



Role of enzyme alkaline phosphatase in parkinsons disease patients

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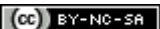
ABSTRACT

Parkinson's Disease (PD) is a debilitating illness associated with considerable impairment of quality of life and substantial cost of Health care systems. Our aim of this present study was to find the levels of alkaline phosphatase enzyme in Parkinson's disease patients for correlation. Methodology: Blood samples were collected from patient of parkinson's disease and also from Healthy volunteers in a government hospital by venipuncture and the serum was separated. The serum was then used for the estimation of Total protein by Lowry's method. The Results indicates the Alkaline phosphatase levels were found to be increased in the view of this present study correlates well with the finding of study. Hence we suggest for the cure of Neurodegenerative Disorder need further Research to confirm the cause of the elevated alkaline phosphatase level in Parkinson's Disease and to develop a newer approach as a novel therapy for Parkinson's Disease.

Keywords: Parkinson's Disease, Alkaline Phosphatase, Neurodegenerative Disease, Lowry's Method, L-DOPA, Spectrophotometer

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INTRODUCTION

The central nervous system consists of the brain and spinal cord. The brain is situated within the cranium and comprises a forebrain (prosencephalon), a midbrain (mesencephalon), and a hindbrain (rhombencephalon). The forebrain is subdivided into a telencephalon consisting of the two cerebral hemispheres laterally and a central part (the diencephalon) composed mainly of two thalami and the hypothalamus. The midbrain (mesencephalon) made up of crus cerebri, substantia nigra, tegmentum, tectum. The hindbrain is subdivided into the Pons, medulla and cerebellum. The midbrain Pons and medulla are collectively known as the brain stem. (1) There are many central nervous system diseases, including infections of the central nervous system such as encephalitis and poliomyelitis, neurodegenerative such as Alzheimer's disease, Parkinsonism, amyotrophic, autoimmune and inflammatory diseases such as multiple or acute disseminated encephalomyelitis, and genetic disorders such as Krabbe's disease, Huntington's disease, or adrenoleukodystrophy.

DEGENERATIVE DISEASE

These are diseases of gray matter characterized principally by the progressive loss of neurons with associated secondary changes in white matter tracts. Two other general characteristics bring them together as a group. First, the pattern of neuronal loss is selective, affecting one or more groups of neurons while leaving others intact. Second, the diseases arise without any clear inciting event in patients without previous neurologic deficits. The neuropathology findings observed in the degenerative diseases differ greatly; in some, there are intracellular abnormalities with some degree of specificity (e.g., Lewy bodies, neurofibrillary tangles), while in others, there is only loss of the affected neurons. It is convenient to group the degenerative diseases according to the anatomic regions of the CNS that are primarily affected. Some degenerative diseases have prominent involvement of the cerebral cortex, such as Alzheimer disease; others are more restricted to subcortical areas and may present with movement disorders such as tremors and dyskinesias. As genetic and molecular studies of these diseases have progressed, there has been recognition of shared features across many of the disorders.

A common theme among the neurodegenerative disorders is the development of protein aggregates that are resistant to normal cellular mechanisms of degradation through the ubiquitin-proteasome system. These aggregates can be recognized histologically as inclusions, which often form the diagnostic hallmarks of these different diseases.

The basis for aggregation varies across diseases. For example, it may be directly related to an intrinsic feature of a mutated protein (e.g., expanded polyglutamine repeat in Huntington disease), a feature of a peptide derived from a larger precursor protein (e.g., A β in Alzheimer disease), or an unexplained alteration of a normal cellular protein (e.g., α -synuclein in sporadic Parkinson disease). The aggregated proteins are generally cytotoxic, but the mechanisms by which protein aggregation is linked to cell death may be different in these various diseases.

DEGENERATIVE DISEASES OF BASAL GANGLIA AND BRAINSTEM

Diseases affecting these regions of the brain are frequently associated with movement disorders, including rigidity, abnormal posturing, and chorea. In general, they can be categorized as manifesting either a reduction of voluntary movement or an abundance of involuntary movement. The basal ganglia and especially the nigrostriatal pathway play an important role in the system of positive and negative regulatory synaptic pathways that serve to modulate feedback from the thalamus to the motor cortex. The most important disorders in this group are those associated with Parkinsonism and Huntington chorea. (2)

ALKALINE PHOSPHATASE

(ALP, ALKP) (EC 3.1.3.1) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called *dephosphorylation*. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. It is sometimes used synonymously as **basic phosphatase**.

PHYSIOLOGY

In humans, alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone, and the placenta. Humans and most other mammals contain the following alkaline phosphatase isozymes:

- ALPI – intestinal
- ALPL – tissue non-specific (liver/bone/kidney)
- ALPP – placental (Regan isozyme)

INTESTINAL ALKALINE PHOSPHATASE

Intestinal alkaline phosphatase is secreted by enterocytes and seems to play a pivotal role in intestinal homeostasis and protection as well as in mediation of inflammation via repression of the downstream toll-like receptor (TLR)-4-dependent and MyD88-dependent inflammatory cascade. It

dephosphorylates toxic/inflammatory microbial ligands like lipopolysaccharides, unmethylated cystosine –guanine dinucleotides, flagellin and extracellular nucleotides such as uridine diphosphate or ATP. Thus, altered IAP expression has been implicated in chronic inflammatory diseases such as IBD. It also seems to regulate lipid absorption and bicarbonate secretion in the duodenal mucosa, which regulates the surface pH.

DIAGNOSTIC USE

The normal range is 20 to 140 IU/L. High ALP levels indicate blocked bile ducts. Levels are significantly higher in children and pregnant women. Also, elevated ALP indicates that there could be active bone formation occurring as ALP is a byproduct of osteoblast activity (such as the case in Paget's disease of bone).

Lowered levels of ALP are less common than elevated levels. Typical use in the lab for alkaline phosphatases includes removing phosphate monoester to prevent self ligation.

ELEVATED LEVELS

If it is unclear why alkaline phosphatase is elevated, isoenzyme studies using electrophoresis can confirm the source of the ALP. Heat stability also distinguishes bone and liver isoenzymes ("bone burns, liver lasts"). Placental alkaline phosphatase is elevated in seminoma and active form of Rickets, as well as in the following diseases and conditions.

Biliary obstruction
 Bone conditions
 Osteoblastic bone tumors
 Osteomalacia
 Osteoporosis
 Hepatitis
 Cirrhosis
 Hyperthyroidism
 Myocardial Infarction
 Pregnancy

LOWERED LEVELS

The following conditions or diseases may lead to reduced levels of alkaline phosphatase:

- Hypophosphatasia, an autosomal recessive disease
- Postmenopausal women receiving estrogen therapy because of osteoporosis
- Men with recent heart surgery, malnutrition, magnesium deficiency, hypothyroidism, or severe anemia
- Children with achondroplasia and cretinism
- Children after a severe episode of enteritis
- Pernicious anemia
- Aplastic anemia
- Chronic myelogenous leukemia
- Wilson's disease

In addition, the following drugs have been demonstrated to reduce alkaline phosphatase:

- Oral contraceptives

Leukocyte alkaline phosphatase:

Leukocyte alkaline phosphatase (LAP) is found within white blood cells. White blood cell levels of LAP can help in the diagnosis of certain conditions.

- Higher levels are seen in polycythemia vera (PV), essential thrombocytosis (ET), primary myelofibrosis (PM), and the leukemoid reaction.
- Lower levels are found in chronic myelogenous leukemia (CML), paroxysmal nocturnal hemoglobinuria (PNH) and acute myelogenous leukaemia (AML).(4)

PARKINSONISM

Parkinsonism is a clinical syndrome known to occur in elderly people and form 1% of neurodegenerative disease. It is characterized by diminished facial expression, stooped posture, slowness of voluntary movement, festinating gait (progressively shortened, accelerated steps), rigidity, and a "pill-rolling" tremor. This type of motor disturbance is seen in a number of conditions that have in common damage to the nigrostriatal dopaminergic system. Parkinsonism may also be induced by drugs that affect this system, particularly dopamine antagonists and toxins. The principal diseases to be discussed here that involve the nigrostriatal system are as follows:

PARKINSON DISEASE (PD)

Multiple system atrophy, a disorder that may have Parkinsonism as a prominent symptom (clinical presentation as striatonigral degeneration) as well as other symptoms (cerebellar ataxia and autonomic dysfunction). Post encephalitic Parkinsonism, which was observed in the wake of the influenza pandemic that occurred between 1914 and 1918 and is now vanishingly rare. Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), which are movement disorders that may also exhibit cognitive impairment; they share some pathologic and genetic features with each other and with other tauopathies (see the discussion above in the section on front temporal dementias).

This diagnosis is made in patients with progressive parkinsonism in the absence of a toxic or other known underlying etiology. Familial forms with autosomal-dominant or autosomal-recessive inheritance exist. Although these make up a limited number of cases, they have contributed to our understanding of the pathogenesis of the disease. In addition to the movement disorder, there are other,

less well-characterized changes in mental function, which may include dementia, in a subset of individuals with Parkinson's disease. Symptoms of Parkinson's disease include manifestation of Dopamine in the substantia nigra in the tremor, rigidity, Bradykinesia, postural instability, Difficulty in swallowing and speech, urinary and other bowel problem leads to other cognitive abnormal problem fatigue is also. The hallmark of Parkinson's disease is neurodegenerative of dopamine in substantia nigra lead to dopamine and cholinergic imbalance.(3)

Pathological the etiopathogenesis of Parkinson's disease characterized by deposition of abnormal α -synuclein in the neuron of substantia nigra which have under neurodegenerative. The pathology of the condition has suggested the use of dopamine in the form of L-DOPA as standard treatment in these patients .other amyloiting drug are also being consider .However treatment of Parkinson's disease with respect to rapid alleviation of symptom remains a difficult. A similar challenge is found is faced in treating patients of younger age group[less than 40 years] ,which have been increasingly observed although free radical changes and metal toxicity have implicated along with the genetic role in which protein implicated are α -synuclein and parkin.

Other treatment modalities include neurosurgical approach of where lesion in the extrapyramidal system has compensatively nigrostatic function by provide motor symptom in Parkinson's disease.

Symptomatic response to l-DOPA therapy is one of the features, in addition to clinical signs and symptoms, which support a diagnosis of PD. While l-DOPA therapy is often extremely effective in symptomatic treatment, it does not significantly alter the intrinsically progressive nature of the disease. Over time, l-DOPA becomes less able to help the patient through symptomatic relief and begins to lead to fluctuations in motor function on its own. As a result, there has been a search for alternative therapies that might alter the disease course. Given the well-characterized biochemical defect in PD, therapy through neural transplantation has been attempted. Clinical improvement has been reported in patients with PD or MPTP-induced Parkinson disease treated with stereotactic implants of fetal mesencephalic tissue into the striatum. Other current treatment modality includes neurosurgical approach where lesion in the extrapyramidal system has compensatively nigrostatic function by placement of stimulative electrode which could provide motor symptoms in Parkinson disease. Alkaline phosphates to occur ubiquitously: its elevated level are more common than lower level .Liver, bile duct, kidney, Bone and

all tissue but in lower concentration .its is also seen to be lower in neurodegeneration disorder Wilson and anemia, plastic anemia, bone disorder and Gastric intestinal tract disorder .the wide range of distribution of this enzyme prompts in it investigation in Parkinson's Disease. (2)

MATERIALS AND METHOD

Sample Collection:

The sample 1ml were collected from patient of Parkinson's disease (5 patients) in a government hospital by venipuncture and kept in a slanting position for one hour for the separation of serum. This serum was then centrifuged at 2000 r.p.m for 5 minutes and then was used for the estimation of total protein by Lowry's method. The samples were collected from healthy volunteers for control (5 samples).

INSTRUMENTS USED:

Various bio-instruments are used in the lab which facilitates the biochemistry test. The bio-instruments used in the laboratory as follows,

1. Centrifuge
2. Spectrophotometer
3. P^H meter

CENTRIFUGE:

A laboratory centrifuge is a piece of laboratory equipment, driven by a motor, which spins liquid samples at high speed. There are various types of centrifuges, depending on the size and the sample capacity.

Centrifuge tubes or centrifuge tips are tapered tubes of various sizes made of glass or plastic. They may vary in capacity from tens of milliliters, too much smaller capacities used in micro centrifuges used extensively in molecular biology laboratories. The most commonly encountered tubes are of about the size and shape of a normal test tube (~ 10 cm long). Micro centrifuges typically accommodate micro centrifuge tubes with capacities from 250 all to 2.0ml. These are exclusively made of plastic.

Glass centrifuge tubes can be used with most solvents, but tend to be more expensive. They can be cleaned like other laboratory glassware, and can be sterilized by autoclaving. Plastic centrifuge tubes, especially micro centrifuge tubes tend to be less expensive. Water is preferred when plastic centrifuge tubes are used. They are more difficult to clean thoroughly, and are usually inexpensive enough to be considered disposable.

The load in a laboratory centrifuge must be carefully balanced. This is achieved by using a combination of samples and balance tubes which all have the same weight or by using various

balancing patterns without balance tubes. Small differences in mass of the load can result in a large force imbalance when the rotor is at high speed. This force imbalance strains the spindle and may result in damage to the centrifuge or personal injury. Some centrifuges have an automatic rotor imbalance detection feature that immediately discontinues the run when an imbalance is detected.

Before starting a centrifuge, an accurate check of the rotor and lid locking mechanisms is mandatory. Centrifuge rotors should never be touched while moving, because a spinning rotor can cause serious injury. Modern centrifuges generally have features that prevent accidental contact with a moving rotor as the main lid is locked during the run.

Centrifuge rotors have tremendous kinetic energy during high speed rotation. Rotor failure, caused by mechanical stress from the high forces imparted by the motor, can occur due to manufacturing defects, routine wear and tear, or improper use and maintenance. Such a failure can be catastrophic failure, especially with larger centrifuges, and generally results in total destruction of the centrifuge. While centrifuges generally have safety shielding to contain these failures, such shielding may be inadequate, especially in older models, or the entire centrifuge unit may be propelled from its position, resulting in damage to nearby personnel and equipment. Uncontained rotor failures have shattered laboratory windows and destroyed refrigerators and cabinetry. To reduce the risk of rotor failures, centrifuge manufacturers specify operating and maintenance procedures to ensure that rotors are regularly inspected and removed from service or derated (only operated at lower speeds) when they are past their expected lifetime.

UV- VISIBLE SPECTROPHOTOMETER: PRINCIPLE:

The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how quickly the absorbance changes with concentration. This can be taken from references (tables of molar extinction coefficients), or more accurately, determined from a calibration curve.

Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. It is more specific than the general term electromagnetic spectroscopy in that spectrophotometry deals with visible light, near-ultraviolet, and near-infrared, but

does not cover time-resolved spectroscopic techniques.

Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer that can measure intensity as a function of the light source wavelength. Important features of spectrophotometers are spectral bandwidth and linear range of absorption or reflectance measurement.

A spectrophotometer is commonly used for the measurement of transmittance or reflectance of solutions, transparent or opaque solids, such as polished glass, or gases. However they can also be designed to measure the diffusivity on any of the listed light ranges that usually cover around 200nm - 2500nm using different controls and calibrations. Within these ranges of light, calibrations are needed on the machine using standards that vary in type depending on the wavelength of the photometric determination. An example of an experiment in which spectrophotometer is used is the determination of the equilibrium constant of a solution. A certain chemical reaction within a solution may occur in a forward and reverse direction where reactants form products and products break down into reactants. At some point, this chemical reaction will reach a point of balance called an equilibrium point. In order to determine the respective concentrations of reactants and products at this point, the light transmittance of the solution can be tested using spectrophotometry. The amount of light that passes through the solution is indicative of the concentration of certain chemicals that do not allow light to pass through.

The use of spectrophotometers spans various scientific fields, such as physics, materials science, chemistry, biochemistry, and molecular biology. They are widely used in many industries including semiconductors, laser and optical manufacturing, printing and forensic examination, and as well in laboratories for the study of chemical substances. Ultimately, a spectrophotometer is able to determine, depending on the control or calibration, what substances are present in a target and exactly how much through calculations of observed wavelengths UV-visible spectrophotometry

The most common spectrophotometers are used in the UV and visible regions of the spectrum, and some of these instruments also operate into the near infrared region as well. Visible region 400–700 nm spectrophotometry is used extensively in colorimetry science. Ink manufacturers, printing companies, textiles vendors, and many more, need the data provided through colorimetry. They take readings in the region of every 5–20 nanometers along the visible region, and produce a spectral

reflectance curve or a data stream for alternative presentations. These curves can be used to test a new batch of colorant to check if it makes a match to specifications, e.g., ISO printing standards.

pH METER:

A pH meter is an electronic instrument used for measuring the pH (acidity or alkalinity) of a liquid (though special probes are sometimes used to measure the pH of semi-solid substances). A typical pH meter consists of a special measuring probe (a glass electrode) connected to an electronic meter that measures and displays the pH reading.

ALKALINE PHOSPHATASE

(ALP, ALKP) (EC 3.1.3.1) which is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. It was estimated on the bases of the below principle.

PRINCIPLE:

Phenol released by enzymatic hydrolysis at alkaline

(pH -10)disodium phenyl phosphatase is measured in spectrophotometer.

REAGENTS:

- 1. Sodium carbonate sodium bicarbonate buffer 0.1 (pH 10):** 3.18 anhydrous Sodium carbonate and 1.68g sodium bicarbonate in 500ml of
- 2. Disodium phenyl phosphate:** 0.001 In distilled water (used as substrate (prepare before use).
- 3. Folin and ciocalteus reagents:** Folin and ciocalteus reagents (2N phenol solutin SRL bamney) distilled water (prepare before use).
- 4. Sodium carbonate:** 20% (w/v) in distilled water.
- 5. Magnesium chloride:** Magnesium chloride 0.1M in distilled water.
- 6. phenol standard:** 100 mg of phenol in 100ml distilled water kept in refrigerated.

Reference standard:

Before use the stock standard diluted to yield 10 to the power-6-moles/ml with distilled water and standard graph constructed.

PROCEDURE:

To 0.05ml of serum and standard phenol (0.05-0.2 micromole) in 15x 150 mm test tubes ,1.5 ml of Sodium carbonate sodium bicarbonate buffer 0.1 (pH 10),1ml of Disodium phenyl phosphate and 0.1 Magnesium chloride were vortex well. The final volume was adjusted to 3ml with distilled water ,after incubation at 37*c for 15min ,1ml of folin and ciocalteus reagent was added to arrest the reaction blank was processed similarly but the serum added after arresting the reaction .The precipitate formed after addition of folins reagents was separated by centrifugation at 1000xg for 10 mins to the supernant 1.0 ml of 20% sodium

carbonate was added and added and left for 20min the intensity of blue color developed was measured at 640nm againt the blank in spectronic 20,spectrophotometer and the serum enzme activity expressed as IU/L.(6)

RESULT

Statistical analysis of the study is shown in Table 1, which showed the response of highly elevated Alkaline Phosphate level in the Test sample group when compared to the Control sample group. whereas the response is $P > 0.001$ with the degree of freedom 4 (Hence the null hypothesis is accepted)

DISCUSSION

In the present study five serum sample from known Parkinson's disease patients where estimated for alkaline phosphatase. There is no current evidence suggesting this enzyme alteration in Parkinson's disease. In addition the classical ethiopathogenic cause like α -synuclein, lewy body, metal, toxin, genetic causes are also implicated(9) .It remains to investigate the role of phosphatases in view of genetic abnormality

The study showed the highly elevated alkaline phosphatase level in the sample group when compared with normal serum levels alkaline phosphatase which is distributed widely and elevated in number of disorder like liver, bone disease and tumor as per previous studies.(10)

In one study (11) the bone density was measured using computerized radiography (CCT) in patients with Parkinson's disease with no other disorder and no other drugs administered .Alkaline phosphatase levels where found to be increased in view of this present study correlates well with the finding of study. It remains to confirm the cause of the elevated alkaline phosphatase levels in parkinson's disease.

CONCLUSION

The current study estimated alkaline phosphatase in a sample group of long standing Parkinson's disease patients with symptom and on treatment .However estimated showed elevated serum alkaline phosphatase level when compared to normal control. The increased alkaline phosphatase value was found to be more than the normal range of alkaline phosphatase in human serum ;20 to 140 IU/L. This suggest gross increase in the enzyme level and therefore requires consideration in view of the abnormality detected by alkaline phosphatase estimation and in relation to bone pathology in Parkinson's disease patients.

This study includes small population, in absence of other abnormality (includes drugs that could alter the serum alkaline phosphatase levels). Relevance of alkaline phosphatase in parkinson's disease

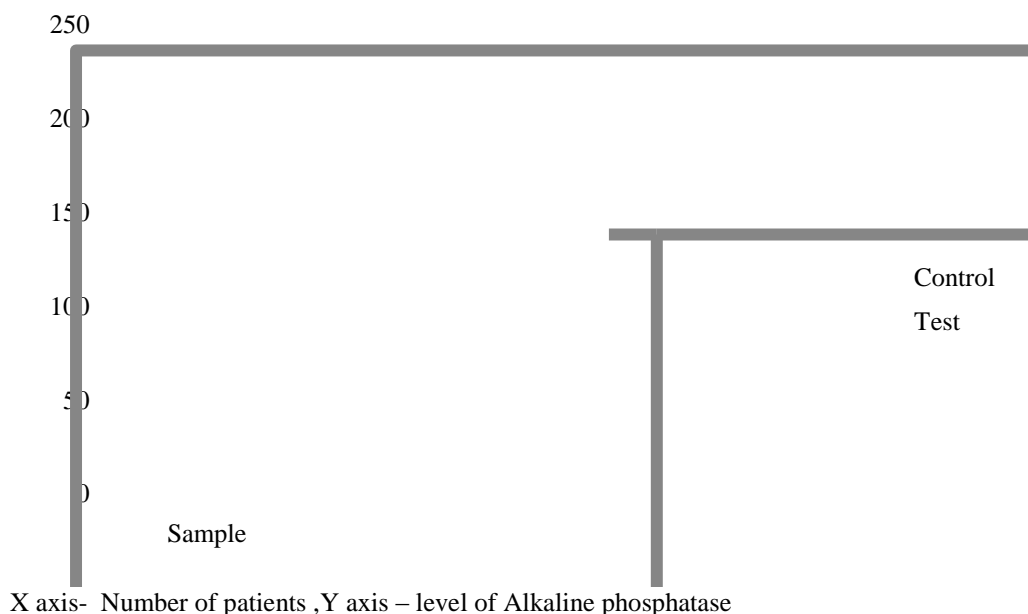
patients is doubtful but the altered levels (increase) suggests its estimation in the disorder in consideration with statistical correlation.

Table:1 Showing the Concentration Of Alkaline Phosphatase(IU/L) in control sample

S.NO	SAMPLE	Conc of alkaline phosphatase in IU/l
1.	Control-1	61.7647 IU/L
2.	Control-2	76.190 IU/L
3.	Control-3	71.428 IU/L
4.	Control-4	63.80 IU/L
5.	Control-5	60.952 IU/L

Table:2 Showing the Concentration Of Alkaline Phosphatase(IU/L) in test sample

S.NO	Sample ID	Conc of alkaline phosphatase in IU/L
1.	OPNO 121106	240 IU/L
2.	OPNO 25293	235.55 IU/L
3.	OPNO 17298	200 IU/L
4.	OPNO 3792	177.77 IU/L
5.	CDM NO72/11	248.88 IU/L



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