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**ORIGINAL ARTICLE**

# Determination of Proliferative and Cytostatic Characteristics of Methylcobalamin on Distrorted Purkinje Cell Layer of Cerebellum

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## ABSTRACT

**Background:** The cerebellum principally the motor organ is involved in the regulation of muscular tone and skilled motor movements. The cerebellar histology consists of three layers and the middle is the Purkinje cell layer which consists of pyramidal shaped purkinje cells. Clinical research shows scanty literature on the beneficial effects of Methylcobalamin on Purkinje cells layer.
**Aim:** Our aim was to bring to light the need for prescribing Methylcobalamin in the masses and patient suffering from motor incoordination.
**Method:** 15 animals were given Methylcobalamin and the changes in the thickness of Purkinje cell layer ware recorded at twelve weeks
**Result:** The morphometric analysis showed restored thickness of Purkinje cell layer
**Conclusion:** The recorded data of the regenerated purinje cell layer thickness proved that the use of Methylcobalamin is mandatory as protective drug in damaged neuronal tissue.
**Key words:** Proliferative, Cytostatic, Purkine cell layer

## INTRODUCTION

Reversal of cerebellar atrophy[1] by metals like Zinc, Aluminum, and Lithium[2] can be achieved by the use of vitamins[3]. It is mandatory for clinicians to bring to knowledge the use of Vitamin B analog[4] Methylcobalamin in patients suffering from the deleterious brain damage[5] caused by Metals.

The cerebellar hemispheres[6] are located in posterior cranial fossa[7] under cover of tentorium Cerebelli.[8]It's cortex is divided into gray and white matter[9].

The Cerebellar Gray matter consists of three layers, the molecular layer, Purkinje cell layer and the Granule cell layer and the middle Purkinje cell layer has Purkinje cells which are the excitatory and inhibitory output of cerebellum[10.]The purkinje cells layer is easily distorted by the use of metals[11.]

Vitamin B 12 analog Mecobal or Methylcobalamin due to its antioxidant property and its efficacy in DNA repair[12] has made its use compulsory in patients of Nervous system diseases. Enough literature is not available in relation to the use of Methylcobalamin in injures of Central nervous system.

Our study was carried out to broaden the horizons of knowledge of use of Methylcobalamin in our population suffering from Neuronal disease so as to increase the clinical use of Vitamin B12 in such patients.

## METHODS

Our present research was carried out in the anatomy department and Animal House of Basic Medical Sciences Institute, JPMC, Fifteen animals weighing 160- 165 grams were selected and divided into three groups .Five in control group A, on lab diet for twelve weeks, for the purpose of documentation of degenerated Purkinje cells and damaged Purkinje cell layer, five animals in Group B were administered Lithium at a dose of 52.5mg/kg/day OD[13] in 1 ml of Nacl for twelve weeks and Group C five albinos were given injection Methylcobalamin 250 µg[14] OD i.p.daily for three months along with lithium carbonate 52.5 mg dissolved in 1ml of Nacl OD for twelve weeks. At the completion of three months animals were sacrificed, brain was separated; the cerebellum was removed and fixed in formaldehyde[15] for 24 hours.

Ascending grades of alcohol was used to dehydrate the cerebellum and it was cleared by xylene and infiltrated by paraffin. The fixed tissue blocks were sectioned and obtained on glass slides four micron thick sections were collected for staining with Haematoxylin and Eosin[16].

The changes of the thickness of Purkinje cells layer were observed under light microscope in all groups. Observations were recorded at the end of twelve weeks. Measurement of thickness of Purkinje layer was recorded under 40 x objectives in selected fields of the tissue. The data was subjected to statistical analysis by using software SPSS 2007 version-16. A statistical difference between means and experimental data was carried out by student 'T' test.

**Statistical Analysis:** Statistical analysis of the thickness of Purkinje cells layer was documented in major group-B (Lithium carbonate treated) shows a highly significant decrease of the thickness of purkinje layer at 12 weeks' time interval as compared to the major group-A (control) but a highly significantly increased thickness of purkinje cells layer was visualized and recorded in Group C as compared to Group B animals.

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## RESULTS

**Group-A (Lab diet for 12 weeks)** which was 20.7± 0.94µm at 12 weeks was observed and showed normal histology and thickness of Purkinje cell layer Table: 1 Observations showed a highly increased thickness of Purkinje cell layer P value <.001 Tables 2 in Group A, Animals

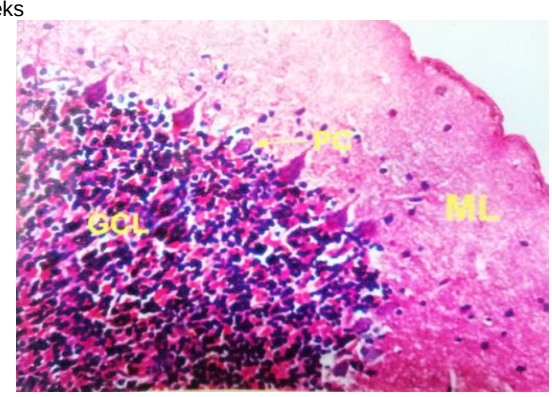
**Group- B (Lithium treated Group at 12 weeks):** A highly significantly (P<0.001) decreased in the mean values of thickness of Purkinje cells layer thickness was observed in Group B **(8.6±0.43µm)**,due to ingestion of Lithium carbonate as compared to group A and Group C animals

**Group-C (Lithium and Methylcobalamin treated group at 12 weeks):** A highly significantly P value< (.001) increased thickness of Purkinje cells layer 17.1±0.40 was recorded in Group C Lithium and Methylcobalamin treated group at twelve weeks as compared to Group B animals. Results proved that Methylcobalamin restored thickness of Purkinje cell layer.

Table 1: Measurement of the thickness of cerebellar purkinje cells layer (µM) of albino rats at 12 weeks in Group A, B AND C

| Groups | n | 12th week | |
|---|---|---|---|
| | | Mean | SEM |
| A Normal Diet | 5 | 20.7 µm | 0.94 |
| B Normal Diet + Lithium Carbonate | 5 | 8.6 µm | 0.43 |
| C Normal Diet + Lithium Carbonate+ Inj.Methylcobalamin | 5 | 17.1 µm | 0.40 |

Cerebellar cortex of Group A animals on Lab Diet for four weeks showing Purkinje cell layer thickness of 20.7±0.94µm at twelve weeks




Distorted Purkinje Cell Layer of 8.6±0.43µm in albinos of Group B on Lithium carbonate for twelve weeks

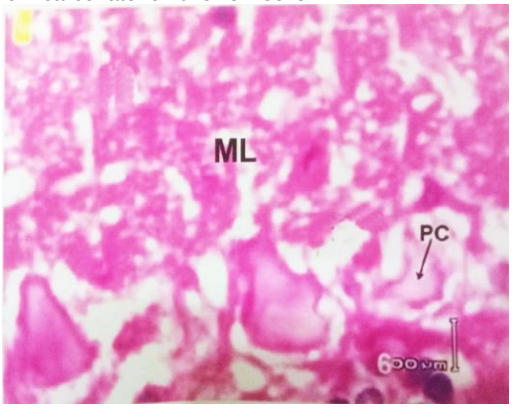
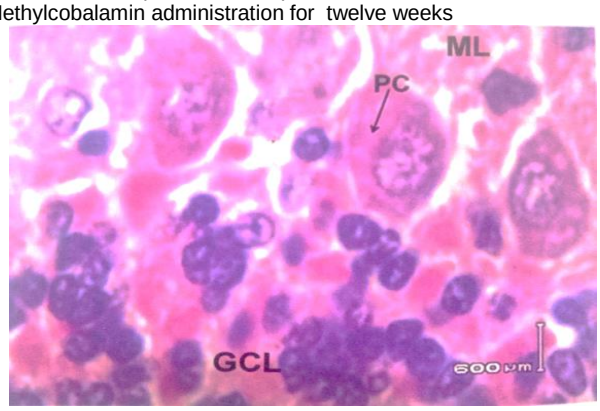



Table 2: P value in Group A, B and C

| Groups | Weeks | P value |
|---|---|---|
| A | 12th Week | P<0.001*** |
| B | 12th Week | P<0.001*** |
| C | 12th Week | P<0.001*** |

Proliferated and regenerated purkinje cell layer with thickness of PCL 17.1±0.40µm in Group C animals on Lithium and Methylcobalamin administration for twelve weeks




## DISCUSSION

The role of deficiency of Methylcobalamin causes Oxidative stress which results in abnormal functioning of brain and Cerebellum[17].

Purkinje cell layer thickness due to purkinje cell death,was evident by increased space between cells, this damage to cerebellar purkinje layer was observed by the use of inorganic substances like Mercury[18], also the dysfunction of Cerebellar Purkinje cells[19] and cerebellum was reported by Banwari in 2016[20], who in their study had reported that the use of Lithium caused cerebellar degeneration and the same documented results are in accordance with our study which showed that metal use leads to Cerebellar gray matter deterioration.

The above observed detrimental effects by use of soft metal [18]and hazardous substances on nervous tissue particularly cerebellar gray matter component that is Purkinje cell layer were reversed by use of micronutrients such as Methylcobalamin[21].

Our research is in accordance with the above observations this may be due to the fact that Methylcobalamin causes DNA synthesis, Methylation and its beneficial effects in brain injury are both neuron regenerative and leads to enhancement of neurotransmitter synthesis.

## CONCLUSION

My study had proved for neurologists that Methylcobalamin administration should be a mandatory ingredient in brain injured patients.

**Conflict of interest:** Nil

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