**Statistical Method**

Differences in normalized right and left habenula volume between PD and CN groups were compared using independent samples t-tests. Normality of the volume values was confirmed visually and using Shapiro-Wilk tests. Levene's test was used to assess homogeneity of variances between groups. Cohen's d effect sizes were calculated to quantify the magnitude of group differences.

For analyzing total habenula volume, prior to the test normality of the volumes were confirmed visually and using Shapiro-Wilk test, and as the as the assumptions of homogeneity of variance were assessed the results were interpreted.

Lateralization of habenula volume was assessed by comparing normalized right and left habenula volumes within each group (PD and CN) using paired-samples t-tests. Prior to analyses, data was checked for normality using Shapiro-Wilk tests and visual inspection of histograms. For each group separately, a paired-samples t-test was conducted between right and left habenula volumes. Cohen's d effect sizes were calculated to quantify the magnitude of lateralization.

For all of the tests 95% confidence intervals (CIs) around the mean differences were computed and alpha was set at 0.05 for statistical significance.

**Results**

**Volumetric Analysis:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **DX** | **Number** | **Mean** | **Std.** |
| **Right Habenula** | CN | 100 | 24.41335238 | 4.793529962 |
| PD | 100 | 18.53799950 | 5.840101147 |
| **Left Habenula** | CN | 100 | 23.54044756 | 4.041445475 |
| PD | 100 | 17.59760558 | 5.156075658 |

**Table 1.** Group Statistic of Patients in right and left habenula region volume.

For right habenula volume, the t-test showed a significant difference between PD (mean = 18.54 , SD = 5.84) and CN (mean = 24.41 , SD = 4.79) groups (t(198) = 7.78, p < 0.001). The effect size was large (Cohen's d = 1.10).

Similarly, for left habenula volume, the t-test revealed a significant difference between PD (mean = 17.60 , SD = 5.16) and CN (mean = 23.54 , SD = 4.04) groups (t(198) = 9.07, p < 0.001). The effect size was very large (Cohen's d = 1.28).

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| --- | --- | --- | --- | --- |
|  | **t-value** | **df** | **Mean Difference** | **p-value** |
| **Right Habenula** | 7.776 | 190.750 | 5.875 | < 0.001 |
| **Left Habenula** | 9.071 | 187.312 | 5.942 | < 0.001 |

**Table 2.** Result of independent sample t-test for comparing left and right Habenula normalized to TIV volume of Parkinson’s patients to control normal.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Standardizer** | **Point Estimate** | **Lower 95% CI** | **Upper 95% CI** |
| **Right Habenula** | 5.342 | 1.100 | 0.801 | 1.396 |
| **Left Habenula** | 4.632 | 1.283 | 0.977 | 1.586 |

**Table 3.** Result of Cohen’s d independent samples effect size for comparing left and right Habenula normalized to TIV volume of Parkinson’s patients to control normal.

Taken together, these results demonstrate significantly reduced normalized right and left habenula volume in PD patients compared to healthy controls. The large effect sizes signify that these differences are highly clinically meaningful. The findings suggest substantial habenula atrophy is present in PD.

**Total Habenula Volume Analysis :**

The total (left + right) habenula volume was compared between PD and CN groups using an independent samples t-test. Total habenula volume was calculated by summing the left and right habenula volumes for each participant.

Prior to analysis, assumptions of normality and homogeneity of variance were assessed. As seen in the stem-and-leaf plots and Q-Q plots, total habenula volume was normally distributed in both groups (p > .05). Levene's test indicated unequal variances between groups (p = .010), so the unequal variances t-test output was interpreted.

The t-test revealed that total habenula volume was significantly smaller in PD (mean = 36.14 , SD = 10.53) compared to CN (mean = 47.95 , SD = 8.24), t(187.19) = 8.84, p < .001. The mean difference was 11.82 mm3 and the effect size was very large (Cohen's d = 1.25).’

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Mean Volume of CN Group** | **Mean Volume of PD Group** | **t-value** | **df** | **Mean Difference** | **p-value** |
| **Total Volume of Habenula** | 47.953 | 36.135 | 7.776 | 187.189 | 11.818 | < 0.001 |

**Table 5.** Total Volume of Habenula analysis results.

**Laterality Analysis :**

For the control group, a paired samples t-test indicated there was a statistically significant difference between left (mean=23.54 , SD=4.04) and right (mean = 24.41 , SD=4.79) habenula volumes (t(df = 99)=-2.661, p=0.009). The mean difference between left and right volume was -0.87 (95% CI [-1.52, -0.22]). The effect size was small to medium (Cohen's d=-0.27).

Similarly for the Parkinson's disease group, there was a significant difference between left (mean=17.60 , SD=5.16) and right (M=18.54, SD=5.84) habenula volumes (t(df = 99)=-2.890, p=0.005). The mean difference was -0.94 (95% CI [-1.59, -0.29]) with a small to medium effect size (Cohen's d=-0.29).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Mean Left Habenula Volume** | **Mean Right Habenula Volume** | **t-value** | **p-value** | **Mean Difference (95% CI)** | **Cohen’s d** |
| **Control Group** | 23.54 ± 4.04 | 24.41 ± 4.79 | - 2.661 | 0.009 | - 0.87 (-1.52, -0.22) | - 0.27 |
| **Parkinson’s Group** | 17.60 ± 5.16 | 18.54 ± 5.84 | - 2.890 | 0.005 | - 0.94 ( -1.59, -0.29) | - 0.29 |

**Table 5.** Laterality analysis of the habenula volume in groups.

In both groups, the left habenula volume was significantly smaller than the right. These results demonstrate lateralization of habenula volume in both healthy controls and Parkinson's disease patients, though the magnitude of lateralization appears slightly greater in Parkinson's disease based on the effect sizes.