Original Article

Progressive Histomorphometric Analysis of the Lateral Ventricle and Cerebral Cortex of Wistar Rats Following Kaolin-Induced Hydrocephalus

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ABSTRACT

Context: Hydrocephalus results in ventriculomegaly following excess production and/or impaired drainage of cerebrospinal fluid. The lateral ventricle (LV) is surrounded by critical structures such as hippocampus and thalamus; its enlargement will adversely impact surrounding brain structures including the cerebral cortex.
Aims: This study aims to evaluate the morphometry of cerebral cortex and LV in hydrocephalus over 4 weeks.
Settings and Design: Fifty-one 3-week-old rats were divided into Groups A (experimental = 6; control = 5), B (experimental = 8; control = 6), C (experimental = 8; control = 6) and D (experimental = 6; control = 6), sacrificed at the end of 1, 2, 3 and 4 weeks, respectively.
Materials and Methods: Experimental rats were induced by injection of 0.04 ml of 200 mg/ml kaolin suspension into the cisterna magnum under ketamine (90 mg/kbw) and diazepam (12.5 mg/kbw) anaesthesia. Rats were sacrificed by cervical dislocation and brain fixed in 10% formal saline. Brain slices at the level of the optic chiasma were processed and stained with haematoxylin and eosin.
Statistical Analysis Used: One-way ANOVA and Student–Newman–Keuls test.
Results: In experimental rats, lethargy, poor feeding, globular head and exophthalmos were noted. The LV width and the LV/cortical thickness (CT) ratio were significantly increased from the 1st to the 4th post-induction week ($P < 0.0001$ across the weeks). CT was significantly reduced from the 2nd to 4th week ($P < 0.0001$ across the weeks). The subcortical white matter (SWM)/CT ratio was significantly reduced from 1st to 3rd week ($P < 0.0001$ across the weeks), but increased in the 4th week ($P = 0.0003$). Thinning/detachment of the choroid plexus was noted from the 3rd to the 4th week.
Conclusions: White matter/cortical thinning and ventriculomegaly are acute-phase features, although cortical thinning lags behind others. Detachment of the choroid plexus and reversal of SWM thinning are features of chronicity.

Key words: Cerebral cortex, hydrocephalus, lateral ventricle, ventriculomegaly, white matter

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INTRODUCTION

Kaolin-induced hydrocephalus is an obstructive animal model of hydrocephalus in rat following the blockage of the outflow tract of the cerebrospinal fluid at the cisterna magnum region. Hydrocephalus is associated with the enlargement of the lateral and 3rd ventricle with attendant structural deformity of contiguous structures such as the thalamus, hypothalamus and hippocampus. Linear measurement has been found to correlate adequately with ventricular enlargement.[1] Loss of cortical tissue had been demonstrated in a post-haemorrhagic chronic hydrocephalus alongside neurocognitive deficit.[2] Widespread cortical thinning in different parts of the human brain has been demonstrated in idiopathic normal pressure hydrocephalus and Alzheimer's disease.[3] Loss of oligodendrocyte alongside astrogliosis is known features of kaolin-induced hydrocephalus that are in direct relation to the severity of ventriculomegaly.[4] Ventriculomegaly is one of the classical structural manifestations of schizophrenia.[5] Many

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neurodevelopmental disorders such as autism, schizophrenia and attention deficit disorder are associated with some degree of ventriculomegaly which usually begins in utero foreshadowing a significant post-natal ventricular enlargement.[6] The pre-natal increase in brain size is associated with a stable ventricular size in the second and third trimester of human gestation.[7] Specifically, lateral ventricle (LV) size increase occurs in the first 20 weeks of human gestation following which the size of the ventricle stabilises or declines despite the continuous increase in brain size throughout gestation.[8] The increase in ventricular size is usually associated with reduction in adjoining grey matter volume.[9] The enlargement of the ventricle within a fixed skull is associated with a reduction in the global size of the cortex in schizophrenia.[10] Fetal LV enlargement is associated with several brain disorder in the pediatric age group such as progressive hydrocephalus, agenesis of the corpus callosum and microcephaly.[11-14] The enlarging ventricles in hydrocephalus have been found to compress surrounding structures in a one-time study.[15] The pattern of the enlargement of the LV over a period of time will further help in explaining the progressive manifestation of hydrocephalus.

MATERIALS AND METHODS

Rat groupings and induction of hydrocephalus

Fifty-one 3-week-old rats of both sexes weighing between 19 and 36 g were used for this research work. Rats were housed in well-aerated animal holding and kept in plastic cages. Natural light/dark cycle between July and August persisted throughout the study; water and rat chow were provided *ad libitum*. Ethical approval for the work was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee. Rats were randomly divided into Groups A, B, C and D each having experimental and control subgroups as follows:

1. Group A, 11 rats (Experimental = 6; Control = 5), sacrificed 1-week post-induction
2. Group B, 14 rats (Experimental = 8; Control = 6), sacrificed 2-week post-induction
3. Group C, 14 rats (Experimental = 8; Control = 6), sacrificed 3-week post-induction
4. Group D, 12 rats (Experimental = 6; Control = 6), sacrificed 4-week post-induction.

Hydrocephalus was induced by the injection of 0.04 ml of 200 mg/ml kaolin suspension in normal saline into the cisterna magnum percutaneously under intramuscular anaesthesia (90 mg/kg body weight of ketamine and 12.5 mg/kg body weight of diazepam). The kaolin suspension was prepared under strict sterile condition from acid-washed kaolin powder obtained from Hopkins and Williams, England. Rats' movement and feeding were closely monitored after induction.

Sacrifice and tissue processing

At the completion of the above-stated number of weeks for each group, experimental and control rats were sacrificed by cervical dislocation. Rats were cardiac-perfused with 10% formal saline following irrigation with normal saline. Rats were decapitated, and the head was further fixed in 10% formal saline overnight. The brain was harvested with a blunt forceps following a sagittal incision on the dorsal surface of the fixed head. The brain was blotted dry and weighed with Mettler Toledo AB204. Coronal brain slices were obtained at the level of the optic chiasma and further fixed in 10% formal saline. Brain slices were processed for histology and embedded in paraffin wax. Five micrometre thick coronal sections were obtained using Leica RM 2125 microtome and stained with haematoxylin and eosin.

Microscopy, measurements and statistics

The slides were viewed under the microscope (×10 objective) to measure the dimensions of the LV and the thickness of the cortex. All photomicrographs were taken with Leica DM 750 microscope interfaced with Leica ICC50 digital camera. All measurements were done with the use of Leica application suite (LAS EZ version 1.8.0 2003–2009). Measurement of the width of the LV and thickness of the dorsolateral cortex was done at the Cornus ammonis 1 (CA1) region of the hippocampus. Results are presented as mean and standard error of mean (SEM) ± SEM. Data were analysed with one-way and Student–Newman–Keuls *post hoc* test.

RESULTS

General observation

Following recovery of the experimental and control rats from the effect of anaesthesia which lasted for about 24 h, the control rats were very active and feed normally. However, the experimental rats appeared lethargic. Within 1 week of induction, there was no difference in the appearances of the control and experimental rats aside from the above-stated lethargy. In the 2nd-week post-induction, the lethargic posture of the experimental rats became more pronounced with reduced feeding activities. The experimental rats of the group exhibited abnormal movement, and toward the end of the 2nd week, the globular appearance of the head of the experimental rats compared with those of the control became apparent. By the 3rd post-induction week, the experimental rats demonstrated an apparent bulge of the eyeball which was less pronounced in the 4th week experimental subgroup.

Corticoventricular morphometry

The thickness of the cerebral cortex, measured at the level of CA1 consistently across the groups and subgroups, increased in a progressed manner as the chronological ages of the rats increases. The apparent natural increase in the cortical thickness (CT) as demonstrated by the control rats' cortical photomicrographs was significantly slowed down in the experimental group. The differences between the experimental and control CT was not significant in the 1-week group ($P = 0.069$). However, in the 2nd to the 4th week post-induction groups, the differences were significant ($P < 0.0001$ across the groups) [Figure 1].

The width of the LV measured at the level of the CA1 demonstrated a consistent significant differences between the experimental and control subgroups of each week ($P < 0.0001$ across the weeks 1–4) [Figure 2]. The control rats demonstrated a consistent increase in the width of the LV as the age of the rats increases. The LV/CT ratio showed a significant difference between the experimental and control subgroups across the 4‑week period ($P < 0.0001$ across the groups) [Figure 3]. Table I showed a significant difference between the control and experimental subgroups across the 4‑week period ($P < 0.0001$ for weeks 1–3; $P = 0.0003$ for week 4). Figure 4 showed a progressive enlargement of the LV in the experimental rats' cerebral cortex compared with the corresponding control. In addition, while the choroid plexus appeared attached to the undersurface of the subcortical white matter (SWM) bundle in the control rats across the groups, similar arrangement was only noted in the experimental subgroup of week 1. Week 2 experimental rat showed an elongated and stretched choroid plexus still attached to the undersurface of the SWM bundle. The choroid plexuses were

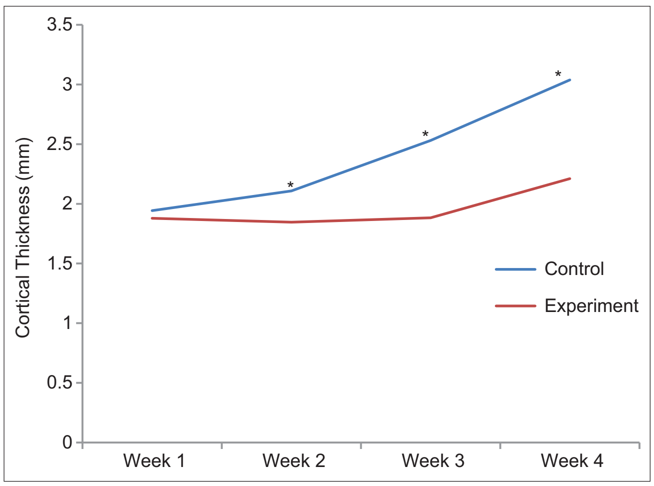

Figure 1: A line graph showing the comparison of cortical thickness (mm) between the experimental and control subsets of 1–4 weeks' post-induction. *Statistically significant difference at $P < 0.05$

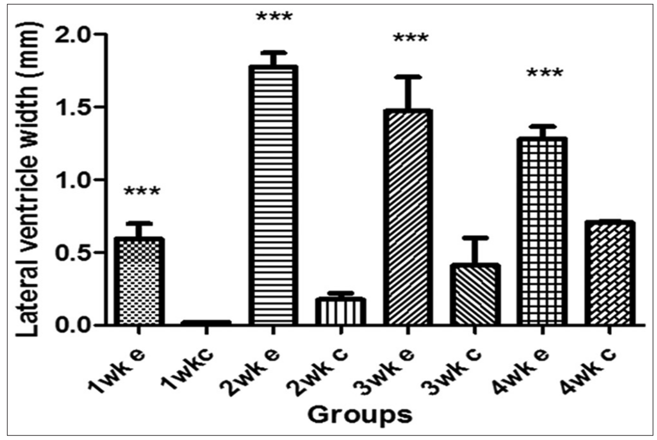

Figure 2: Comparison of lateral ventricle width between the experimental and control subsets of 1–4 post-induction weeks. C; control, E; experimental (***Statistically significant at $P < 0.05$)

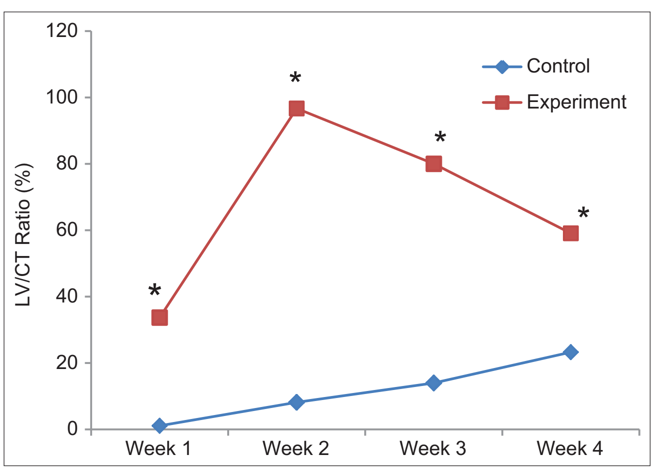

Figure 3: A graph showing the comparison of lateral ventricle/cortical thickness ratio (%) between the experimental and control subsets of 1–4 weeks' post-induction groups. *Statistically significant difference at $P < 0.05$. Lateral ventricle width; cortical thickness

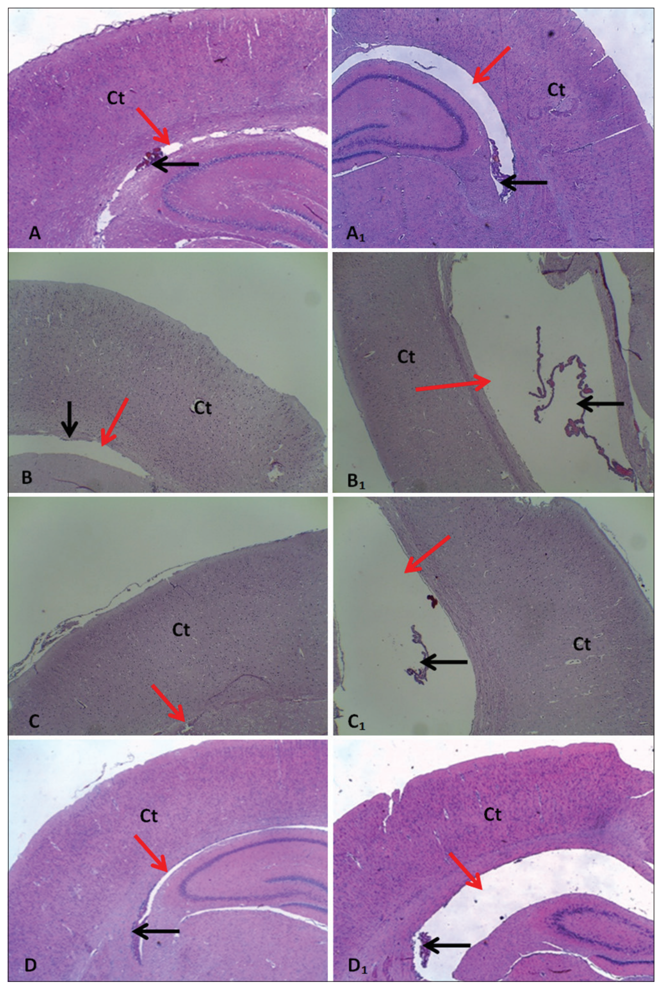

Figure 4: Photomicrographs of the brain of 1, 2, 3 and 4 weeks control rats (A, B, C and D, respectively) and 1, 2, 3 and 4 weeks experimental rats (A1, B1, C1 and D1, respectively) showing ventriculomegaly in the experimental groups. The lateral ventricle (red arrow), cortex (Ct). Choroid plexus (black arrow) (H and E, ×40)

Table I: The comparison of the control and experimental subgroups' subcortical white matter/cortical thickness ratio across the 4-week period

	Control (%)	Experimental (%)	*P*
Week 1	18.25±0.819	13.61±1.129	<0.0001
Week 2	16.61±1.246	11.63±1.031	<0.0001
Week 3	26.51±4.795	12.96±0.809	<0.0001
Week 4	15.67±0.876	18.68±1.011	0.0003

completely detached in the experimental rats of the 3rd and 4th week group.

DISCUSSION

Kaolin-induced hydrocephalus in 3-week-old Wistar rats is a model of obstructive hydrocephalus in young adult rats. Following recovery from anaesthesia which lasted for about 24 h with significant reduction in activities of the rats, the control subgroups of the rat became very active and started feeding normally. However, the experimental subgroup rats remained lethargic with reduced food consumption. This might be the earliest consequence of the blockage of cerebrospinal fluid drainage without obvious increase in head size. This assertion is further corroborated by the finding that globular appearance of the head did not become apparent till the end of the 2nd week of induction. Therefore, the sustained lethargy in the experimental rats might indicate that very slight ventricular enlargement might be sufficient to have mechanical effects on the structures surrounding the lateral, 3rd and 4th ventricle. Since the induction is via the cisterna magnum, it is therefore expected that the obstruction will first of all reflect in the enlargement of the 4th ventricle. This might result in mechanical compression of the medullary part of the brain stem which surrounds the 4th ventricle, thereby resulting in a lethargy. The delay in globular enlargement of the head might be explained by the later timing of the enlargement of the LV. However, the presentation of the 4th ventricle in an obstructive model of hydrocephalus will be a subject of further investigation.

The thickness of the cerebral cortex was affected by the enlarging LV. This is in tandem with previous findings in human and animal models of hydrocephalus.[16] In this present study, the control rats across the 4-week period revealed that a natural increase of the thickness of the cerebral cortex is a progressive phenomenon in developing rats. The rate of increase of the CT appears to be constant on weekly basis particularly from the 2nd to the 4th week post-induction. The non-significant difference in the 1st week of the induction is due to the point of obstruction in the model of hydrocephalus used in this study. As earlier mentioned, injection of kaolin at the foramen magnum will likely result in obstruction caudal to the 4th ventricle thereby causing an enlargement of the 4th ventricle ahead of the LV which is in consonance with earlier studies.[17] The significant enlargement of the LV from the 2nd to the 4th week coincides with the globular enlargement of the head from the 2nd week. This points to the fact that the globular head that characterises obstructive hydrocephalus[18] in rat is secondary to the enlargement of the LV as against other ventricles. Although the significant increase in size of the LV remains till the 4th week, a reduction in the degree of LV enlargement in the 3rd and 4th week post-induction was noted when compared with the degree of enlargement in the 2nd week. Although when compared with the corresponding control, a significant increase in the width of the LV is still maintained. The reduction in the degree of ventriculomegaly is attributable to a possible reduction in the production of cerebrospinal fluid by the choroid plexuses of the lateral, 3rd and 4th ventricle due to the buildup of pressure within the ventricular system. While the state of the choroid plexus of the 3rd and 4th ventricle is not within the scope of this study, it is known that a significant amount of the cerebrospinal fluid is produced by the choroid plexus of the LV. Therefore, the stretching in the 2nd week and subsequent detachment (3rd and 4th weeks) of the choroid plexus of the LVs might be indicative of a damaged choroid plexus resulting in reduced production of the cerebrospinal fluid. While the choroid plexus of the control rats were subcortical in position, none of the experimental rats' micrograph presented a similar position. The change in position might also be a reflection of damage to the choroid plexus thereby resulting in reduced cerebrospinal fluid production. The trend of differences between the experimental and control rats was similar in the width of the LV and the LV-CT ratio. It is known that the size of the cerebral cortex has direct effect on cognitive capacity;[19] therefore, changes in the cognitive capacity in the scenario of hydrocephalus and ventriculomegaly of the LV are major factors to be considered in any model of obstructive hydrocephalus. The LV-CT ratio is meant to eliminate the bias that is likely to be created by the differences in the original brain size in the rats that constitute each group which might be reflected in differences in the CT. However, the pattern of the differences between the experimental and control subgroups in LV-CT ratio is similar to that of the LV. This showed that the initial size of the brain reflective in the thickness of the cerebral cortex does not significantly affect the degree of the enlargement of the LV. Even after the removal of the bias of the cortical size, the degree of the LV-CT ratio peaked at the 2nd week and dropped subsequently, although the differences between the experimental and control subgroups remain significant throughout the 4-week timeline. This is in consonance with the pattern shown by the LV width. The initial thickness of the cerebral cortex does not therefore alter the degree of ventriculomegaly in the LV in this model of hydrocephalus. The progressive reduction in the degree of LV enlargement from the 3rd week might indicate a timing to this phenomenon in obstructive hydrocephalus. While the scope of this study is limited to a 4-week period of ventriculomegaly, the finding about the LV enlargement suggests a continuous reduction of the rate of ventriculomegaly. It is possible that after a period of ventriculomegaly beyond 4 weeks, ventricular size might be similar between the experimental and control rats; however, it is known that even when ventriculomegaly slows down, cellular and molecular damage would have been done to the constituents of the cerebral cortex thereby making recovery almost impossible.[20] The subventricular zone of the cerebral cortex is occupied mainly by the white matter bundle emanating from the various neuronal constituents of the cerebral cortex. The SWM bundle thickness-CT ratio showed a consistently significant difference between the experimental rats and their corresponding control subgroup in the first 3 weeks of ventriculomegaly. This shows that the subcortical white matter bundle will consistently suffer diminution in the face of increasing ventriculomegaly. The fact that there was a reversal of the difference between the experimental and control subgroups in the 4th week post-induction is indicative of a change in the factors that led to the earlier reduction of the thickness of the subcortical white matter in the experimental subgroup. Denudation of the ependymal layer of the LV has been reported as a consequence of hydrocephalus over a period of time. Such denudation usually results in the sipping of the cerebrospinal fluid into the subcortical white matter region of the cortex resulting in a false increase in the thickness of the subcortical white matter bundle. Since ependymal denudation in the LV is a product of chronicity in obstructive hydrocephalus, this might have occurred in the 3rd or 4th week of ventriculomegaly resulting in an apparent increase in the thickness of the subcortical white matter in the experimental subgroup when compared with the control subgroup in the 4th week of ventriculomegaly. The 3rd week witnessed more than double the thickness of the subcortical white matter bundle in the control rats compared with the experimental just before the sudden reversal of the thickness of the subcortical white matter bundle in the 4th week. The sudden nature of this phenomenon lay credence to the thinking that the change might be secondary to a mechanical breech of the ependymal layer. Whether the increasing thickness of the SWM will continue after the 4 weeks of ventriculomegaly covered by the scope of this study will be a worthwhile venture in the future.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Reinard K, Basheer A, Phillips S, Snyder A, Agarwal A, Jafari-Khouzani K, *et al.* Simple and reproducible linear measurements to determine ventricular enlargement in adults. Surg Neurol Int 2015;6:59.
2. Chen S, Feng H, Sherchan P, Klebe D, Zhao G, Sun X, *et al.* Controversies and evolving new mechanisms in subarachnoid hemorrhage. Prog Neurobiol 2014;115:64-91.
3. Kang K, Hwang SK, Lee HW. Shunt-responsive idiopathic normal pressure hydrocephalus patient with delayed improvement after tap test. J Korean Neurosurg Soc 2013;54:437-40.
4. Olopade FE, Shokunbi MT, Sirén AL. The relationship between ventricular dilatation, neuropathological and neurobehavioural changes in hydrocephalic rats. Fluids Barriers CNS 2012;9:19.
5. Gilmore JH, van Tol J, Kliewer MA, Silva SG, Cohen SB, Hertzberg BS, *et al.* Mild ventriculomegaly detected *in utero* with ultrasound: Clinical associations and implications for schizophrenia. Schizophr Res 1998;33:133-40.
6. Gilmore JH, Smith LC, Wolfe HM, Hertzberg BS, Smith JK, Chescheir NC, *et al.* Prenatal mild ventriculomegaly predicts abnormal development of the neonatal brain. Biol Psychiatry 2008;64:1069-76.
7. Patel MD, Goldstein RB, Tung S, Filly RA. Fetal cerebral ventricular atrium: Difference in size according to sex. Radiology 1995;194:713-5.
8. Kinoshita Y, Okudera T, Tsuru E, Yokota A. Volumetric analysis of the germinal matrix and lateral ventricles performed using MR images of postmortem fetuses. AJNR Am J Neuroradiol 2001;22:382-8.
9. Horga G, Bernacer J, Dusi N, Entis J, Chu K, Hazlett EA, *et al.* Correlations between ventricular enlargement and gray and white matter volumes of cortex, thalamus, striatum, and internal capsule in schizophrenia. Eur Arch Psychiatry Clin Neurosci 2011;261:467-76.
10. Lawrie SM, McIntosh AM, Hall J, Owens DG, Johnstone EC. Brain structure and function changes during the development of schizophrenia: The evidence from studies of subjects at increased genetic risk. Schizophr Bull 2008;34:330-40.
11. Wilson RD, Hitchman D, Wittman BK. Clinical follow-up of prenatally diagnosed isolated ventriculomegaly, microcephaly and encephalocele. Fetal Ther 1989;4:49-57.
12. Bromley B, Frigoletto FD Jr., Benacerraf BR. Mild fetal lateral cerebral ventriculomegaly: Clinical course and outcome. Am J Obstet Gynecol 1991;164:863-7.
13. Hertzberg BS, Lile R, Foosaner DE, Kliewer MA, Paine SS, Paulson EK, *et al.* Choroid plexus-ventricular wall separation in fetuses with normal-sized cerebral ventricles at sonography: Postnatal outcome. AJR Am J Roentgenol 1994;163:405-10.
14. Patel MD, Filly AL, Hersh DR, Goldstein RB. Isolated mild fetal cerebral ventriculomegaly: Clinical course and outcome. Radiology 1994;192:759-64.
15. Jugé L, Pong AC, Bongers A, Sinkus R, Bilston LE, Cheng S. Changes in rat brain tissue microstructure and stiffness during the development of experimental obstructive hydrocephalus. PLoS One 2016;11:e0148652.
16. Zhang S, Ye X, Bai G, Fu Y, Mao C, Wu A, *et al.* Alterations in cortical thickness and white matter integrity in mild-to-moderate communicating hydrocephalic school-aged children measured by whole-brain cortical thickness mapping and DTI. Neural Plast 2017;2017:5167973.
17. Knol DS, van Gijn J, Kruitwagen CL, Rinkel GJ. Size of third and fourth ventricle in obstructive and communicating acute hydrocephalus after aneurysmal subarachnoid hemorrhage. J Neurol 2011;258:44-9.
18. Gordon DS, Taylor AR. Obstructive hydrocephalus in childhood. Arch Dis Child 1956;31:191-4.
19. Fernández V, Llinares-Benadero C, Borrell V. Cerebral cortex expansion and folding: What have we learned? EMBO J 2016;35:1021-44.
20. Campos-Ordoñez T, Herranz-Pérez V, Chaichana KL, Rincon-Torroella J, Rigamonti D, García-Verdugo JM, *et al.* Long-term hydrocephalus alters the cytoarchitecture of the adult subventricular zone. Exp Neurol 2014;261:236-44.