or suicidal, one of the reasons being easy to access and ensuring that it was fatal.

In most cases (94.12%), organophosphate toxins were used orally and 5.88% of cases by inhalation. It should be noted that all cases of occupational poisoning occurred through inhalation, which could be due to non-use or misuse of safety devices while working with organophosphate toxins and insufficient knowledge of the dangers of the poison. The most common pulmonary complications were increased pulmonary secretion (45.10%) and acute pulmonary edema (Rawls) (33%).

The effects of organophosphate toxins on the electrical activity of the heart and conduction system showed that sinus bradycardia occurred in 57 cases (35.62%) and sinus tachycardia in 43 cases (26.87%); in 60 cases (37.5%), the pulse rate was normal. Table 1 assesses serum levels of hs-CRP, Vitronectin, and NT-proBNP and their association with electrical activity and the heart's conduction system.

In this study, only 9.37% of cases (n = 15) showed an increase in P-R distance, which is characteristic of the first-degree ventricular atrial block. Table 2 shows the association of hs-CRP, Vitronectin, and NT-proBNP serum levels with P-R distance in patients and the control group.

In the patient group, 89 patients (55.62%) had long QT_C interval (>450 msec), 43 cases (26.87%) had possible long QT_C (431-450 msec), and 28 cases (17.5%) had normal QT_C (<430 msec). Long QT_C intervals can cause fatal cardiac arrhythmias and cardiac arrest (25). Table 3 shows the relation of hs-CRP, vitronectin, and NT-proBNP serum levels with QT_C interval.

Smooth T-wave changes were observed in 9.8% of patients and reverse T-wave was observed in 17.6%. A long T-wave was not reported in any case. In only two cases (1.25%) was grade 1 ventricular atrial block and grade 2 and 3 blocks were not observed. Organophosphate compounds undergo many biotransformation reactions after absorption into the body. Because organophosphate compounds are lipophilic, they are readily permeable to the skin (2). Biotransformation reactions are mainly directed towards forming a more polarized compound so that the kidneys can excrete the compound.

Table 1. Association of hs-CRP, Vitronectin, and NT-proBNP serum levels with electrical activity and conduction system of the heart in patient and control groups

Variable	Patient Group (n=160)			Control group (n=40)	P-value
	Sinus Bradycardia	Sinus Tachycardia	Normal Pulse Rate		
The hs-CRP (mg/l)	5.4±0.4[b]	7.6±0.8[a]	4.8±0.6[b]	1.9±0.3[c]	0.04
Vitronectin (ng/l)	390±28[b]	430±42[a]	405±31[ab]	228±32[c]	<0.001
NT-proBNP (pg/ml)	1624±647[a]	1547±638[b]	1603±529[ab]	469±221[c]	<0.0001

In the Tukey test, common letters mean no significant difference. The significant level is P<0.05

Table 2. Association of hs-CRP, Vitronectin, and NT-proBNP serum levels with P-R distance in patient and control groups

Variable	Patient Group (n=160)		Control group (n=40)	P-value
	With P-R distance	Without P-R Distance		
The hs-CRP (mg/l)	8.4±4.1[a]	4.3±1.7[b]	1.9±0.3[c]	<0.001
Vitronectin (ng/l)	388±33[a]	372.27[a]	228±32[b]	<0.001
NT-proBNP (pg/ml)	1924±701[a]	1414±554[b]	469±221[c]	<0.0001

The significant level is P<0.05

Table 3. Association of hs-CRP, Vitronectin, and NT-proBNP serum levels with QT_C interval in patient and control groups

Variable	Patient Group			Control group	P-value
	QTc>450 msec	431≤QTc<450 msec	QTc<430 msec	QTc<430 msec	
The hs-CRP (mg/l)	7.8±1.1[a]	6.9±0.9[ab]	4.5±0.7[b]	1.9±0.3[c]	<0.001
Vitronectin (ng/l)	431±29[a]	388±22[ab]	335±33[b]	228±32[c]	<0.001
NT-proBNP (pg/ml)	1865±206[a]	1881±405[a]	1368±343[b]	469±221[c]	<0.0001

In the Tukey test, common letters mean no significant difference. The significant level is P<0.05

In biological terms, organophosphate compounds may be converted to specific metabolites whose toxicity is modifiable (4). Except for phosphates and phosphonates, most organophosphate compounds must be metabolized to act or exhibit deficient inhibition toward acetylcholinesterase (AChE) (1). Although in the living organism, the power of anticholinesterase can be significantly increased and lead to toxic effects, overall, it is biochemically beneficial for detoxification. Sometimes biotransformation can activate organophosphate compounds or convert them to other more active compounds (26). But the primary purpose of detoxification of organophosphate compounds will be to convert them into non-toxic metabolites (27). Sometimes biotransformation of organophosphate compounds can produce highly toxic metabolites even if the amount of metabolites formed in these reactions is low. They will have a special place in terms of toxicology (28).

The effects of organophosphate compounds on human physiology are numerous and complex (28). These compounds inhibit several enzymes, including esterases (28, 29). Inhibition of acetylcholinesterase leads to the accumulation of acetylcholine in cholinergic synapses and interferes with the normal functioning of the autonomic, somatic, and central nervous systems (29). It will cause a range of clinical manifestations, called Acute Cholinergic Crisis. Special features manifest as stimuli of muscarinic, nicotine, and central nervous system (30). These symptoms can appear five minutes after ingesting large amounts of toxins and almost always occur within the first 12 hours. Muscarinic symptoms rarely appear more than 24 hours after eating (31). The findings in muscarinic stimulation are commonly referred to as "wet findings." Its characteristics are increased salivation, secretion, and shedding of tears, excessive secretion from the bronchi, incontinence of urine and feces, and vomiting (30). Bronchial contraction, meiosis, and cardiac complications are

the most critical signs of organophosphate poisoning (26, 30).

In the current study, the effects of organophosphate toxins on the electrical activity of the heart and conduction system showed that sinus bradycardia occurred in 57 cases (35.62%) and sinus tachycardia in 43 cases (26.87%); in 60 cases (37.5%), the pulse rate was normal. In terms of serum hs-CRP level, there was a significant difference between the patient and control groups (P = 0.04). Intragroup comparison of patients showed that the highest value was related to individuals with sinus tachycardia (7.6±0.8mg/l), which was significantly different from the two groups of sinus bradycardia (5.4±0.4mg/l) and normal pulse rate (4.8±0.6mg/l)(P <0.05). Most studies showed that hs-CRP is a sensitive systematic marker for inflammation and an important prognostic marker for cardiovascular risk (32). Recent research suggests that CRP can be produced within the smooth muscle cells of the coronary arteries. This event may directly lead to the expression of several mediators for the progression of the atherosclerotic process. Numerous articles have shown that increased CRP is a good predictor of a heart attack. Zhou *et al*. (33) in a meta-analysis study reported a significant increase in the hs-CRP serum level of the patient group compared to controls, which is consistent with the present study results.

Also, the results of the current study showed that there was a significant difference between the patient group and the control group in terms of serum vitronectin level (<0.001). Intragroup comparison of patients showed that the highest value was related to individuals with sinus tachycardia (430±42ng/l) and normal pulse rate 405±31ng/l). There was a significant difference between Sinus Bradycardia (390±28ng/l) and sinus tachycardia (P <0.05). Vitronectin is an indicator of blood platelet formation and adherent (34). Therefore, because heart rate rises during platelet formation, people with higher heart rates had higher vitronectin serum levels. Vitronectin is observed in the compression of human atherosclerotic plaques and is placed in atherosclerotic with pvc5 and pvc3 receptors (14). Damage to endothelial cell adhesion and proliferation as a result of vitronectin glycosylation may also cause endothelial dysfunction and vascular disease (35). In a study by Ekmekci *et al*. (36), 62 patients with CAD showed a significant increase in VN in patients compared to controls. A survey by Derer *et al*. (37) showed that an increase in vitronectin might be associated with recurrence in patients with coronary heart disease undergoing coronary artery disease. Our study also showed that the plasma level of vitronectin increased in coronary artery disease and was associated with the extent of the disease, i.e., the highest increase in serum VN levels was in patients with sinus bradycardia.

About NT-proBNP serum level, our results showed that there was a significant difference between the patient group and control group in terms of electrical activity and conduction system of the heart. Also, there was a significant difference between Sinus bradycardia (1624±647pg/ml) and sinus tachycardia (1547±638pg/ml) (P<0.05). In heart failure, NT-pro BNP is secreted from ventricular myocardial tissue in response to increased myocardial infarction. Thus, the higher level of this peptide causes a higher risk of a heart attack (38). The results of our study also showed that the rate of this factor increases with decreasing heart rate. BNP protein is a polypeptide with 32 amino acids and is secreted from the muscles of the heart and blood vessels. A small amount of a precursor protein called pro-BNP is constantly produced by the heart (39). The pro-BNP is then broken down by an enzyme called Corin to an active protein fragment called BNP and an inactive fragment, NT-pro BNP, released into the bloodstream (38). The Brain Natriuretic Peptide (BNP) test is a blood test that measures the level of a protein called Brain Natriuretic Peptide (BNP). When a person has heart failure, the level of BNP in their blood is higher than usual (40). BNP levels include diastolic dysfunction, acute coronary syndromes (very sensitive but nonspecific), hypertension with LVH, heart disease, atrial fibrillation, pulmonary embolism, pulmonary hypertension, sepsis, COPD, or hyperthyroidism (41). BNP was originally called Brain Natriuretic Peptide because it was first found in brain tissue. But it was later discovered that BNP is mainly produced by the left ventricle of the heart (the central part of the heart that is important in heart pumping), and its amount depends on pressure, blood volume, and the heart rate that pumps blood throughout the body (42, 43). The NT-pro BNP protein is a marker of the hormone BNP in the blood, which increases during strain and heart tension. When

the heart wall expands too much due to too much blood or the heart is damaged due to lack of blood flow, BNP levels rise, and NT-pro BNP rises (38).

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