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Original Article

Analyses of Histomorphological Changes in Cerebellar Cortex after Exposure to Fine Particle Matter in Animal Model

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ABSTRACT

Objective: To determine the histology of morphological changes in cerebellar cortex after exposure to fine particulate matter in mice.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Anatomy Department, Army Medical College in collaboration with Pak Emirates Military Hospital, Rawalpindi, Pakistan and National Institute of Health (NIH) Islamabad, Pakistan, from Jun to Sep 2020.

Methodology: Thirty C57BL/6 male and female mice (8 weeks of age) procured from NIH, Islamabad. There were two groups of fifteen mice each, consisting of seven female and eight males. As the reference, Group-A was given an ordinary food and unlimited water. Group-B, the test group, spent 12 weeks receiving dynamic inhalation of 3 mg/m³ fine particles (soot) through air circulation, six hours a day. The treatment took place in a 2 x 2 x 2-foot plastic cabin equipped with two tiny fans to distribute particulate matter equally. Following the period of exposure, the animals were slaughtered. The tissue sectioned, stained, and then the microscopic investigation was done both qualitatively for spongiosis, inflammation and quantitatively for cortical thickness.

Results: Animals in Group-B showed presence of spongiosis in grey and white matter with (p<0.001, p=0.017, respectively) inflammatory changes (p<0.001) and changes in cortical thickness with (p<0.001, p<0.001 respectively).

Conclusion: The current study found that mice's cerebellar tissue histomorphological traits, such as spongiosis and cortical thickness, are altered by fine particulate matter.

Keywords: Air Pollution, Cerebellum, Grey Matter, Inflammation, Particulate Matter, Spongiosis.

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INTRODUCTION

Particulate matter (PM), also called aerodynamic diameter, can be coarse (2.5 - 10µm PM10), micro (<2.5µm PM2.5), or nano (<100nm or 0.1µm; UFP). Presence of PM causes greater harm to human health as compared to ground level ozone.[1,2] The biggest source of ambient UFPs is automotive emissions.[3,4] Over 80% of the ecosphere's inhabitants are subjected to contaminant concentrations above World Health Organization's recommended level, with nearly,[3,6] million deaths attributable to ecological contamination.[5] PM is generated from gaseous precursors (subordinate PM) or discharged directly into the atmosphere (primary PM). PM species, like sulfates and organic compounds reflect sunlight back, cooling the surrounding air; PM like black carbon absorbs visible light and warms the environment.[6]

Air pollution affects the central nervous system (CNS) through inflammation and oxidative stress.[3,7] Neuroinflammation damages neurons and activates microglia, producing cytokines and reactive oxygen species. This damages the blood brain barrier causing astrogliosis, lipid peroxidation, DNA damage, and CNS disorders.[8] Particulate matter was found in human brain parenchyma and capillaries, indicating the possibility of it interacting with blood brain barrier cells and passing past it via as-yet-unidentified pathways.[9] PM has extremely detrimental impacts on the brain including cerebral edema, manifested as memory loss, mental dysfunction, and behavioral and cognitive abnormalities.[10]

Studies have focused the histological effect of fine particulate matter on multiple organs for example lungs, heart, nasal mucosa, maternal exposure and some components of brain. Despite considerable literature there is still a need for studies, which should investigate the histomorphological changes in brain because of rapidly increasing rate of dispersion of air pollutants, especially in the developing country like Pakistan. Considering this, we planned a study to

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observe the effects of particulate matter on cortical thickness and presence of spongiosis.

METHODOLOGY

The laboratory-based experimental study was conducted at the Anatomy Department, Army Medical College in collaboration with Pak Emirates Military Hospital, Rawalpindi, Pakistan and National Institute of Health (NIH) Islamabad, Pakistan, from Jun to Sep 2020, after receiving approval from the Ethical Review Committees (dated: 2nd Feb 2020, letter no. ERC/ ID/10).

Inclusion Criteria: Healthy male and female mice of 8 weeks of age having an average weight of 37 gram.

Exclusion Criteria: Mice with any obvious injury and disease.

Thirty C57BL/6 male and female mice (average weight 37 grams) at 8 weeks of age were obtained from NIH, Islamabad using non-probability convenience sampling technique. Within the NIH animal house, they were housed in individual cages with conventional laboratory settings, including a 12-hour light/dark cycle and a temperature of 22±2°C. The rats were given unlimited water and regular laboratory rat food.

There were two groups of 15 mice each, consisting of 7 female and 8 male mice. Group-A was the control group and Group-B was experimental group (Figure-1). They were fed a regular meal and water ad libitum. For a period of 12 weeks, mice in experimental group B were subjected to a dynamic inhalation of carbon soot, containing 3 mg/m^3 fine particles, via air circulation six hours a day.[11,12] In two distinct cages, 15 mice (8 males and 7 females) were housed in a 2x2x2 foot plastic cabin at NIH Islamabad, equipped with two little fans facing each other. Fan operation was kept to a moderate speed to maintain air circulation. Using a fan, the particulate matter was distributed uniformly around the cabin. Particulate matter dosage was determined in milligrams per minute of respiration.[13,14] The dose was measured with a precision digital balance. With a refractive index of 2.4 and a normal sensitivity, the particle was identified as carbon powder. Particle size varied between 0.020 and 2000.000µm, having a specific surface range of 0.555 m2/g.

The animals were put to death, dissected, and beheaded by severing the neck near the head at the end of three months, eighteen hours after their final exposure. The cranium was opened, revealing the brain and identifying the optic nerve. The cerebellum was recognized after the brain was detached and put on the dissection board. After identifying the middle cerebellar peduncle, the cerebellum was sliced through to detach it from the brain stem. After fixation, three transverse sections of cerebellum were obtained. After processing tissue was embedded with paraffin wax. The slabs were permitted on a cold plate to harden, cross sections made and placed on glass slides. Hematoxylin and Eosin (H & E) stains were applied to the sections using the Leica autoStainer in order to examine the histology of the cerebellum.[15] Qualitative parameter like spongiosis (brain edema) and inflammation in grey and white matter was assessed by taking ten successive high-power fields from left to right per specimen and was graded as absent or present.[16] Quantitative parameter like thickness of molecular and granular layer was recorded by considering 3 separate fields from left to right and measuring the thickness from periphery to the center at 10X magnification by using Micrometry. Three slides per specimen were examined and mean was calculated.[17,18]

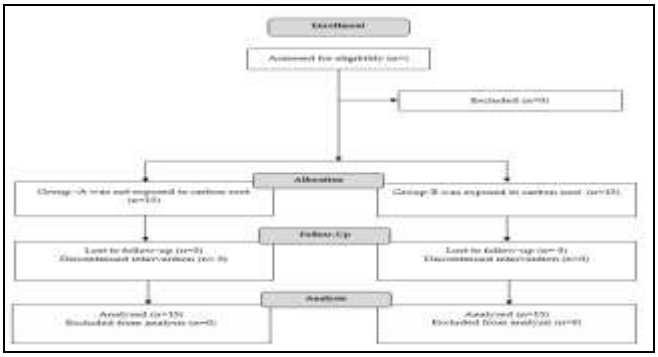


Figure-1: Flow Diagram (n=30)

Statistical Package for Social Sciences version 23 was employed for data analysis. Mean±SD were used to present quantitative data. Frequencies and percentages were used to display qualitative factors. The independent samples t-test was used to compare cortical thickness between groups. A p-value ≤0.05 was deemed significant. The intergroup comparison between A and B was done by Fisher's exact test to show the presence and absence of spongiosis in grey and white matter and inflammation. To compare the qualitative variables, Chi-square test was used.

RESULTS

A total of 30 mice with average weight of 37 grams, and 08 months of age were included in the

study. Gender wise distribution of Group-A and Group-B was 8(4.8%) male and 7(4.2%) female. The spongiosis (edema) in grey matter showed a frequency of 100% in Group-A, whereas in Group-B it was present in 11 mice (73%) and absent in 4 mice (27%) as explained in Figure-2. The presence of spongiosis can be seen histologically in Figure-3. The frequency of spongiosis (edema) in white matter in Group-A was 100%, whereas in Group-B it was present in 6 mice (40%) and absent in 9(60%). The evaluation of spongiosis between groups was done by Fisher's exact test which displayed substantial results with (p<0.001, 0.017 respectively) as documented in Table-I. The inflammation present in Group-A was 100% whereas in Group-B inflammatory signs were present in 10(67%) and absent in 5,(33%) with a p-value of <0.001. Signs of inflammation can be seen in Figure-4. Mean thicknesses of molecular layer in Group-A was 14.8±1.6 µm and in Group-B mean thickness of molecular layer was 12.6±1.8 um, whereas mean thickness of granular layer in Group-A was 13.4±2.1 µm and for experimental Group-B the mean thickness of granular layer was 8.7±1.4µm. The intergroup comparison was done by independent sample t-test which yielded significant result with a p-value <0.001 as depicted in Table-II.

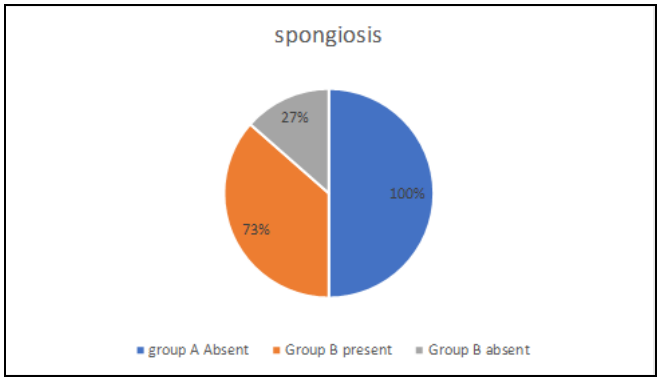


Figure-2: Pie Chart Showing Percentage of Spongiosis in Grey Matter among Group-A (100%) and Group-B, present in 73% and Absent in 27%. (n=30)

DISCUSSION

Thirty C57BL/6 mice, each eight weeks of age, were chosen for this study in order to evaluate the outcome of exposure to fine particulate matter on the morphology of the mouse cerebellum histology.[19] Each of the subjects survived during the course of the research. Compared to control group, the experimental group showed signs of spongiosis (cerebellar edema) in both the grey and white matter of the cerebellum, (73% in the grey matter and 40% in the white matter), with the difference being statistically significant (p≤0.05).

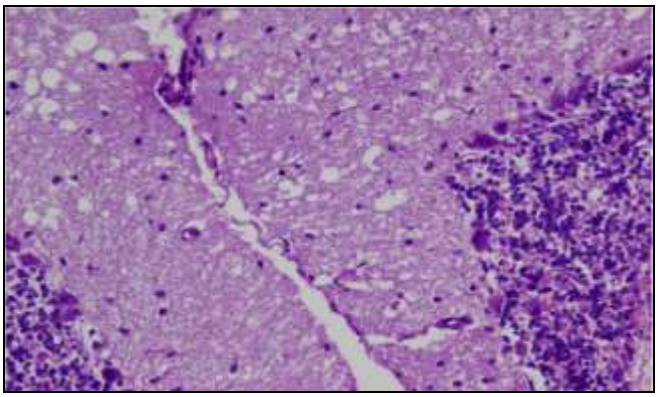


Figure-3: Micrograph of Histology Section of Cerebellum in Group-B showing layers of Grey Matter. Red Arrowhead Indicates Spongiosis in Molecular Layer while yellow Arrowhead Shows Spongiosis in Purkinje cell layer (n=30)

Table-I Comparison of Inflammation, Spongiosis in Grey and White Matter across Groups (n=30)

Parameters	Findings	Group-A (n=15) n (%)	Group-B (n=15) n (%)	p-value
Spongiosis in grey matter	Present	-----	11 (73)	< 0.001
	Absent	15 (100)	4 (27)	
Spongiosis in white matter	Present	-----	6 (40)	0.017
	Absent	15 (100)	9 (60)	
Inflammatory cells	Present	----	10 (67)	< 0.001
	Absent	15 (100)	5 (33)	

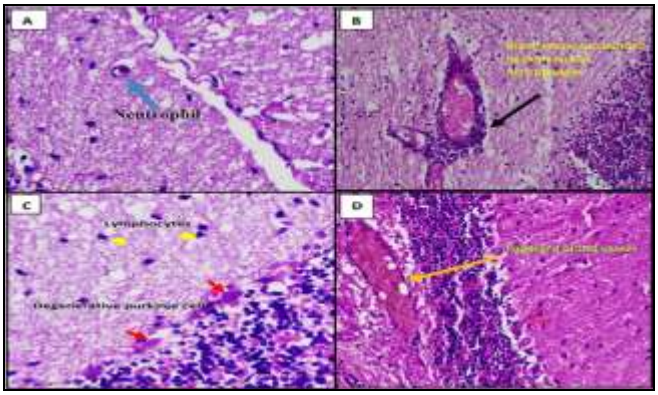


Figure-4: Photomicrograph of Histological Section of Cerebellum of B2F, B3F& B8M Showing Signs of Inflammation. (A) Neutrophil blue Arrowhead; (B) blood vessel Surrounded by Perivascular Macrophages black Arrowhead; (C) Lymphocytes yellow Arrowhead; (D) Engorged Blood Vessel orange Arrowhead (n=30)

In contrast to deeper levels, such as the granular layer, and less noticeable in the white matter, the spongiosis is more noticeable in the molecular and

Purkinje cell layers of the grey matter. This might be because the current study lasted for 12 weeks, and longer exposure times might be necessary for deeper penetration. These findings are consistent with research that found that deep layers of the cerebellum develop spongiosis as a result of FPM exposure.[20] Inhaled particle air pollution has been shown to cause inflammation and oxidative stress in the brain, which can impair central nervous system function. Brain edema develops as a result of the extracellular buildup of fluid brought on by the BBB's enhanced permeability.

Table-II Comparison of Means Thickness of Molecular Layer and Granule Cell Layer across Groups (n=30)

Parameter	Group-A n =15 Mean±SD	Group-B n =15 Mean±SD	*p*-value
Thickness of molecular layer	14.8 ± 1.6µm	12.6±1.8µm	0.001
Thickness of granular layer	13.4±2.1µm	8.7±1.4µm	< 0.001

In contrast to Group-A, which shows no evidence of inflammation (*p*-value 0.000), Group-B had 67% more signs of inflammation, notably early signs of necrosis and dispersed lymphocytes in the grey matter. These findings are comparable to a study that shows that oxidative stress and inflammation are the common and fundamental mechanisms through which air pollution causes damage, including negative effects in the central nervous system (CNS) through the activation of pro-inflammatory signals that stimulate cytokines that cause neuroinflammation.[22] Following induction with diesel exhaust particles, microglia in another investigation directly boosted the production and release of pro-inflammatory cytokines.[23]

Compared to Group-A, Group-B's molecular layer thickness was substantially lower ($p<0.05$). The development of spongiosis and stress brought on by exposure to fine particulate matter are most likely the causes of the thickness decrease. These findings are consistent with research that found children who were exposed to PM from traffic had thinner cortical walls.[21]

With a *p*-value of <0.001, Group-B's granule cell layer thickness was reduced in comparison to control group A. The development of spongiosis and stress brought on by exposure to fine particulate matter could be the cause. This outcome is consistent with research, which found that exposure to air pollution from traffic reduced cortical thickness.[17] Another study found that stress from radiation exposure causes the granular layer to thin.[24]

LIMITATIONS OF STUDY

The study could have been more elaborate, but unfortunately due to lack of modern equipment for particulate matter dispersion, advanced microscopy and staining and shortage of time, we were unable to address many aspects including evaluating the effects of particulate matter, especially PM2.5, associated with traffic related air pollution (TRAP) because it is one of the most hazardous of the outdoor pollutants.

CONCLUSION

The current study found that mice's cerebellum tissue exhibits morphological changes in histology when exposed to fine particulate matter in the form of carbon soot. The main consequences of exposure were appearance of spongiosis, inflammation and alteration in thickness of cerebellar cortex.

Conflict of Interest: None.

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Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

SS & SH: Conception, study design, drafting the manuscript, approval of the final version to be published.

SMK & SM: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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