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RESEARCH ARTICLE

TRANSPORT FUNCTION: WHICH CHOROID PLEXUS IS MORE ACTIVE?

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Abstract

The choroid plexus (CP) is present in brain ventricles. It is responsible for cerebrospinal fluid (CSF) secretion and various vital functions. Special proteins present in choroidal epithelium play important roles in CSF production and energy metabolism.This study aims to compare between the lateral and fourth ventricles CPs using monocarboxylate transporter 1 (MCT1), transport marker, to evaluate the functional activity of this tissue in the two regions.Ten adult male albino rats were used to study the histological features of the CPs and to study the functional activity by quantitative immunohistochemical labeling with MCT1.Reactions intensities for MCT1 marker in the fourth ventricle CP was significantly higher with a wider spectrum of transport activity than those in the lateral ventricle. Both CPs displayed "non-reactive" cells to this marker.The CP of the fourth ventricle had more functional activity than the CP of the lateral ventricle. Immunohistochemical detection of transport marker went along with findings of other histological and biochemical studies to define the CP as a highly dynamic structure with regional variations forming a continuum of one entity tissue capable of functional adaptation according to body needs.

Introduction:-

The choroid plexuses (CPs) are leaf-like highly vascular structures (1). Four CPs floating inside the ventricular cavities of the brain: one in each of the two lateral ventricles, one in the third, and one in the fourth ventricle (2).The CPs are extensions of the ependymal lining of the ventricular walls and consist of a fenestrated vasculature core surrounded by a single layer of polarized cuboidal epithelium with an interstitial stromal layer of connective tissue rich in fibroblasts and cells of the immune system in between (3). Adjacent CP epithelial cells are joined together by tight junctions to form the blood-cerebrospinal fluid (CSF) barrier. Together with adherens junctions (4). The main known function of the CP epithelium is to produce CSF (5).Monocarboxylate transporter 1 (MCT1) is one member of monocarboxylate transporter family or SLC16 (solute carrier 16) that is composed of 14 members based on sequence homologies (6). The MCT isoforms whose distribution within and between cells can be quite distinct from MCT1 (7). The major physiological role of MCT1 is to facilitate L-lactic acid entry into or efflux out of cells depending on their metabolic state. MCT1 is expressed in almost all tissues, in liver and kidney and found in CP, ependyma (8) and endothelial cells (6). MCT1 may be used to transport L-lactate into the cells for gluconeogenesis for which it is a major substrate (9).

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This study aims to compare the regional differences between the lateral and 4th ventricles CPs in terms of transporters system through the quantitative evaluation of MCT1 histochemical detection, in order to highlight the functional variations between the CPs of the cerebral ventricles and reach better understanding of the activity in this tissue and answer the question of: Are the CPs one entity?

Materials and methods:-

Animals and tissue preparation:-

A sample of 10 adult male rats (*Rattusnorvegicusalbinus*). The animals aged 3-6 months, with 300 ± 50 g body weight, and were fed with standard pellet diet. Animals were euthanized with chloroform soaked cotton in an air tight chamber for 5 minutes, then the brains were removed from the skulls and fixed for 18 hours in 4% paraformaldehyde at room temperature (22°C).

The brains were cut in coronal planes rostral to the optic chiasma and caudal to the midbrain in order to obtain lateral and third ventricles specimens, while fourth ventricle samples were made by trimming the remaining caudal part of the brain (cerebellum and brainstem). The specimens were then left in the fixative for another 18 hours and finally transferred into commercial 70% methanol where they were kept until further processing. Paraffin blocks were made and 5 µm thickness sections were cut for immunohistochemical labelling (10).

Immunohistochemistry Labeling:-

The Super Sensitive IHC for Detection Kit MCT1 antibody was found in CP by following all subsequent steps, which carried out at room temperature in a humidified chamber. Super Sensitive IHC Detection Kit was used. Sectioning at 5 µm were used and deparafinization, Incubate tissue in appropriate pretreatment or digestive enzyme for primary antibody and PBS/TBS wash 3 times for 2 minutes. Then incubate slide in Hydrogen Peroxide Blocking Reagent for 10 minutes, PBS/TBS wash 3 times for 2 minutes. Apply Blocking Reagent and incubate for 5 minutes, PBS/TBS wash 3 times for 2 minutes. Apply primary antibody and incubate according to manufacturer's recommended protocol (overnight) incubation, PBS/TBS wash 3 times for 2 minutes. Apply HRP Polymer and incubate for 10 minutes, PBS/TBS wash 3 times for 2 minutes. Add 20µl of DAB Chromogen to 1 ml of DAB Substrate, mix by swirling and apply to tissue. Incubate for about 3 - 5 minutes, PBS/TBS wash 3 times for 2 minutes. Finally counter stain and cover slip using a permanent mounting media.

Controls

For positive controls, adult male rat kidney sections were labelled for MCT1 in the same procedure, while for negative controls adult male rat brain and kidney sections were labelled in the same procedure except that primary antibodies of both MCT1 was replaced by PBS.

Immunohistochemical Reaction Assessment

Forty field images of immunohistochemically labelled slides were captured from the lateral ventricle CP, and a similar number of fields were captured from the 4th ventricle CP. A LEICA DM 750 light microscope equipped with Digital Microscopic Camera 5 Mega pixel digital camera were used to capture the fields. Images were processed with AperioImageScope v.11 program for total positivity. Microsoft office Excel® 2013 program was used to describe the collected data by calculating the Descriptive Statistics and t-Test were used to compare between means in this study.

Results:-

Immunohistochemical Labelling of the Choroid Plexus

Monocarboxylate Transporter 1 (MCT1)

Under light microscope, sections of rat brain labeled with anti-MCT1 showed high reactivity in choroidal epithelium in comparison to other brain regions. Similar reactivity was detected in the choroidal epithelium of both lateral and fourth ventricles (Figures 1-3). Ependyma showed weaker reactivity to MCT1 marker, still less than that of CP epithelium, but higher than the outer surrounding of brain substance (Figure 3). In all sections, some epithelial cells did not label with MCT1 marker, rather they showed their spherical or ovoid nuclei with hematoxylin counter stain. Endothelium of choroidal vessels were highly reactive to MCT1 marker whereas blood cells inside these vessels were non-reactive with this marker (Figure 4).

Controls

External positive and negative controls, and internal negative controls are seen in figure 5.

AperioImageScope Software and Statistical Analyses

Assessment of Anti-MCT1 Reactivity

Statistical analysis of anti-MCT1 reactivity in the lateral ventricle and fourth ventricle CPs gave mean values of 0.253088 ± 0.096446 pixel/micron² and 0.323152 ± 0.106912 pixel/micron², respectively, with a wider range of reaction intensity in the fourth ventricle CP than that in the lateral ventricle CP. Two-sample assuming equal variances t-Test revealed a significant difference between these values ($p<0.05$) (Tables 1-2).

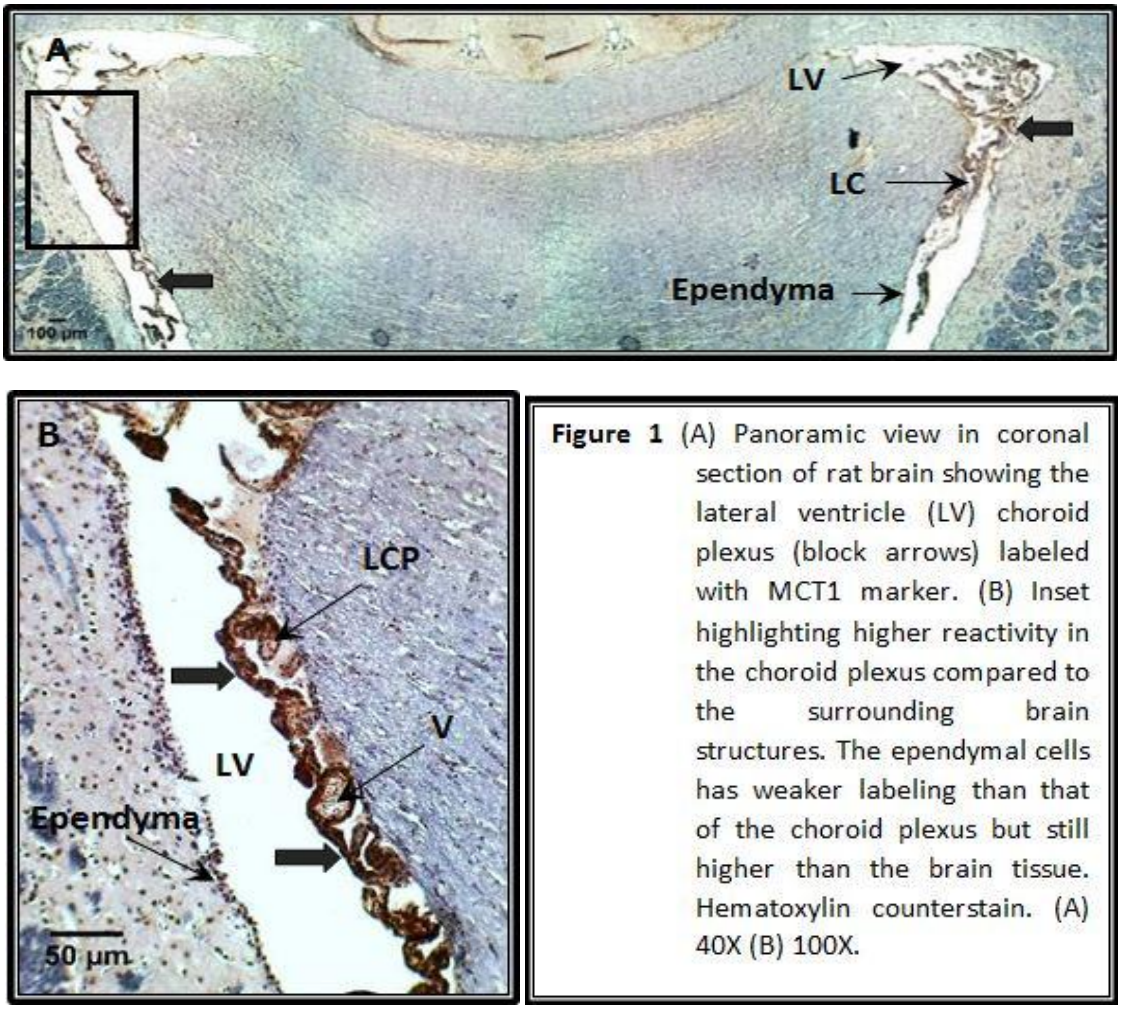


Figure 1 (A) Panoramic view in coronal section of rat brain showing the lateral ventricle (LV) choroid plexus (block arrows) labeled with MCT1 marker. (B) Inset highlighting higher reactivity in the choroid plexus compared to the surrounding brain structures. The ependymal cells has weaker labeling than that of the choroid plexus but still higher than the brain tissue. Hematoxylin counterstain. (A) 40X (B) 100X.

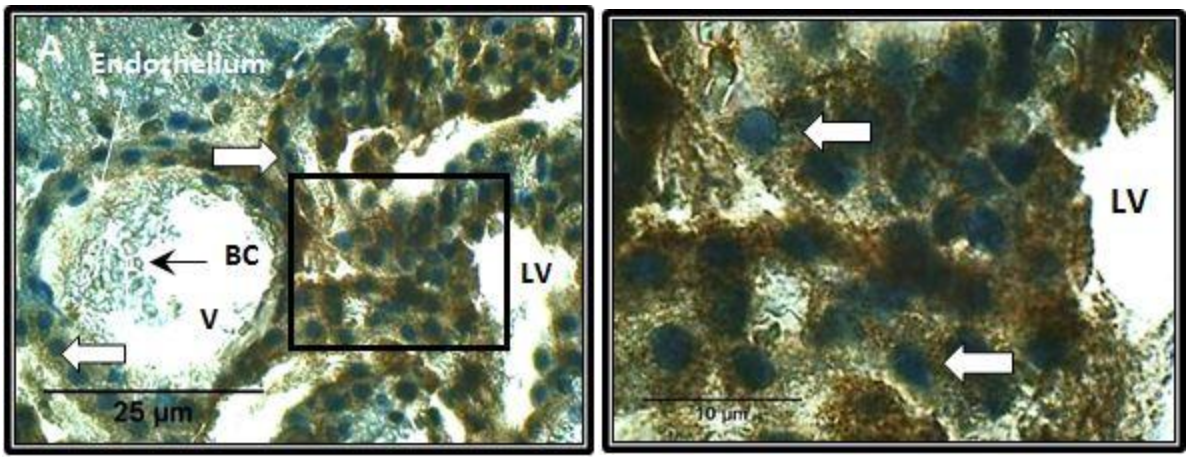
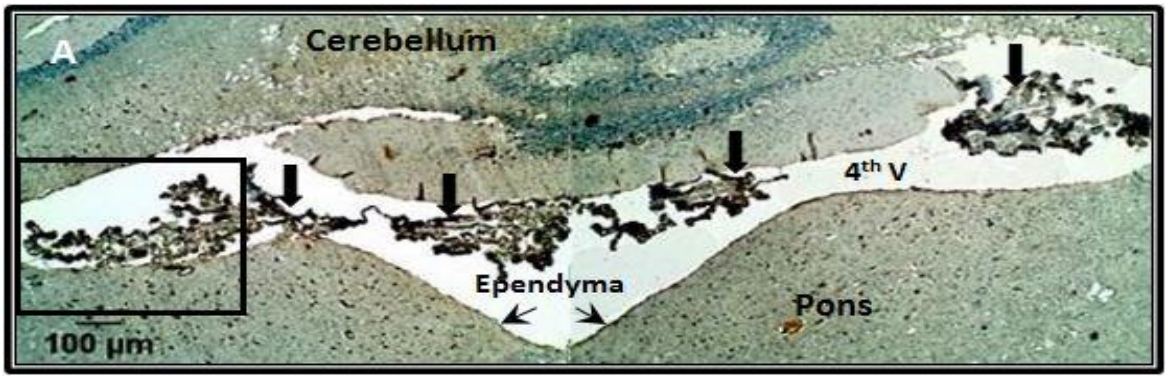


Figure 2 (A) Higher magnification of lateral ventricle (LV) choroid plexus (block arrows) in rat brain showing dark brown color labeling with MCT1 marker. (B) Inset showing large choroidal epithelium nuclei stained with hematoxylin counterstain whereas blood cells (BC) inside blood vessels (V) do not stain. Hematoxylin counterstain. (A) 400X (B) 1000X.



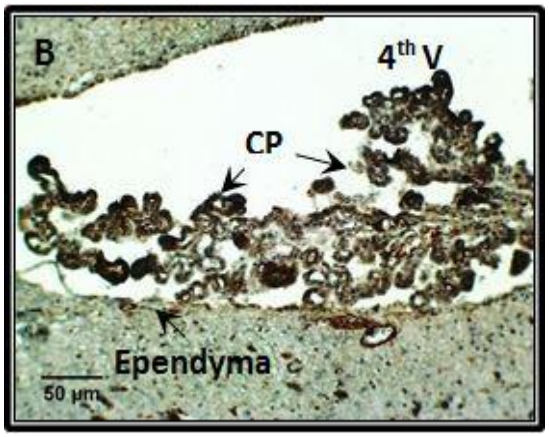


Figure 3 (A) Panoramic view in coronal section through the fourth ventricle (4th V) of rat brain where the choroid plexus (block arrows) is labeled with anti-MCT1 (dark brown color) compared to other parts of the brain. (B) The single-layer ependymal lining of the fourth ventricle has weaker labeling with this marker compared to that of the choroid plexus. Hematoxylin counterstain. (A) 40X (B)

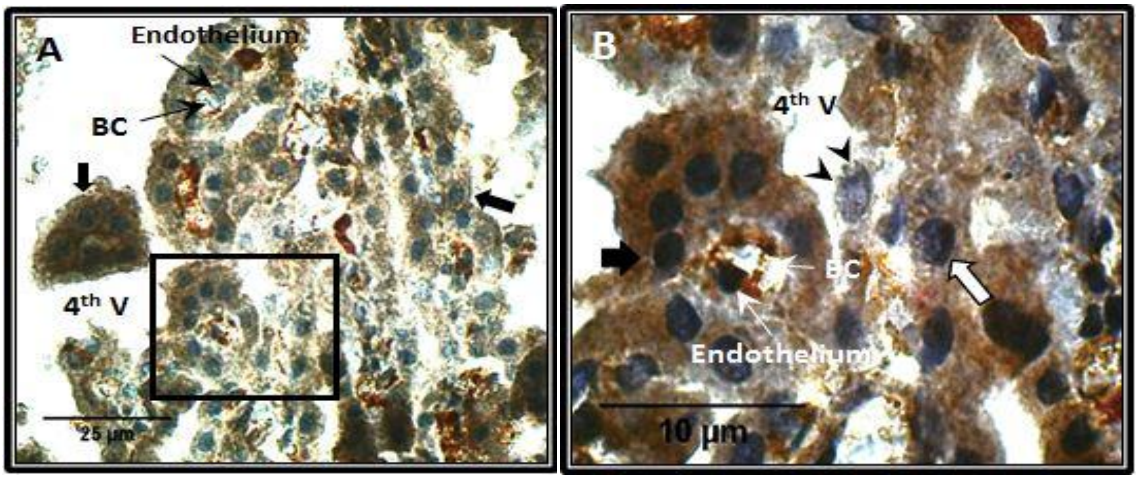


Figure 4 (A) Fourth ventricle (4th V) choroid plexus (block arrows) showing dark brown color reactivity with anti-MCT1. (B) The endothelium of blood vessels is also intensely labeled with MCT1 marker whereas blood cells (BC) inside these vessels do not show such reactivity. Some cells of the choroidal epithelium are not reactive to MCT1 marker (arrow heads). Choroidal epithelium nuclei are seen blue in color as they stain with hematoxylin counterstain. (A) 400X (B) 1000X.

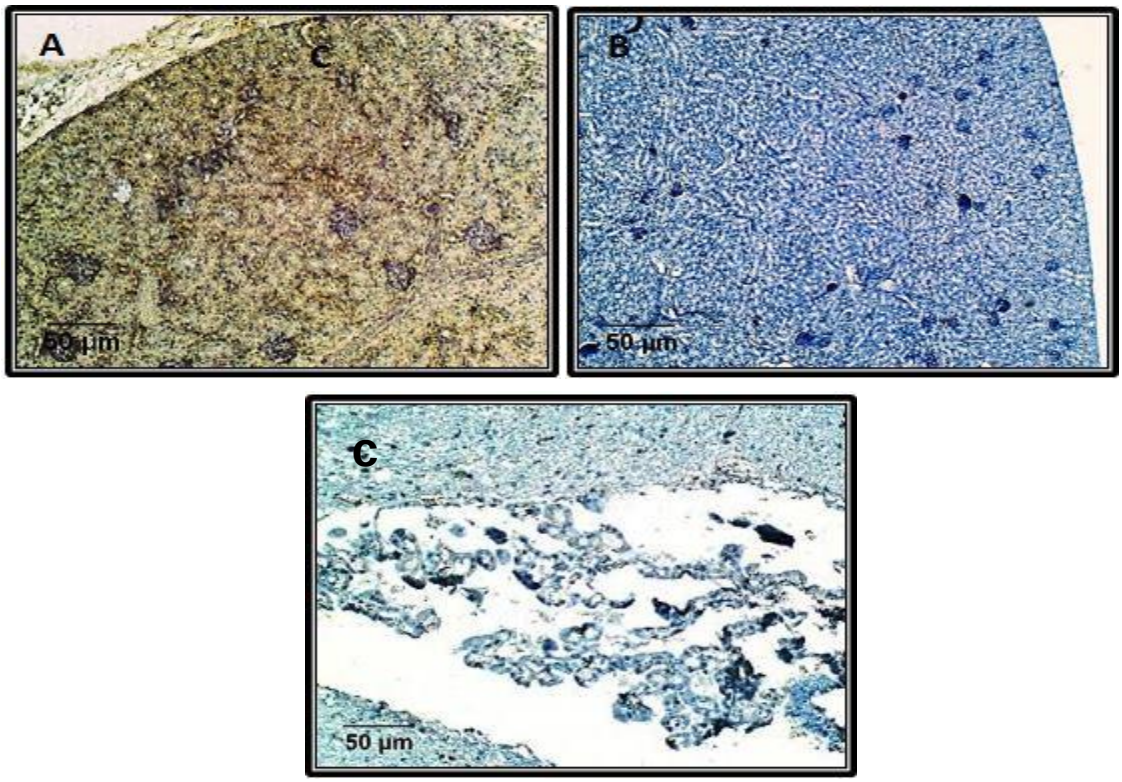


Figure 5 Controls used for immune histochemical labeling. Kidney external positive controls for MCT1 (A) marker, reveal brown labeling of renal tubules with nuclei counterstained with hematoxylin. Kidney external negative controls for MCT1 (B) marker, show no brown labeling. Nuclei are counterstained with hematoxylin. Choroid plexus internal negative controls for MCT1 (C) marker, highlight no immune histochemical labeling while the nuclei are counterstained with hematoxylin. 100X.

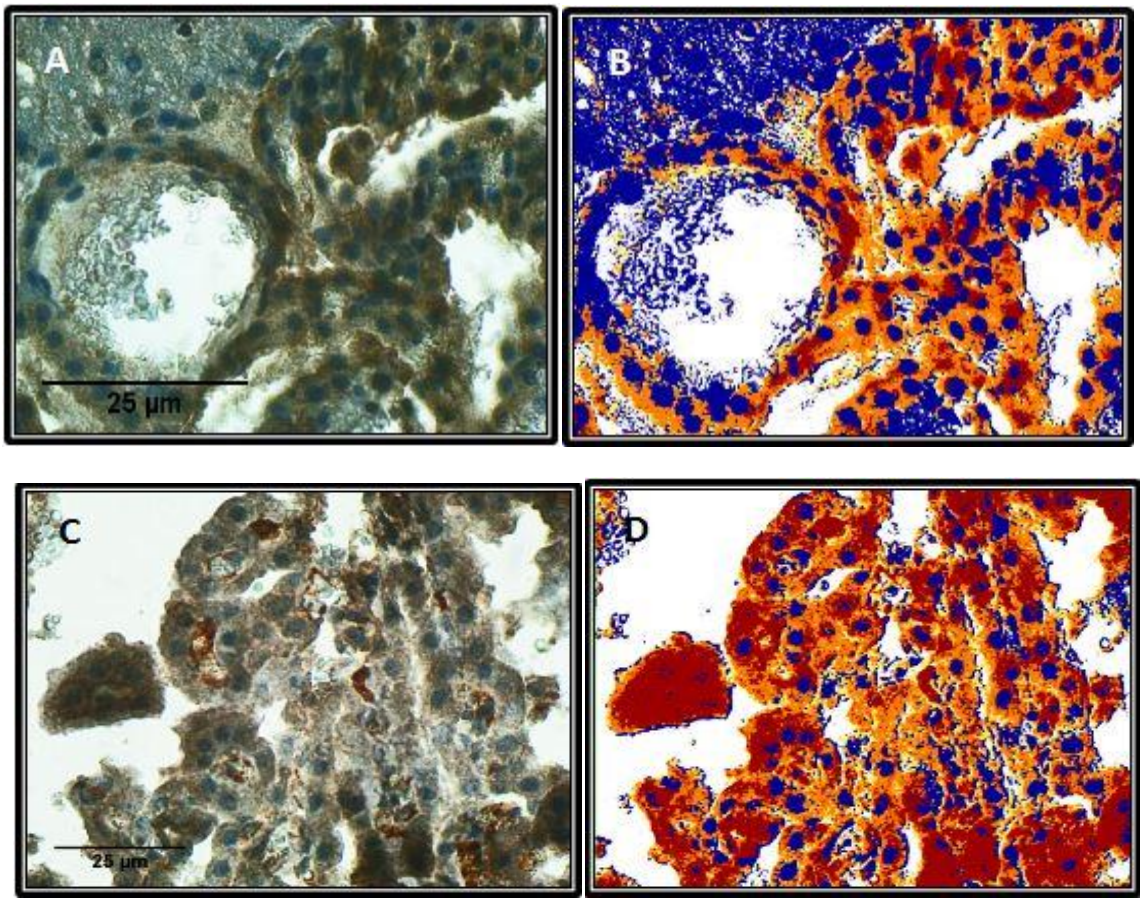


Figure 6 Immune histochemical labeling of the choroid plexuses with MCT1 in the lateral ventricle (A) and fourth ventricles (C) with their respective makeover images (B) and (D) obtained via Aperio ImageScope software. Reaction strength is detected visually where the red color represents areas of strong positivity, orange color represents positivity, yellow color represents weak positivity, and blue color indicates negative or no reaction. 400X.

Table 1:-Descriptive statistics of MCT1 marker labeling in terms of positive pixel algorithm obtained with AperioImageScope software analysis in the lateral and fourth ventricles CPs.

Descriptive Statistics	Lateral ventricle CP MCT1	Fourth ventricle CP MCT1
Mean	0.253088	0.323152
Standard Error	0.015249	0.016904
Median	0.223155	0.290929
Mode	0.186849	0.283605
Standard Deviation	0.096446	0.106912
Sample Variance	0.009302	0.01143
Kurtosis	-0.49597	-0.44496
Skewness	0.664083	0.416701
Range	0.354758	0.441043
Minimum	0.126999	0.130483
Maximum	0.481757	0.571526
Sum	10.1235	12.92607
Count	40	40

Table 2:-Two-sample assuming equal variances *t*-Test of MCT1 marker labeling in terms of positive pixel algorithm obtained with AperioImageScope software analysis in the lateral and fourth ventricles CPs.

Variable	Lateral ventricle CP MCT1	Fourth ventricle CP MCT1
Mean	0.253088	0.323152
Variance	0.009302	0.01143
Observations	40	40
Pooled Variance	0.010366	
Hypothesized Mean Difference	0.010366	
df	0	
t Stat	78	
P(T<=t) one-tail	-3.07755	
t Critical one-tail	0.00144	
P(T<=t) two-tail	0.002879	
t Critical two-tail	1.990847	

Discussion:-

In this study, immunohistochemicallabeling of the lateral and fourth ventricles CPs with MCT1 was analyzed with the AperioImageScope software in terms of reaction intensity of cells which were stained with MCT1 marker. The reaction intensity was categorized into three groups: strongly positive, positive and weakly positive, while areas that did not show any staining were reported as negative (no reactivity). Ependymal cells of CP and endothelial cells, but not blood cells, were stained with MCT1 marker (Figures 1-4). This is in agreement with other immunohistochemical methodologies performed in rat brain where MCT1 protein is detected in brain microvessels, neuropil, ependyma, and glial-limiting membranes, the CP, glial elements of the corpus callosum (8) and in neurons of the thalamus (11). The results of this study showed higher expression of MCT1 in the fourth ventricle CP compared to the lateral ventricle CP (Figure 6) (Tables 1-2). This might be due to the higher activity of the former CP than the latter as it is documented that energy metabolic enzymes (both aerobic and anaerobic) and CSF-linked secretory enzymes are higher in the fourth ventricle CP (12). On the other hand, having a wider range of MCT1 reactivity in the fourth ventricle CP might reflect a spectrum of functional state in the choroidal epithelium in terms of transport mechanism, and thus having “active cells” and “resting cells” mosaic pattern in a higher proportion than in the lateral ventricle CP. On the ultrastructural level, this study also agrees with Al-Kaabi (12) who found that transmission electron microscope study of the fourth ventricle CP marked overall more mitochondrial content in the choroidal epithelium than in the specimens derived from the lateral and third ventricles CPs. This observation reinforces the histochemical finding of MCT1 labelling in this study that the activity of the choroidal epithelium of the fourth ventricle is in more energetic metabolism than that of the lateral and third ventricles. Studies of shark brain suggest that it metabolizes ketone bodies (13). Prior to that study, MCT expression in the brain had not been analyzed in non-mammalian vertebrates where Western blot analysis using antibodies specific for MCT1 detected a protein similar to that observed in the positive controls. In addition, MCT1 was detected within the mitochondria,

apical and basolateral membranes of the CP, indicating that MCT1 can be involved in the movement of ketone bodies at the blood-CSF barrier. If lactate was generated by radial glial cells, the gradient of this metabolite would be oriented from the CSF to the blood such that the plexus would be involved in the depuration of the excess lactate generated by the cerebral parenchyma. In addition, MCT1 has also been described in mammalian endothelial cells involved in the transport of ketone bodies through the BBB (14), and MCT1 and MCT4 could be related to the influx or efflux of lactate (15). The MCT1 is highly expressed in endothelial cells. The strategic location at the blood-brain and CSF-brain interfaces indicates that MCT1 is major factor in the regulation of lactate movement into and out of the brain (16). In that aspect, if the blood vessels present in the lateral and fourth ventricle CPs were assumed to be of equal density, the immunohistochemical reaction intensity detected in this study from these blood vessels would not affect the results of labelling of the CP. However, a quantitative study of CP vasculature density is advised. Such morphological characteristics of the choroidal epithelium are in fact functional adaptations for the role of these cells in CSF secretion. Studies on CSF secretion showed that about 70% is produced by the CP, while the remaining 30% is added by non-choroidal sources, including the ependymal lining of the cerebral ventricles and the spinal canal (12). This means that the function of ependymal cells in terms of CSF secretion (and eventually MCT1 expression) is less than that of the CP, as it was seen in this study, though further assessment through quantitative measurement of labelling intensity is recommended. Previous studies on the CPs of the lateral, third and fourth ventricles considered them as one entity but some authors reported differences in activities of certain metabolic enzymes of the various CPs (12).

Conclusion:-

While carrying the same name as a CP, that part in the fourth ventricle proved distinct functional characteristics from that in the lateral ventricle despite the structural similarities of their cells. In terms of transport system, this study showed preponderance in favour of the fourth ventricle CP.

These findings might add to previous works that showed higher functional activity in the CP of the fourth ventricle compared to that of the lateral ventricle, however short of addressing the two regions as distinct entities. Rather, they form a continuum of tissue capable of functional adaptation according to the body needs.

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Author contribution

Salman: collection, assembly and interpretation of data, manuscript writing; Al-Kaabi: conception and design, interpretation of data, manuscript writing.

Conflict of interest

Authors declare no conflict of interests.

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