



## “ASSESSMENT OF SERUM BIOMARKERS WITH TREATMENT OUTCOME AMONG PATIENTS WITH IDIOPATHIC PARKINSON'S DISEASE IN A TERTIARY CARE HOSPITAL, KOLKATA”

### Biochemistry

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### ABSTRACT

**Objectives:** There is a pressing need for robust diagnostic & prognostic biomarkers in idiopathic Parkinson's disease. So far most of potential biomarkers have been studied in isolation. No study has yet been done taking all the biomarkers into consideration. Our study thus aimed to investigate the diagnostic & prognostic potential of a panel of biomarkers in IPD patients of West Bengal.

**Methods:** 55 untreated patients of clinically diagnosed idiopathic Parkinson's disease within the age group 50–80 years visiting in the neurology out-patient department of R. G. Kar Medical College and Hospital, were taken as cases, included in the study, based on a set of fixed inclusion & exclusion criteria. Serum TSH, fT3, Uric acid, Homocysteine were measured in diagnosed untreated cases and 3 & 6 months post treated cases of Idiopathic Parkinson's disease. Also correlation of above-mentioned biomarkers with disease severity (UPDRS 2) were also assayed. Thin layer chromatography was done with aromatic amino acids (Phenylalanine, Tyrosine and Tryptophan) in untreated cases of IPD as well as 3 & 6 months post treated too.

**Results:** The results of statistical analysis showed that levels of serum Uric acid (P value < 0.0001) and serum homocysteine (P value = 0.0003) were significantly raised in patients of IPD compared to their apparently healthy age and sex matched controls. We also found significantly raised value of serum level of TSH (P < 0.0001) & Uric acid (P value < 0.0001) and significantly lowered value of serum fT3 (P < 0.0001) in 3 months and 6 months post treated cases compared to untreated cases of IPD. A significant correlation was found between serum levels of fT3 (P value = 0.0344, Pearson r = 0.2857) with disease severity (UPDRS 2) of IPD.

**Interpretation:** Serum Uric acid and Homocysteine level is associated with increase risk factor of IPD. Serum TSH, Uric acid and fT3 would be a better upcoming diagnostic and prognostic marker for diagnosis of IPD patients.

### KEYWORDS

Idiopathic Parkinson's Disease, Updrs 2 Scoring System, statistically Significant P Value

### INTRODUCTION

Sporadic or Idiopathic Parkinson's disease (PD) is an age-related neurodegenerative disorder of unknown origin that ranks only second behind Alzheimer's disease (AD) in prevalence. Idiopathic Parkinson's Disease neuropathology is characterized by a selective loss of dopaminergic neurons in the substantia nigra pars compacta; however, more widespread involvement of other CNS structures and peripheral tissues now is widely documented. When proteins break down, elevated levels of amino acids like homocysteine may be found in the bloodstream. Changes in serum or plasma concentration of aromatic amino acids such as glutamate, large neutral amino acid (Phenylalanine, Tryptophan, Tyrosine) have been reported. Idiopathic Parkinson's disease (IPD) is an age-related neurodegenerative disorder of unknown origin that ranks only second behind Alzheimer's disease (AD) in prevalence. In Idiopathic Parkinson's Disease there is a pressing need for robust diagnostic & prognostic biomarkers. So far most of potential biomarkers have been studied in isolation. No study has yet been done taking all the biomarkers into consideration.

Our study thus aimed to investigate the diagnostic & prognostic potential of a panel of biomarkers in IPD patients of West Bengal. [1]. A recent study by Hirayama et al revealed high phenylalanine levels in de novo Parkinson's disease patients, but not in treated ones. Homocysteine levels increase in the body when the growing number of studies have correlated higher urate levels with a lower risk of developing Parkinson's disease (PD) and with a favorable rate of disease progression, indicating that urate could be an important biomarker of the pathophysiology underlying PD. [2] Dietary and genetic determinants of urate have also been linked to a reduced risk or delayed onset of PD. Based on the known antioxidant and metal complexing properties of urate, together with evidence for oxidative stress as a contributor to neurodegeneration in PD, urate may serve as an endogenous neuroprotectant that helps reduce the risk and rate of the disease. [3] metabolism to cysteine of methionine to cysteine is impaired. This may be due to dietary deficiencies in vitamin B6, vitamin B12, and folic acid. An increase in plasma Homocysteine levels has been reported in Parkinson's disease (PD) patients who were using levodopa. Total Homocysteine concentrations in the cerebrospinal fluid were also higher following levodopa therapy than

before treatment and than in controls. The catalysis of levodopa with the catechol-O-methyltransferase (COMT) enzyme results in the formation of S-adenosylhomocysteine (SAH), which hydrolyses to form Homocysteine. [4]

### MATERIALS AND METHODOLOGY

#### Experimental Design Interpretation –

A cross-sectional and case control study.

#### Study Setting Parameters And Timelines -

The study was conducted in the Department of Biochemistry after getting approval from the University of Health Sciences and the Ethical committee in collaboration with the Department of Neuromedicine of R. G. Kar Medical College and Hospital, Kolkata, over a period of 18 months from January 2019 to June 2020.

#### Study Population:

55 untreated patients of clinically diagnosed idiopathic Parkinson's disease within the age group 50–80 years visiting in the neurology out-patient department of R. G. Kar Medical College and Hospital, were taken as cases, included in the study, based on a set of fixed inclusion & exclusion criteria. 100 age and sex matched apparently healthy individuals without any evidence of ischemic heart disease were taken as controls.

#### Study Design:

Detailed history was taken and disease severity was assessed from UPDRS 2 (Unified Parkinson disease Rating Scale 2) Scoring system. 5 ml Blood was collected from both categories using aseptic precautions. Serum was separated by centrifugation @ 3000 rpm and used for biochemical estimation for levels of TSH, fT3, Homocysteine, Uric acid, and Serum aromatic amino acids (Phenylalanine, Tyrosine & Tryptophan) by Thin Layer Chromatography. Uric acid estimation was done by Immunoturbidimetry assay, serum homocysteine, TSH and fT3 was estimated by chemiluminescence assay & present of free aromatic amino acids estimation was done by Thin Layer Chromatography Technique. The data were tabulated in Microsoft Excel spreadsheets. Obtained data were analyzed using Graph Pad Prism (v5.0). Same methodology was applied for all cases available for

first follow up 3 months and 6 months post initiation of treatment.

#### Study Tools And Techniques:

##### A) FOR SERUM HOMOCYSTEINE MEASUREMENT

**Principle:** estimated by ADVIA CENTAUR Homocysteine assay, a competitive immunoassay using direct chemiluminescent technology. Homocysteine in the patient sample are reduced to free homocysteine by the dithiothreitol, a reducing agent in citrate buffer with preservative. Free homocysteine is then converted to S-adenosyl homocysteine (SAH) by the enzyme reagent. Converted SAH from the patient sample competes with SAH covalently coupled to paramagnetic particles in solid phase (bovine serum albumin and preservatives in phosphate buffer) for a limited amount of acridinium ester-labeled anti-SAH antibody approximately 0.4 microgram/ml.

##### B) FOR MEASUREMENT OF SERUM URIC ACID

**Principle:** Uricase is a very specific enzyme acting on uric acid, end products being allantoin and peroxide. Peroxidase is used to utilize hydrogen peroxide [proportional to the uric acid concentration] to convert chromogen to colored complex. The intensity of color produced is proportional to uric acid concentration and is measured photometrically at 520 nm [500-550nm or green filter]

##### C) FOR MEASUREMENT OF SERUM TSH:-

**Principle:** The ADVIA Centaur TSH3- Ultra assay is a third generation assay that employs anti-FITC monoclonal antibody covalently bound to paramagnetic particles, (an FITC-labeled-anti-TSH capture monoclonal antibody) a tracer proprietary acridinium ester & an anti-TSH mab antibody conjugated to bovine serum albumin (BSA) for chemiluminescent detection.

##### D) MEASUREMENT OF SERUM fT3 :

**Principle:-** The ADVIA Centaur fT3 assay is a competitive immunoassay using direct chemiluminescent technology. fT3 in the sample competes with a T3 analog, which is coupled to paramagnetic particles in the solid phase for a limited amount of a combination of acridinium ester-labeled monoclonal mouse anti-T3 antibodies in the LITE reagent.

##### E) FOR AROMATIC AMINO ACIDS THIN LAYER CHROMATOGRAPHY WAS DONE THIN LAYER CHROMATOGRAPHY

###### Principle:

- Stationary phase (silica or alumina gel) attached to suitable matrix coated thinly onto a glass, plastic or metal foil plate
- Mixture of analytes applied as spot near edge of coated plate
- Liquid mobile phase (ethanol/n-butanol/hexane) passed across plate held horizontally/vertically, by capillary action.

###### F) Statistical Analysis

For analysis of results, the method, test and diagram we used were Mean and Standard deviation, D'Agostino and Pearson omnibus normality test, Paired T test, Unpaired T test, Mann-Whitney U test, Pearson Rank Order Correlation Coefficient test, Wilcoxon signed rank test, Spearman Rank Order Correlation Coefficient test, Bar diagrams and Scatter diagrams. The results of the analysis were showed in following tables and diagrams. The D'Agostino and Pearson omnibus normality test was applied across the cases and control groups to test normality of distribution of data. On the basis of whether the data followed a Gaussian distribution or not appropriate parametric and non parametric tests were applied. Spearman's and Pearson's rank order Correlation Coefficients test was applied according to the test correlation between the various serum parameters under the study (TSH, fT3, Uric acid, homocysteine, serum free aromatic amino acids) with disease severity of IPD as assessed by UPDRS 2.

## RESULTS

### 1. Comparison Between Serum TSH, fT3, Uric Acid And Homocysteine Levels Among 55 Untreated Cases Along With 55 Apparently Healthy Age & Sex Matched Controls In Idiopathic Parkinson's Disease:-

Data found in comparison among the serum TSH [Table 1.a, Figure 1.a] and fT3 values [Table 1.b, Figure 1.b] of same no of untreated diagnosed cases and apparently healthy age & sex matched controls showed that serum TSH and fT3 values is lower in diagnosed untreated cases than controls. Comparison among the Uric acid levels among same no of untreated cases and controls [Table 1.c, Figure 1.c] showed that a statistically significant lower (P value < 0.0001)

values in untreated cases than controls. Comparison among the serum Homocysteine levels among same no of untreated cases and controls [Table 1.d, Figure 1.d] showed that a statistically significant (P value = 0.0003) higher values in cases than controls.

### 2. Comparison Between Serum fT3, TSH, Uric Acid And Homocysteine Levels Among 55 Untreated Cases, And 3 Months Post Treatment Cases Along With 55 Apparently Healthy Age & Sex Matched Controls In Idiopathic Parkinson's Disease:-

Comparison among the serum TSH value [Table 2.a, Figure 2.a] of same no of untreated cases with 3 months post treatment cases showed a significantly higher values in post treatment cases. (P < 0.0001), whereas comparison among the serum fT3 value [Table 2.b, Figure 2.b] of same no of untreated cases with 3 months post treatment cases showed a significantly lower values in post treatment cases. In cases of uric acid levels [Table 2.c, Figure 2.c] comparison among the serum levels of untreated cases with 3 months post treatment cases showed a significantly lower (P < 0.0001) levels in post-treated cases.

Comparison between serum homocysteine levels [Table 2.d, Figure 2.d] among diagnosed untreated cases and 3 months post treatment cases showed a higher value in untreated cases than in 3 months post-treated cases.

### 3. Comparison Between Serum TSH, fT3, Uric Acid And Homocysteine Levels Among 55 Untreated Cases And 6 Months Post Treatment Cases Along With 55 Apparently Healthy Age & Sex Matched Controls In Idiopathic Parkinson's Disease:-

Comparison among the serum TSH value [Table 3.a, Figure 3.a] of same no of untreated cases with 6 months post treatment cases showed a significantly higher values in post treatment cases. (P < 0.0001), whereas comparison among the serum fT3 value [Table 3.b, Figure 3.b] of same no of untreated cases with 6 months post treatment cases showed a significantly lower (P value < 0.0001) values in post treatment cases. In cases of uric acid levels [Table 3.c, Figure 3.c] comparison among the serum levels of untreated cases with 6 months post treatment cases showed a significantly higher (P < 0.0001) levels in post-treated cases. Comparison between serum homocysteine levels [Table 3.d, Figure 3.d] among diagnosed untreated cases and 6 months post treatment cases showed a higher value in untreated cases than in 6 months post-treated cases.

### 4. Correlation Of All The Biomarkers (Serum TSH, fT3, uric Acid And Homocysteine) With Disease Severity (UPDRS 2 Scoring System):

A significant correlation (P Value = 0.0344, Spearman r = 0.2857) [Table 4.b, Figure 4.b] was found between serum fT3 values and idiopathic Parkinson's disease severity. (UPDRS 2 scoring system). No significant correlation was found between serum TSH [Table 4.a, Figure 4.a] Uric acid levels [Table 4.c, Figure 4.c] & serum Homocysteine levels [Table 4.d, Figure 4.d] and idiopathic Parkinson's disease severity. (UPDRS 2 scoring system)

### 5. Thin Layer Chromatography Findings

Prototype chromatogram [Figure 5.a] was done with three aromatic amino acids (Phenylalanine, Tyrosine & Tryptophan). We also done thin layer chromatography between untreated cases of idiopathic Parkinson's disease compared to their apparently healthy age & sex matched controls. [Figure 5.b [A], Figure 5.b [B]]. When we done Thin layer chromatography in both 3 months [Figure 6.a [A], Figure 6.b [B]] & 6 months [Figure 7.a [A], Figure 7.b [B]] significant finding were observed.

## DISCUSSION

Idiopathic Parkinson's disease is a chronic, progressive neurodegenerative disease characterized by both motor and nonmotor features. The disease has a significant clinical impact on patients, families, and caregivers through its progressive degenerative effects on mobility and muscle control. The motor symptoms of PD are attributed to the loss of striatal dopaminergic neurons, although the presence of nonmotor symptoms supports neuronal loss in non dopaminergic areas as well. The term Parkinsonism is a symptom complex used to describe the motor features of PD, which include resting tremor, bradykinesia, and muscular rigidity. Early in the disease the diagnostic error rate can be as high as 25% among practitioners with limited clinical experience in Parkinson's disease. This high level of misdiagnosis affirms the strong need for a diagnostic biomarker of PD. The Biomarker definitions working group has defined a biomarker

as a characteristic that is objectively measured and evaluated as an indicator of normal biologic process, pathogenic process or pharmacological responses to a therapeutic intervention. So there is a pressing need for a robust biomarker to diagnose Idiopathic Parkinson's disease and assess disease severity and prognosis. So far the potential biomarkers have been studied in isolation. No study has yet been done taking all the biomarkers into consideration. Furthermore, very few studies have been conducted on the prognostic role of these biomarkers and practically no study pinpointing the diagnostic and prognostic potential of biomarkers have been conducted in West Bengal. Aim of Our study is to investigate the diagnostic and prognostic potential of a panel of biomarkers in patients of idiopathic Parkinson's disease of West Bengal. We analyzed four serum biomarkers ( TSH, ft3, uric acid and homocysteine) and focused on three aromatic amino acids ( Phenylalanine, Tyrosine and Tryptophan) in serum of freshly diagnosed untreated, 3 months post treatment and 6 months post treatment cases of idiopathic Parkinson's disease by thin layer chromatography. We also compared all the above mentioned serum biomarkers in untreated as well as 3& 6 months post treatment cases along with healthy age and sex matched controls. Correlation of above biomarkers with disease severity scale (UPDRS 2 scoring system) was also observed. In the present study we focused on changes in serum TSH values of 55 cases of idiopathic Parkinson's disease with healthy age& sex matched controls. We found that serum values of TSH in cases were lower than controls. When comparison among the serum TSH value of same no of untreated and 3 months post treatment [ Mean  $\pm$  SD = 2.496 $\pm$ 0.4706 Vs Mean  $\pm$  SD = 5.915 $\pm$ 0.5933] cases were done a significantly higher (p value < 0.0001) value found in 3 months post treatment cases. Again same no of untreated and 6 months post treatment cases were compared and data shown that serum TSH values in 6 months post treatment cases were significantly higher (p < 0.0001) than untreated cases. [ Mean  $\pm$  SD = 2.496 $\pm$ 0.4706 Vs Mean  $\pm$  SD = 6.264 $\pm$ 0.4668] . So after post treatment serum TSH values increased than untreated cases of idiopathic Parkinson's disease. From this observation it was concluded that serum TSH value can be a Prognostic biomarker for follow up of idiopathic Parkinson's disease. No significant correlation ( Table 5.5) found between serum TSH with severity scale ( UPDRS 2 Scoring system) ( P value = 0.7902). Newly diagnosed and untreated cases of idiopathic Parkinson's disease had low TSH value compared to their age and sex matched controls. When we got the 3 months and 6 months follow up values of serum TSH, it was significantly higher (p value < 0.0001) than untreated cases.

As hypothyroidism and idiopathic Parkinson's disease masks each other symptoms this study has great help to follow up the thyroid hormone status of the patients which was neglected frequently. In case of elderly patients symptoms of hypothyroidism e.g. locomotors slowness and hypomimia, can be easily overlooked in patients with Parkinson's disease (PD). [6] There is a disagreement, whether hypothyroidism is or is not more prevalent in PD patients. Parkinsonism and hypothyroidism may have some common clinical features, such as bradykinesia and hypomimia. Consequently, the diagnosis of thyroid dysfunction may be difficult in PD patients. [7] Thyroid hormones (thyroxin and triiodothyronine) increase the expression of calcium channels in beta adrenergic receptor of all excitable cells, including the central and peripheral nervous systems. The enhanced neuronal excitability or hypersensitivity in the oscillatory loops possibly exacerbates the tremor. [8] In this study we did not measure the calcium channel expression in excitable cell in central & peripheral nervous system. In our study all the findings indicates that Serum ft3 values of 55 cases of idiopathic Parkinson's disease were lower than healthy age& sex matched controls. when comparison among the serum ft3 value of same no of untreated cases with 3 months post treatment was done a significantly lower [Mean  $\pm$  SD = 82.82 $\pm$ 6.022 Vs Mean  $\pm$  SD = 62.73 $\pm$ 6.067] value found in 3 months post treatment cases. (P < 0.0001) when same no of untreated and 6 months post treatment cases were compared and it was found that [ Mean  $\pm$  SD = 82.82 $\pm$ 6.022 Vs Mean  $\pm$  SD = 58.71 $\pm$ 6.097] that serum ft3 values in 6 months post treatment cases were significantly lower (p < 0.0001) than untreated cases. From this observation it was concluded that cases of idiopathic Parkinson's disease become hypothyroid after getting treatment. The decreased free triiodothyronine level was associated with akinetic-rigid motor subtype of Parkinson's disease. [9] Our study demonstrated decreased level of ft3 in untreated cases compared to control as well as 3 & 6 months post treatment cases. A positive correlation was found between serum ft3 value with severity index (UPDRS 2 Scoring system) also.

p value = 0.0344, Pearson's r = 0.2857). So serum ft3 is a good prognostic marker for follow up in idiopathic Parkinson's disease. In the present study all finding showed that significantly lower Serum uric acid values [Mean  $\pm$  SD = 3.971 $\pm$ 1.045 mg/dl Vs Mean  $\pm$  SD = 5.262 $\pm$ 0.7605 mg/dl.] in idiopathic Parkinson's disease cases compared with healthy age& sex matched controls. ( P value = < 0.0001). When we compared between untreated cases and 3 months [Mean  $\pm$  SD = 3.971 $\pm$ 1.045 mg/dl Vs Mean  $\pm$  SD = 5.300 $\pm$ 0.3756 mg/dl & 6 months [Mean  $\pm$  SD = 3.971 $\pm$ 1.045 mg/dl Vs Mean  $\pm$  SD = 5.805 $\pm$ 0.2621 mg/dl] post treatment cases a significantly higher serum uric acid (p value < 0.0001) value was found. . So after treatment values of serum uric acid increases than pre treatment cases. From this observation it was concluded that higher values of serum uric acid was protective for patients in idiopathic Parkinson's disease. A significant positive correlation was found between serum uric acid and severity index (UPDRS 2 Scoring system) ( p value = 0.0344, Pearson's r = 0.2857). So from this study it was postulated that serum uric acid has a prognostic potential in idiopathic Parkinson's disease.. Another study with uric acid showed that with early Parkinson's disease higher concentrations of urate in serum and cerebrospinal fluid could delay PD progression and the relationship was robust in men, but weak and nonsignificant among women. [10] We did not measure the level of uric acid in CSF and saw the correlation between disease severity with sex in idiopathic Parkinson's disease. In the present study all finding showed that significantly higher Serum homocysteine values [Mean  $\pm$  SD = 19.01 $\pm$ 5.183  $\mu$ mol/L Vs Mean  $\pm$  SD = 15.78 $\pm$ 2.500  $\mu$ mol/L. ] in idiopathic Parkinson's disease cases compared with healthy age& sex matched controls. (P value = < 0.0001). When comparison between untreated cases and 3 months [Mean  $\pm$  SD = 19.01  $\pm$ 5.183  $\mu$ mol/L Vs Mean  $\pm$  SD = 17.80 $\pm$ 2.067  $\mu$ mol/L post treatment cases were done it was found lower value in post treatment cases. Same comparison between untreated cases and 6 months post treatment cases showed slightly higher values in post treatment cases than untreated cases. So after treatment values of serum homocysteine increases than untreated one.. From this observation it was concluded that higher values of serum homocysteine accentuates the progression of disease. During treatment with levodopa serum value of homocysteine increases due to levodopa metabolism. [11] Negative correlation was found between serum homocysteine levels and severity index ( UPDRS 2 Scoring system) [ Spearman r = -0.1811]. From this observation it was concluded that plasma homocysteine levels was positively associated with disease duration and levodopa usage, So it can be used as a diagnostic marker in idiopathic Parkinson's disease.

Hyperhomocysteinemia is neurotoxic to the substantia nigra by damaging neuronal DNA and stimulating NMDA receptors, contributing to accelerated death of dopaminergic neurons. [12] When correlation ( Table 4.d, Figure 4.d) of serum homocysteine with severity index ( UPDRS 2 Scoring system) was done, Spearman Rank Order correlation coefficient shows, no significant correlation between two parameters. (p value = 0.1857 Spearman r = - 0.1811). So from this study it was postulated that serum homocysteine has no prognostic value in idiopathic Parkinson's disease. An increase in plasma homocysteine levels has been found in idiopathic Parkinson's disease (PD) patients who were using levodopa. [13] We did thin layer chromatography for qualitative measurement of three long chain neutral aromatic amino acids ( Phenylalanine, Tyrosine and Tryptophan) in untreated, along with 3& 6 months post treatment cases of idiopathic Parkinson's disease. In untreated cases no significant positive finding is present in chromatogram. There is loss of nigrostriatal dopaminergic neurone in Parkinson's disease leading to depletion of dopamine. Also there is defective hydroxylation of tyrosine ( due to low level of tyrosine hydroxylase in serum of idiopathic Parkinson's disease patients) to 3, 4-dihydroxyphenylalanine, a decreased absorption of phenylalanine which effects Tyrosine production and a deficient tyrosine hydroxylation in IPD patients. [14] The possible mechanisms of low amino acid in serum of idiopathic Parkinson's disease include 1) malabsorption and changes in amino acid metabolism, 2) effects of mitochondrial dysfunction and oxidative stress, 3) reflection of progressive neurodegenerative processes in the brain, and 4) effect of dopaminergic medications and aromatic L-amino decarboxylase inhibitors. Tryptophan catabolism pathway in plasma in idiopathic parkinson's disease patients were aggravated with inflammatory activation of the kynurenine pathway. Specifically, IPD cases had higher amounts of the neurotoxic metabolite 3-HK in plasma. [15] In 3 months post treatment idiopathic parkinson's disease cases also there is absence of any of

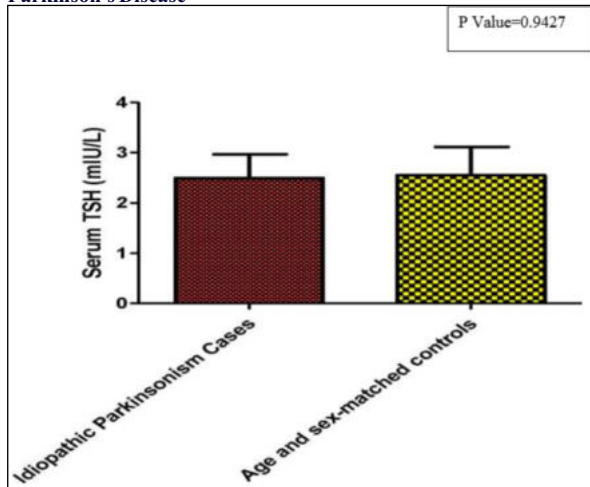
three amino acid in chromatogram. Patients with idiopathic Parkinson's disease suffer from low levels of tyrosine hydroxylase. The decreased levels of tyrosine hydroxylase inhibit conversion of l-tyrosine to l-dopa; l-dopa depletes l-tyrosine. So there was absence of tyrosine in serum. Defective Phenylalanine absorption and increase in tryptophan catabolism also leads to their absence in serum of Idiopathic Parkinson's disease patients. In 6 months post treatment cases there is presence of tyrosine in chromatogram. Levodopa competes with tyrosine and the other large neutral amino acids at the BBB; So there is accumulation of tyrosine in serum of IPD patients. Phenylalanine had defective absorption and Tryptophan catabolism pathway exaggerated in IPD patients.

**CONCLUSION**

Hence from our study we concluded that serum TSH, FT3 and uric acid had a good diagnostic potential in Idiopathic Parkinson's disease. Also serum FT3 may be used as a prognostic marker too.

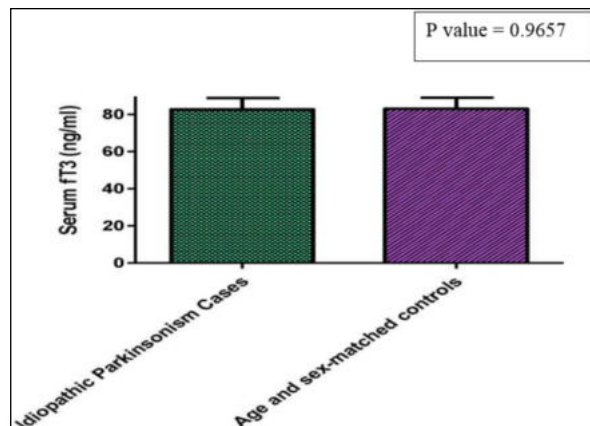
**IMAGES**

**1a). Comparison Of Serum TSH Levels Among Untreated Cases And Apparently Healthy Age Sex Matched Controls In Idiopathic Parkinson's Disease**



**Figure 1a:-**Bar diagram showing the distribution of serum TSH levels in patients of idiopathic Parkinson's disease and their healthy age and sex matched controls[ Serum TSH range in cases 1.4-3.8 ( mIU/L) with mean ±SD= 2.496 ±0.4706 & range in controls 4-7.5 ( mIU/L) with mean± SD= 2.553±0.5670].Mann Whitney U test applied, showed that serum TSH value in cases were lower than controls (P= 0.9427).

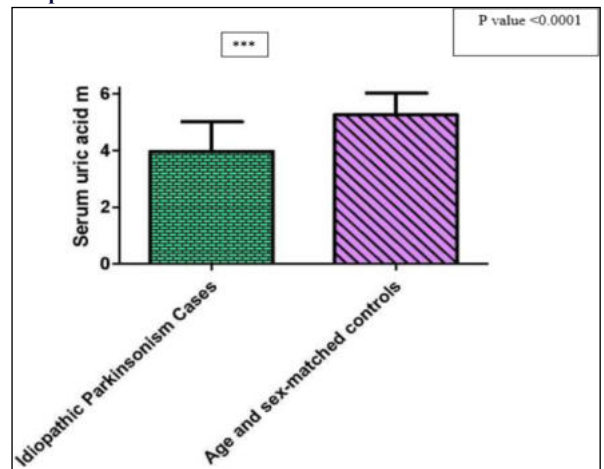
**1b). Comparison Of Serum FT3 Levels Among Untreated Cases And Apparently Healthy Age Sex Matched Controls In Idiopathic Parkinson's Disease**



**Figure 1b):-** Bar diagram depicting the distribution of serum FT3 levels in patients of idiopathic Parkinson's disease and their age and sex matched healthy controls[ Serum FT3 range in case 55-95( ng/dl) with mean ±SD=82.82 ± 0.8120& range in controls 70-100 (ng/dl) with mean± SD= 83.11±0.8168] .Unpaired T test showed a lower

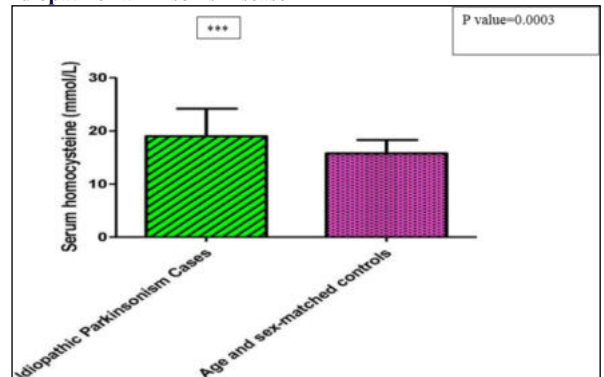
value of FT3 in cases than controls.(P value= 0.9657)

**1c) Comparison Of Serum Uric Acid Levels Among Untreated Cases And Apparently Healthy Age Sex Matched Controls In Idiopathic Parkinson's Disease**



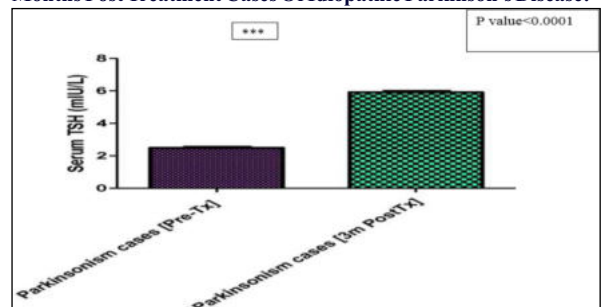
**Figure 1c):-**Bar diagram showing the comparison of serum uric acid levels in patients of idiopathic Parkinson's disease and their age and sex matched healthy controls[ Serum uric acid range in case 2.3-5.6 ( mg/dl) with mean ±SD =82.82 ± 0.8120& range in controls 3.5-6.5 (mg/dl) with mean± SD= 83.11±0.8168]. Mann Whitney U test showed a statistically significant( P value< 0.0001) changes in serum uric acid levels in untreated cases compared to their apparently healthy age sex matched controls.

**1d) Comparison Of Serum Uric Acid Levels Among Untreated Cases And Apparently Healthy Age Sex Matched Controls In Idiopathic Parkinson's Disease**



**Figure 1d.:-**Bar diagram depicting the distribution of serum homocysteine levels in 55 patients of idiopathic Parkinson's disease and their age and sex matched apparently healthy controls[ Serum homocysteine range in case 10-32 ( µmol/L) with mean ±SD =19.01 ± 5.183 & range in controls 10-20 (µmol/L) with mean± SD= 15.78±2.500]. Mann Whitney U test showed a statistically significant P value (0.0003)

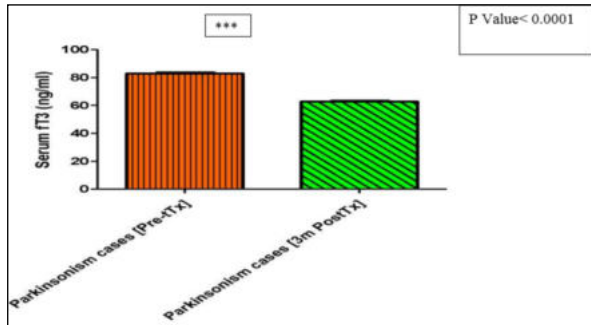
**2a) Comparison Of Serum TSH Values Among Untreated And 3 Months Post Treatment Cases Of Idiopathic Parkinson's Disease:**



**Figure 2a:-** Bar diagram showing the distribution of serum TSH levels in un treated and 3 months post treatment cases of idiopathic

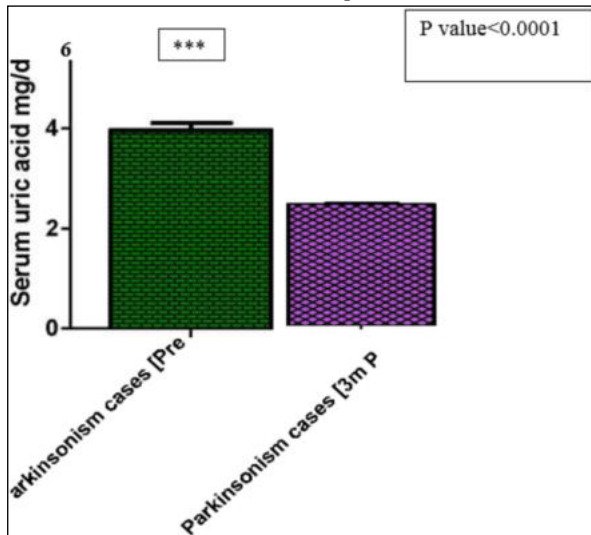
Parkinson's disease [ Serum TSH range in untreated cases 0.5-4 (mIU/L) with mean  $\pm$ SD =2.496  $\pm$ 0.4706 & range in 3 months post treatment cases 4.5-7 (mIU/L) with mean $\pm$  SD=5.915  $\pm$ 0.5933]. Wilcoxon signed rank test showed a statistically significant P value (<0.0001)

**2b) Comparison Of Serum FT3 Levels Among Untreated And 3 Months Post Treatment Cases Of Idiopathic Parkinson's Disease:-**



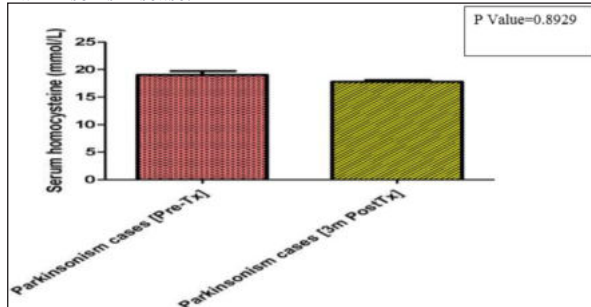
**Figure 2.b:-** Bar diagram showing the distribution of serum ft3 levels in untreated and 3 months post treatment cases of idiopathic Parkinson's disease [ Serum ft3 range in untreated cases 70-90 (ng/dl) with mean  $\pm$ SD =82.82  $\pm$ 6.022 & range in 3 months post treatment cases 50-75 (ng/dl) with mean $\pm$  SD=62.73  $\pm$ 4.767]. Paired T test showed a statistically significant P value (<0.0001)

**2c) Comparison Of Serum Uric Acid Levels Among Untreated And 3 Months Post Treatment Cases Of Idiopathic Parkinson's Disease:-**



**Figure 2.c:-** Bar diagram showing the distribution of serum uric acid levels in untreated and 3 months post treatment cases of idiopathic Parkinson's disease [ Serum uric acid range in untreated cases 3-6 (mg/dl) with mean  $\pm$ SD =3.971  $\pm$ 1.045 & range in 3 months post treatment cases 4-6 (mg/dl) with mean $\pm$  SD=5.300 $\pm$ 0.3756]. Wilcoxon signed rank test showed a statistically significant P value (<0.0001)

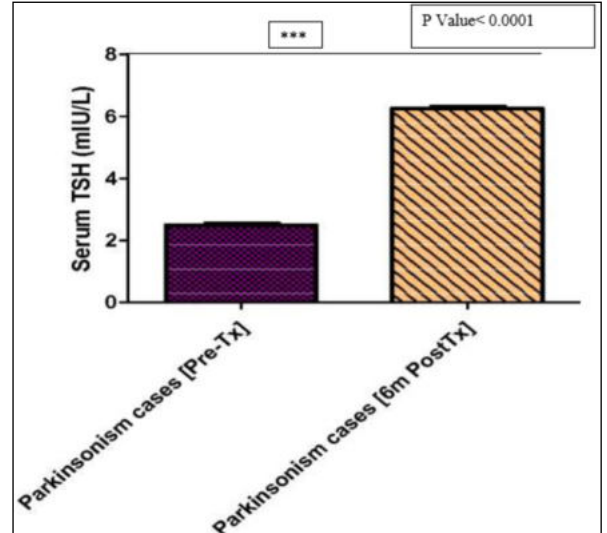
**2d) Comparison Of Serum Homocysteine Levels Among Untreated And 3 Months Post Treatment Cases Of Idiopathic Parkinson's Disease:-**



**Figure 2d:-** Bar diagram showing the distribution of serum

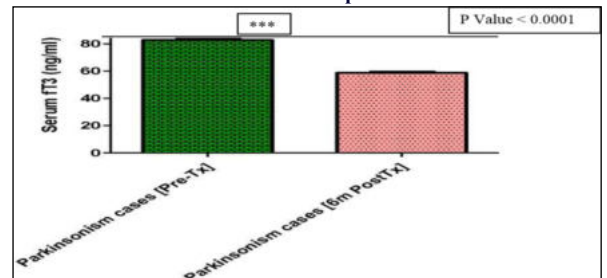
homocysteine levels in untreated and 3 months post treatment cases of idiopathic Parkinson's disease [ Serum homocysteine range in pre treatment cases 17-32 ( $\mu$ mol/L) with mean  $\pm$ SD =19.01  $\pm$ 5.183 & range in 3 months post treatment cases 15-22 ( $\mu$ mol/L) with mean $\pm$  SD=17.80  $\pm$ 2.067]. Wilcoxon signed rank test showed a higher values of homocysteine in untreated cases than 3 months post treatment case

**3a) Comparison Of Serum TSH Levels Among Untreated And 6 Months Post Treatment Cases Of Idiopathic Parkinson's Disease:-**



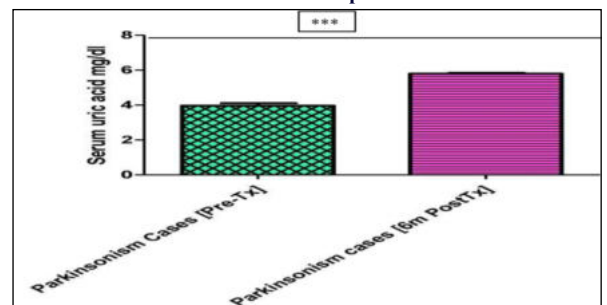
**Figure 3.a:-** Bar diagram showing the comparison of serum TSH levels in untreated and 6 months post treatment cases of idiopathic Parkinson's disease [ Serum TSH range in pre untreated cases 0.5-4 (mIU/L) with mean  $\pm$ SD = 2.496 $\pm$ 0.4706 & range in 6 months post treatment cases 4.8-7 (mIU//L) with mean $\pm$  SD=6.264 $\pm$ 0.4668]. Wilcoxon signed rank test showed a statistically significant p value<0.0001

**3.b) Comparison Of Serum ft3 Levels Among Untreated And 6 Months Post Treatment Cases Of Idiopathic Parkinson's Disease:-**



**Figure 3.b:-** Bar diagram showing the comparison of serum ft3 levels in untreated and 6 months post treatment cases of idiopathic Parkinson's disease [ Serum ft3 range in untreated cases 70-94 (ng/dL) with mean  $\pm$ SD =82.82  $\pm$ 6.022 & range in 6 months post treatment cases 48-76 (ng/dL) with mean $\pm$  SD=58.71  $\pm$  6.097]. Paired T test showed a statistically significant p value<0.0001.

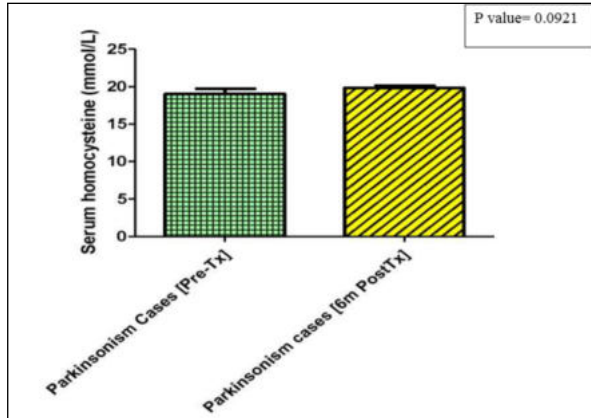
**3.c) Comparison Of Serum Uric Acid Levels Among Untreated And 6 Months Post Treatment Cases Of Idiopathic Parkinson's Disease**



**Figure 3.c :-**Bar diagram showing the comparison of serum uric acid levels in untreated and 6 months post treatment cases of idiopathic

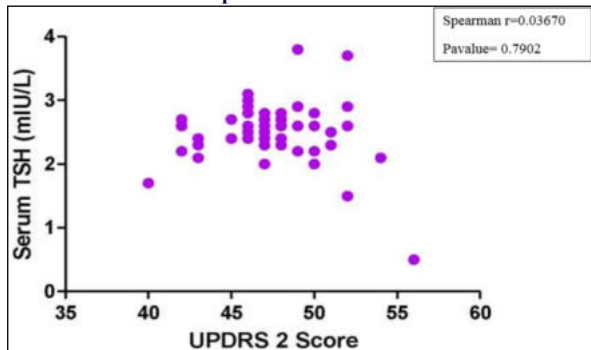
Parkinson's disease [ Serum uric acid range in pre treatment cases 2.4-6 (mg/dL) with mean  $\pm$ SD =3.971  $\pm$  1.045 & range in 6 months post treatment cases 4.5-6.5 (mg/dL) with mean $\pm$  SD=5.801  $\pm$ 0.2621 ]. Wilcoxon signed rank test was applied and showed a statistically significant p value <0.0001.

**3.d) Comparison Of Serum Homocysteine Levels Among Untreated And 6 Months Post Treatment Cases Of Idiopathic Parkinson's Disease:-**



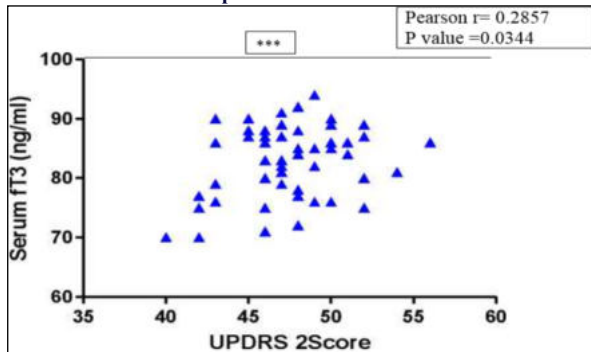
**Figure 3.d:-** Bar diagram showing the comparison of serum homocysteine levels in untreated cases and 6 months post treatment cases of idiopathic Parkinson's disease [ Serum homocysteine range in untreated cases 10-31 ( $\mu$ mol/L) with mean  $\pm$ SD =19.01  $\pm$ 1.083 & range in 6 months post treatment cases 16-24 ( $\mu$ mol/L) with mean $\pm$  SD= 19.82 $\pm$ 2.161 ]. Wilcoxon signed rank test showed a lower values of homocysteine among untreated cases compared to 6 months post treatment cases. (p value=0.0921)

**4.a) Correlation Of Serum TSH Values And UPDRS 2 Score In Untreated Cases Of Idiopathic Parkinson's Disease:-**



**Figure 4.a:-** Scatter diagram showing distribution of serum TSH levels with respect to UPDRS 2 score in case of idiopathic Parkinson's disease. Spearman Rank Order Correlation test applied, showed no significant correlation between the two parameters. (P value=0.7902, Spearman r=0.03670)

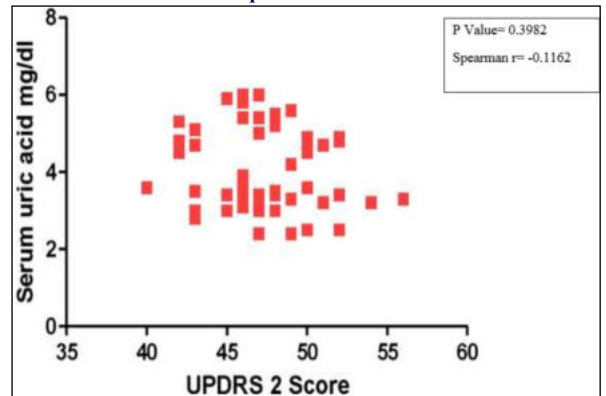
**4.b) Correlation Of Serum ft3 Levels And UPDRS 2 Score In Untreated Cases Of Idiopathic Parkinson's Disease:-**



**Figure 4.b:-** Scatter diagram showing distribution of serum ft3 levels with respect to UPDRS 2 score in case of idiopathic Parkinson's

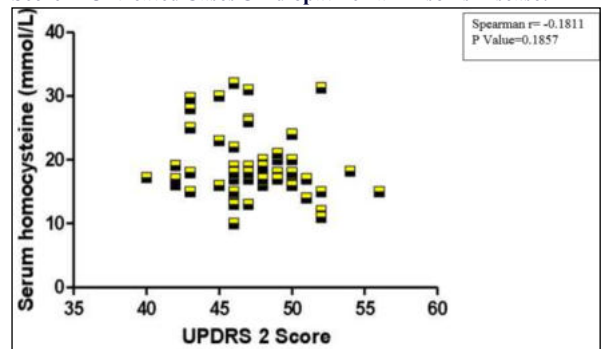
disease. Pearson's Rank Order Correlation test was applied and it showed significant correlation between serum ft3 levels and UPDRS 2 score. (P value=0.0344, Pearson r=0.2857)

**4.c) Correlation Of Serum Uric Acid Levels And UPDRS 2 Score In Untreated Cases Of Idiopathic Parkinson's Disease:-**

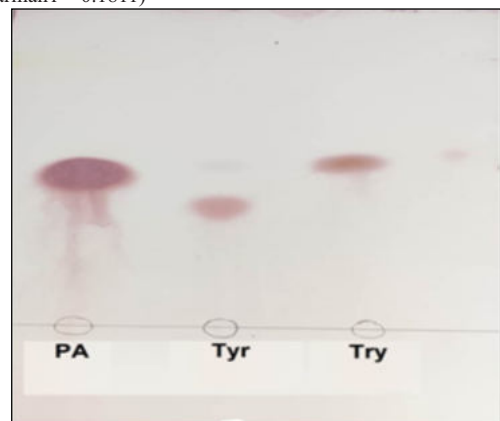


**Figure 4.c:-** Scatter diagram showing distribution of serum uric acid levels with respect to UPDRS 2 score in case of idiopathic Parkinson's disease. Spearman Rank Order Correlation test was applied and it showed no significant correlation between serum uric acid value and UPDRS 2 score. (P value=0.3982, Spearman r=-0.1162)

**4.d) Correlation Of Serum Homocysteine Levels And UPDRS 2 Score In Untreated Cases Of Idiopathic Parkinson's Disease:-**

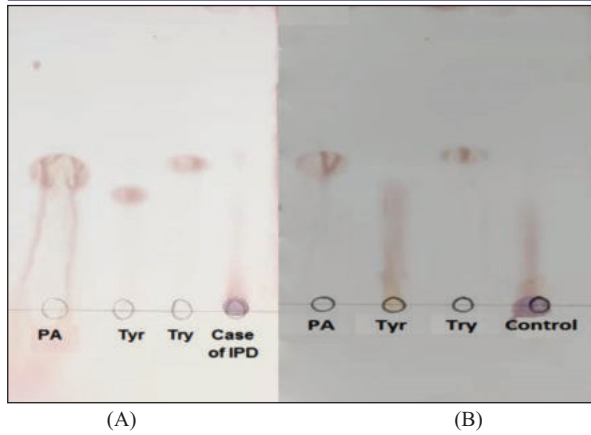


**Figure 4.d:-** Scatter diagram showing distribution of serum homocysteine levels with respect to UPDRS 2 score in case of idiopathic Parkinson's disease. Spearman Rank Order Correlation test was applied and it showed no significant correlation between the serum homocysteine levels and UPDRS 2 score. (P value=0.1857, Spearman r=-0.1811)



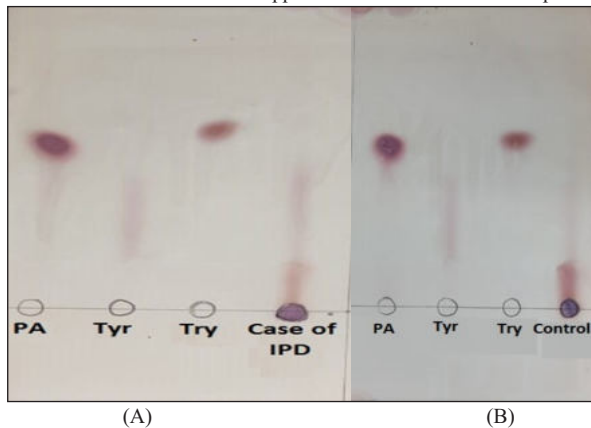
PA= Phenylalanine (Rf value=0.68), Tyr= Tyrosine (Rf value=0.45),Try=Tryptophan (Rf value=0.66), Rf= movement of solute from the baseline / distance travelled by solvent front from the baseline.

**Figure 5.a)** Thin layer chromatography findings of aromatic amino acids( Phenylalanine, Tyrosine and Tryptophan) in Idiopathic Parkinson's disease

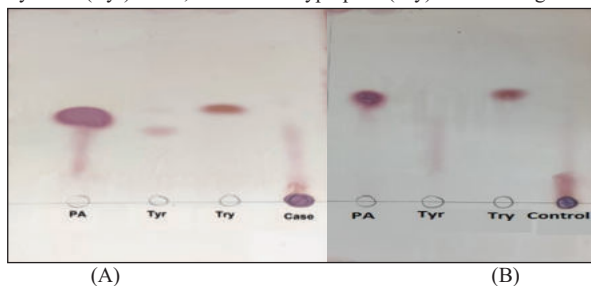


**Figure 5.b):-** Prototype chromatogram of reference aromatic amino acid. (Phenylalanine, Tyrosine and Tryptophan)

(A) = Prototype chromatogram of untreated case of idiopathic Parkinson's disease along with reference aromatic amino acids applied on three different circular spots. [ Rf value in case=0.14, Rf value for Phenylalanine (PA)=0.43, Rf value for Tyrosine (Tyr)=0.35 ,Rf value for Tryptophan ( Try)=0.50] (B)= Prototype chromatogram of apparently healthy age & sex matched control [ Rf value in controls=0.28 , Rf value of Phenylalanine (PA)=0.35, Rf value in Tyrosine ( Tyr)= 0.35, Rf value in Tryptophan (Try)=0.35] along with reference aromatic amino acids applied on three different circular spots.



**Figure 5.c):-** (A) = Prototype chromatogram of 3 months post treatment case of idiopathic Parkinson's disease [ Rf value in case=0.21, Rf value in Phenylalanine(PA)= 0.28, Rf value in Tyrosine (Tyr)=0.21, Rf value in Tryptophan ( Try)=0.43] along with reference amino acids applied on three different circular spots. 7.(B)= Prototype chromatogram of apparently healthy age sex matched control ( Rf value=0.14 in control, Rf value in Phenylalanine(PA)= 0.35, Rf value in Tyrosine ( Tyr)=0.21, Rf value in Tryptophan (Try)=0.43 along with



**Figure 5.d):-** (A) =Prototype chromatogram of 6 months post treatment case of idiopathic Parkinson's disease [ Rf value of Phenylalanine(PA)=0.40, Rf value of Tyrosine (Tyr)=0.21, Tryptophan ( Try)= 0.29, Rf value in cases =0.28] along with reference aromatic amino acids applied on three different circular spots.

(B)= Prototype chromatogram of apparently healthy age sex matched control ( Rf value in control=0.14, Rf value of Phenylalanine(PA)=

0.35, Rf value Tyrosine (Tyr)= 0.21, Rf value of Tryptophan ( Try)=0.42] along with reference aromatic amino acids applied on three different circular spots.

**Table 1.a:- Table Showing Mean And Standard Deviation Of Serum TSH Levels Of Cases And Controls In Idiopathic Parkinson's Disease.**

Serum TSH levels of cases and controls ( Miu/L)		
Groups	Mean	Standard deviation
Cases	2.496	0.4706
Controls	2.553	0.5607

**Table 1.b: Table Showing Mean And Standard Deviation Of Serum ft3 Levels Of Cases And Controls.**

Serum ft3 levels of cases & controls( ng/dl)		
Groups	Mean	Standard deviation
Cases	82.82	0.8120
Controls	83.11	0.8168

**Table 1.c: Table Showing Mean And Standard Deviation Of Serum Uric Acid Levels Of Cases And Controls**

Serum Uric Acid Levels Of Cases & Controls( Mg/dl)		
Groups	Mean	Standard deviation
Cases	3.971	5.262
Controls	1.045	0.7605

**Table 1.d: Table Showing Mean And Standard Deviation Of Serum Homocysteine Levels Of Cases And Controls.**

Serum homocysteine levels in cases and controls( μmole/L)		
Groups	Mean	Standard deviation
Cases	19.01	5.183
Controls	15.78	2.500

**Table 2.a :- Table Showing Mean And Standard Deviation Of Serum TSH Levels Of Untreated And 3 Months Post Treatment Cases Of Idiopathic Parkinson's Disease.**

Serum TSH levels in pretreated and 3 months post treated cases of Idiopathic Parkinson's disease		
Groups	Untreated cases	3 months post treated cases
Mean	2.496	5.915
Standard deviation	0.4706	0.5933

**Table 2.b :- Table Showing Mean And Standard Deviation Of Serum Ft3 Levels Of Untreated And 3 Months Post Treated Cases Of Idiopathic Parkinson's Disease.**

Serum ft3 levels in untreated and 3 months post treated cases of Idiopathic Parkinson's disease		
Groups	un treated cases	3 months post treatment cases
Mean	82.82	62.73
Standard deviation	6.022	4.767

**Table 2.c :- Table Showing Mean And Standard Deviation Of Serum Uric Acid Levels Of Untreated And 3 Months Post Treatment Cases Of Idiopathic Parkinson's Disease:-**

Groups	un treated cases of idiopathic Parkinson's disease	3 months post treatment cases
Mean	3.971	5.300
Standard deviation	1.045	0.3756

**Table 2.d:- Table Showing Mean And Standard Deviation Of Serum Homocysteine Levels Of Untreated And 3 Months Post Treatment Cases Of Idiopathic Parkinson's Disease:-**

Serum homocysteine level in untreated and 3 months post treatment cases( micromole/L)		
Groups	untreated cases of idiopathic Parkinson's disease	3 months post treated cases of idiopathic Parkinson's disease
Mean	19.01	17.80
Standard deviation	5.183	2.067

**Table 3.a : Table Showing Mean And Standard Deviation Of Serum Ft3 Levels Of Untreated And 6 Months Post Treatment Cases Of Idiopathic Parkinson's Disease.**

Serum TSH levels in untreated and 6 months post treatment cases of Idiopathic Parkinson's disease(mIU/L)		
Groups	un treated cases of idiopathic Parkinson's disease	6 months post treated cases

Mean	2.496	6.264
Standard deviation	0.4706	0.4668

**Table 3.b : Table Showing Mean And Standard Deviation Of Serum Ft3 Levels Of Untreated And 6 Months Post Treatment Cases Of Idiopathic Parkinson's Disease**

Serum ft3 levels in untreated and 6 months post treatment patients of Idiopathic Parkinson's disease (ng/dl)		
Groups	un treated cases of idiopathic Parkinson's disease	6 months post treated cases
Mean	82.82	58.71
Standard deviation	6.022	6.097

**Table 3.c : Table Showing Mean And Standard Deviation Of Serum Uric Acid Levels Of Untreated And 6 Months Post Treatment Cases Of Idiopathic Parkinson's Disease**

Serum uric acid level in untreated and 6 months post treatment cases of Idiopathic Parkinson's disease ( mg/dl)		
Groups	untreated cases of idiopathic Parkinson's disease	6 months post treatment cases
Mean	3.971	5.801
Standard deviation	1.045	0.2621

**Table 3.d : Table Showing Mean And Standard Deviation Of Serum Homocysteine Levels Of Untreated And 6 Months Post Treatment Cases Of Idiopathic Parkinson's Disease:-**

Serum homocysteine level in untreated and 6 months post treated cases of Idiopathic Parkinson's disease ( μmol/L)		
Groups	un treated cases of idiopathic Parkinson's disease	6 months post treatment cases
Mean	19.01	19.82
Standard deviation	1.083	2.161

**Table 4.a:- Statistical Correlation Between Serum TSH Levels And UPDRS 2 Score In Cases Of Idiopathic Parkinson's Disease:-**

Parameters	Spearman's rank order correlation coefficient	Significant level of correlation ( P value)
Serum TSH levels Vs UPDRS 2 Score	0.03670	0.7902

**Table 4.b :- Statistical Correlation Between Serum Ft3 Levels And UPDRS 2 Score In Cases Of Idiopathic Parkinson's Disease :**

Parameters	Pearson's rank order correlation coefficient	Significant level of correlation ( P value)
Serum ft3 levels Vs UPDRS 2 score	0.2857	0.0344

**Table 4.c:- Statistical Correlation Between Serum Uric Acid Levels And UPDRS 2 Score In Cases Of Idiopathic Parkinson's Disease**

Parameters	Spearman's rank order correlation coefficient	Significant level of correlation ( P value)
Serum uric acid levels Vs UPDRS 2 score	-0.1162	0.3982

**Table 4.d :- Statistical Correlation Between Serum Homocysteine Levels And UPDRS 2 Score In Cases Of Idiopathic Parkinson's Disease**

Parameters	Spearman's rank order correlation coefficient	Significant level of correlation ( P value)
Serum uric acid levels Vs UPDRS 2 score	-0.1811	0.1857

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