

Original Article

Numbers of Purkinje Cell with Increasing Age-A Post Mortem Study

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Abstract

The change in the number of Purkinje cells with increasing age is evident especially in disorders of fine movement, equilibrium, hypotonia, postural changes, and disturbances of voluntary movement. The present study was done to see the changes in the number of Purkinje cells per square mm in different age groups of Bangladeshi people. This cross sectional descriptive type of study was designed and done in the Department of Anatomy, Dhaka Medical College, Dhaka, from January to December 2010, which was performed on the cerebellum of 28 Bangladeshi people, collected during autopsy examination of unclaimed dead bodies from Department of Forensic Medicine. Paraffin blocks of cerebellum were cut at 5 μ m thickness and stained with routine Harris' Haematoxylin and Eosin (H & E) stain. Estimation of number of Purkinje cell was done by using the counting circle and examined under the light microscope. The mean \pm SD of number of Purkinje cell was 160.71 \pm 24.47 in group A (Age 20-29 years) and 152.20 \pm 6.49 in group D (age > 50 years), the mean reduction was 2.5% per decade. Histological studies revealed the number of Purkinje cell per square mm decreased with age which was statistically significant and further cytological study of Purkinje cell with larger sample size is recommended.

Key words: Purkinje cell, Cerebellum.

Introduction :

Galen was the first to give an extensive description of cerebellum and the anatomy of cerebellum was described more thoroughly by Thomas Willis in 1664¹. The cerebellum is the largest part of the hindbrain lies behind the pons and Medulla oblongata in the posterior cranial fossa and is covered by the tentorium cerebelli²⁻⁴. Structurally, the cerebellum consists of an outer lamina of grey matter and inner white matter⁴. Three layers are seen in histological sections, from the surface

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to the white matter of the folium, these are the molecular layer, the Purkinje cells layer and the granule layer³. The Purkinje cells are found in the cerebellar cortex throughout the vertebrates. Their flattened cell bodies are flask shaped when viewed in a transverse section across a folium⁴⁻⁶. Number of Purkinje cell in male is 6-8% higher than in the female and mean reduction with age is 2.5% per decade and this age related loss of Purkinje cell is uniform across the vermis and hemisphere, it is not related to any vascular degeneration or to any pathological process⁷. In alcoholism there was loss of Purkinje cell 21%^{8,9}. In gait ataxia, there is shrinkage of the cortex of anterior lobe and loss of up to 10% of granule cells, 20% Purkinje cells and 30% reduction of molecular layer. A Quantitative study of the human cerebellum shows that the numbers of Purkinje cells are to be as high as 30.5x10⁶ and number of Purkinje cell is 5-8 per mm^{10,11}.

Materials and Method:

Materials:

A Cross-sectional descriptive type of study was carried out in the Department of Anatomy, Dhaka Medical College, Dhaka, Bangladesh from January 2010 to December 2010. The present study was performed on 28 human cerebellum collected from the morgue of Dhaka Medical College, Dhaka. Among them lowest age was 22 years and highest was 58 years. For convenience of

differentiating the changes of various features of cerebellum in relation to age, the collected samples were divided into four groups; Group-A (20-29 years), Group-B (30-39 years), Group-C (40-49 years), Group-D (>50 years)

Method:

Preservation of brains:

After collection of whole brain, 100ml of 40% formaldehyde solution was injected by using a 50cc syringe into the brain through the surfaces (super lateral and inferior surfaces). Then it was preserved in 40% formaldehyde solution (Origin Germany) for 15 days. After 15 days the cerebellum was collected from the preserved brains and fixed in 10% formol saline solution which Composed 37-40% formaldehyde (100cc), Sodium chloride (9gm), tap water (900cc).

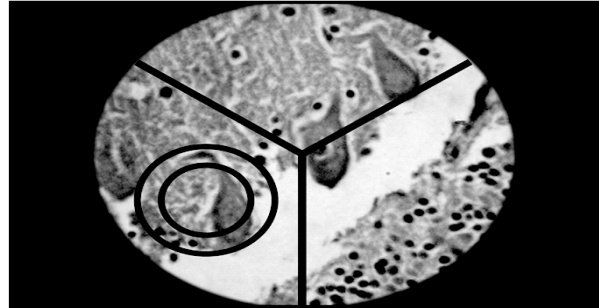
Preparation of the slide:

Tissue blocks were fixed in 10% formol saline in a plastic container for 1 week. The tissues were washed in running tap water, dehydration was done with ascending grades of alcohol, cleared with xylene, infiltrated and embedded in paraffin. Paraffin blocks were cut at 5 μ m thickness and were stained with routine Harris' Haematoxylin and Eosin (H & E) stain. For measurement of number of Purkinje cell of the cerebellar cortex, 7 slides were selected from each group. Total 28 slides were examined. The light compound microscope was used for the microscopic measurement which was OLYMPUS CHB, made in Tokyo, Japan.

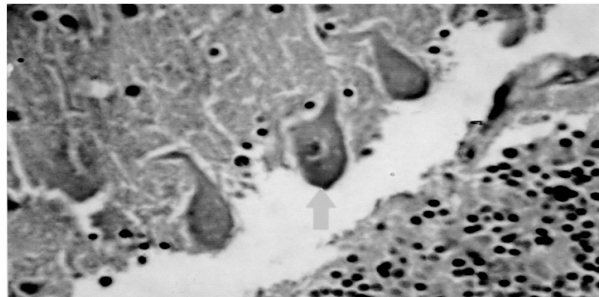
Estimation of number of the purkinje cells per square mm:

For the study of histological parameters, the stained tissue section on the slide was divided into three equal parts by a computer generated, photographically produced equal sized area over a transparent plastic sheet by drawing three lines which radiated from the centre towards the periphery at 10 o'clock, 2 o'clock and 6 o'clock position. Then, this sheet was fixed on the top of the cover slip by an adhesive tape. The centre of this sheet corresponded with the centre of the tissue section. From each triangular area, one microscopic field was selected closer to the centre for study. Thus from each slide, three different fields were chosen for counting the number of purkinje cells. The counting was done within a counting circle specially devised for this purpose. A counting circle of 5 mm diameter was printed on a transparent plastic sheet, which was cut to fit into the eyepiece of the light microscope. Thus a black circular outline was superimposed over the actual microscopic

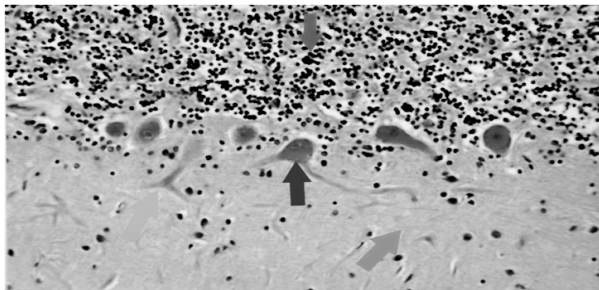
field. The circle encircled some total of the purkinje cells, while some other cells were partly included inside that circle. All the cells within the circle were taken as a count. From the three counts of three different fields of each slide, an average count was calculated for each slide. Thus the average counts of 28 slides from group A, B, C and D were available. The count was then converted into number per square mm by conversion measurement by means of a stage micrometer.



Photograph 1: Photograph showing estimation of number of Purkinje cell by using the counting circle (Under light microscope x 40 objective x 10 eye piece) in H & E stain.



Photograph 2: The sectional view of cerebellum showing Purkinje cell (yellow arrow) taken from group A (20-29 years) under light microscope (x 40 objective x 10 eyepiece) in H & E stain.



Photograph 3: The sectional view of cerebellum showing Purkinje cell (blue arrow), Dendrites of Purkinje cell (yellow arrow) and Molecular layer (green arrow) taken from group B (30-39 years) under light microscope (x 40 objective x 10 eyepiece) in H & E stain.

Statistical Processing of Data:

The data collected from the histological studies were processed to get mean values, standard deviations etc.

as applicable. Statistical analyses were done by unpaired Student's 't' test. All the statistical analyses were done by using the SPSS 15.0 version.

Ethical clearances:

This research work was approved by the Ethical Review Committee of Dhaka Medical College, Dhaka.

Results:

Table-I: Number of Purkinje cell in different age group

Group (n)	Number of Purkinje Cell Per Square mm, Mean \pm SD	Comparison Group	P Value
Group - A	160.71 \pm 24.47	A vs. B	>0.10
Group - B	185.14 \pm 22.51	A vs. C	>0.10
Group - C	157.00 \pm 10.54	A vs. D	>0.10
Group - D	152.20 \pm 6.49	B vs. C	>0.05
		B vs. D	>0.05
		C vs. D	>0.10

The mean \pm SD number of Purkinje cell was found 160.71 \pm 24.47 in group A, 185.14 \pm 22.51 in group B, 157.00 \pm 10.54 in group C, 152.20 \pm 6.49 in group D. The highest mean number of Purkinje cell per sq mm was found in group B and lowest were found in group D. The differences of mean number of Purkinje cell in different age group were not statistically significant in any group except group B & group D.

Discussion:

In the present study, the mean \pm SD number of Purkinje cell was found 160.71 \pm 24.47 in group A, 185.14 \pm 22.51 in group B, 157.00 \pm 10.54 in group C, 152.20 \pm 6.49 in group D. The difference of mean number of Purkinje cell in different age group was statistically significant in group B & group D. Hall et al conducted a study on 128 cerebellum and were found that the mean 6318 nucleolated purkinje cells. There was mean reduction of Purkinje cell 2.5% per decade, which is similar to present study¹¹. Chowdhury et al conducted a comparative anatomical study of the cerebellum of man and fowl and found that the large flask shaped purkinje cells arrange in single row, which observed in both species but population per unit volume was lesser in case of human cerebellum¹². Anderson et al estimates the total numbers of Purkinje cell on cerebellum of 10 men with chronic schizophrenia but no neurological disorder, mean age 57 years in rotator method and found mean numbers of Purkinje cell were 345 for each cerebellum¹. A quantitative histological study was made on cerebellar vermis of 10 male alcoholic and 8 age match control cases. The mean Purkinje cell loss was 21% in the alcoholic group^{8,9}. Anatomy of the cerebellum is necessary for diagnosis and treatment of motor abnormality and other diseases of cerebellum¹²⁻¹⁴. We mostly depend on data and findings available from studies conducted in other countries. It clearly indicates

that there is research vacuum in Bangladesh about cerebellum and demands more studies. The findings of the present study are expected to help neurosurgeon, psychiatrist and sonologist to adopt appropriate plan for the evaluation of morphological and histological changes of cerebellum as well as diagnosis and treatment of cerebellar disorders.

Conclusion:

The histological dimensions of the cerebellum changes in respect of age and sex. In the present study, histological studies revealed the thickness of gray matter and white matter and number of Purkinje cell per sq mm decreased with age which was statistically significant. As the sample size was small, further study with larger sample size is recommended and cytological study of diameter of Purkinje cell, Basket cell, Granular cell by electron microscope are recommended.

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