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Immunohistochemical Profile of Proliferating Cell Nuclear Antigen (PCNA) in the Choroid Plexus of the Rabbit Ventricles with Clinical Implication

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ABSTRACT

The choroid plexus is composed of highly specialized vascularized epithelial tissues present in 4 ventricles of the brain. The central core of the choroid plexus is occupied by blood vessels. The choroid plexus has many functions like CSF production and synthesis of bioactive peptides. Antigen like Proliferating cell nuclear antigen PCNA, a 36 kd DNA polymerase delta auxiliary protein; is involved in the proliferation of neoplastic and non-neoplastic cells. PCNA has a role in DNA synthesis and repair. Previous studies reported that the PCNA expression in the cytoplasm of cells can be demonstrated by histochemical stains. Total 50 adult male New Zealand rabbits (Oryctolagus cuniculus) were sacrificed, a sample of lateral and 4th ventricles was prepared for H and E and immunohistochemical stains for PCNA. PCNA expression was more in lateral ventricle than 4th one indicates the higher involvement in biological function. The higher PCNA expression in the choroidal cells of the lateral ventricle suggests the clinical importance and pharmacological role in many neurological diseases. ***Significance Statement:*** *The study is conducted to study the differences in choroid plexus of the ventricles separately, and to see the clinical importance for that, and also to evaluate the PCNA agent expression in lateral and 4th ventricles.*

Keywords: Choroid plexus, Proliferating cell nuclear antigen, Epithelial tissues

INTRODUCTION

The central canals of spinal cord continue upward into the brain stem. Then canal widens to form 4th ventricle at the posterior aspect of the Pons and medulla. The loose masses of the connective tissue between the cerebral hemispheres form an approximately horizontal plate that constitutes the roof of the third ventricle which is a structurally similar plate which forms the roof of the 4th ventricle [1].

This plate consists of a single layer of ependymal cells with a thin covering of pia matter forming the tela choroidea, a vascularized membrane in which choroid plexus blood vessels are formed. Because of the active proliferation of vascular mesenchyme, a number of sac-like invaginations are projected into the underlying of the ventricular cavity; these invaginations from the choroid plexus [2].

The choroid plexus in human is found in the 4 ventricles of the brain and they are characterized by:

- Lateral ventricles: Choroid plexus is a vascular fringe composed of pia mater covered with the ependymal lining of the ventricular cavity [3]
- Third ventricle: It is a narrow cavity; there are no choroid plexuses in cerebral aqueduct [4]. The choroid plexus is formed from the tela choroidea situated above the roof of the ventricle [3]
- Fourth ventricle: It is a wide flattened space located anterior to the cerebellum

The 4th ventricle is continuous with subarachnoid space via small 3 openings called basal cistern, foramen of Luschka and 2 foramen of Magendie [4] (Figure 1).

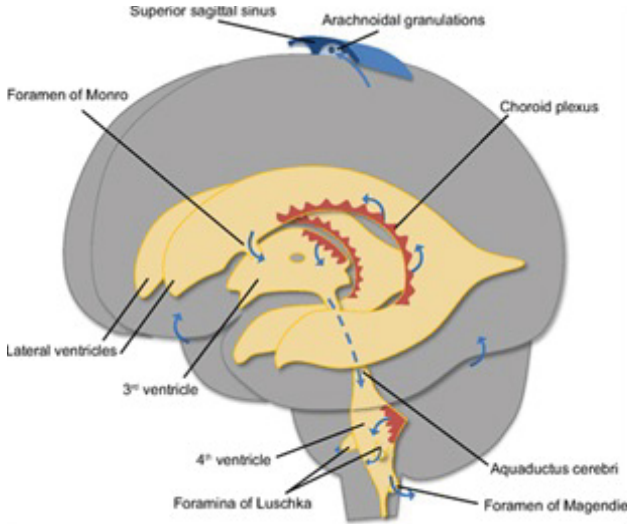

Figure 1 Illustration of the ventricular system with the choroid plexus: Immediately in front of the pineal stalk there is a thin roof of the third ventricle which is pushed into the cavity to form choroid plexus. The 4th ventricle is under the posterior edge of the cerebellar vermis, on each side of the anterior part of vermis there was a thin membranous valve of vieussens, attached anteriorly to the corpora quadrigemina [3]

Embryology of Choroid Plexuses

The proliferative stages of the choroidal cell during the development in human and the rabbit include:

- The first stage: Occurs during the 7th week of gestation when the choroidal cells form columnar pseudostratified with a central nucleus
- The second stage: Occurs in the 9th week of gestation when the cells become larger, short columnar with an apical nucleus, having microvillus and abundant glycogen
- The third stage: Occurs in the 17th-week gestation when the cells become larger, cubical with abundant villi and moderate glycogen
- The fourth stage: Occurs in the 29th week of gestation when the choroid plexus become cuboidal to squamous epithelium with basal or central nuclei and few glycogen [5]

Histological Aspect of the Choroid Plexus

The ependymal is divided into 3 groups of cells:

- Ependymocyte: It is ciliated columnar epithelial cells [3]
- Tanycytes: It is specialized ependymal cells lining the cerebral ventricles [3]
- Also found in the circumventricular organs [6]

The functions of these cells are:

- Transport of substances from the CSF to the hypophyseal portal system
- Participate in the release of gonadotropin-releasing hormones
- Express important functional molecules such as glucose [6]

The choroid plexus is composed of highly specialized vascularized epithelial tissues with elaborate folds and many villi projecting into the 4 large ventricles of the brain [7]. Also, there are 3 types of cells within the choroid plexus that may play a role in controlling the development of immune response within the nervous system.

- The epithelial cells
- Macrophage anatomical studies suggest that these cells mat traffic into the CSF from the choroid plexus [8]
- Dendritic cells in the stromal matrix [8]

Epithelial Part of the Choroid Plexus

It is a cuboidal stratified and pseudostratified epithelium which has apical bulbs microvilli forming the brush border and interdigitation in the basolateral side in the rats that lead to increase the surface area of choroid plexus 10-folds [8,9]. These cells are connected to each other by tight junctions as shown in Figure 2 [10].

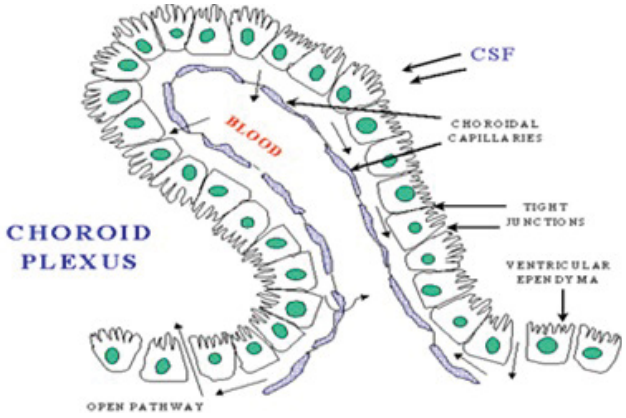

Figure 2 The epithelial cells and the tight junction of choroid plexuses [10]

These cells have mitochondria that are concentrated at apical ends of the cell, it is assumed that the mitochondria are responsible for the high respiratory metabolism of epithelial cells [11].

Vascularized Part of the Choroid Plexus

The central core of the choroid plexus is largely occupied by blood vessels which consist of small arteries and arterioles in addition to a large number of sinuses and capillaries [12]. Previous studies reported that the choroid plexus of the lateral ventricle of some mammals had a large amount of connective tissue with a parallel arrangement of capillaries, while that of 4th ventricle has small and short capillary with little connective tissues in between [13].

The function of the Choroid Plexus

The choroid plexus has many important functions:

1. Formation of the CSF: The CSF contains a high level of sodium and chloride [7,14]. The role of choroid plexus is in transport and clearance of both endogenous molecules and xenobiotic, as well as in drug metabolism it has also been well documented [15]
2. Synthesize a large number of bioactive peptides and secret many proteins like growth factor and transthyretin [16,17]. Many materials are transported across the choroid plexuses epithelium [18]
3. Serves as a gateway for immune cell trafficking to the CSF and provide continuous immune surveillance by CD44+T cell [8], also the choroid plexus has abundant macrophage and dendritic cells that support this immune process [19]
4. Forms blood-CSF-barrier [20]

Previously many authors tried to study the differences between the choroid plexuses of various cerebral ventricles in many aspects to improve the idea that the choroid plexus is not a single entity. The 4th ventricle differs from the lateral ventricle in the severity of inflammation of choroid plexuses in rats [21], also cholinergic nerves and number of sympathetic nerve fibers in choroid plexus of lateral is more than that of the 4th ventricle [22]. Al-kabbi reported that the 4th ventricle contained the higher activity of carbonic anhydrase and esterase activity than the lateral ventricle in adult rabbits [11].

Blood-Brain Barrier and Blood-CSF Barrier

The blood CSF barrier separates the lumen of the capillary from the lumen of the ventricle and composed from the endothelial cells which have a very thin wall and are fenestrated [3]. Many experiments established that permeability barriers existed between the blood and the brain and CSF [23].

The blood-brain barrier is capable of selectively expediting the passage of certain substances while absolutely denying access to others [24]. The presence of tight junctions is largely responsible for the blood-brain barrier [25,26]. The blood-brain barrier is located at the capillary endothelium, these endothelial cells have a low rate of transcytosis and transcellular traffic of solutes [26,27].

A second feature is a large number of mitochondria in cerebral endothelial cells; which reflects the high energy demands to maintain energy-dependent transport mechanisms. The third feature and the most interesting feature of cerebral endothelial is their circumferential enclosure by astrocytic foot processes [11]. The endothelial cell of the blood CSF barrier differs from those forming the blood-brain barrier in being fenestrated [28]. This indicates that the endothelial fenestrations facilitate the movement of fluid out of capillaries.

The lateral surfaces of the choroidal epithelium show zonulae adherents or adhering junctions beneath the tight junctions. The basal lamina of the choroidal epithelium is somewhat a selective barrier which surrounds the relatively smooth basal surface of the cells [23]. There are membrane-bound dense bodies probably lysosomes [27].

In general, the blood-CSF and blood-brain barrier are highly permeable to water, carbon dioxide, oxygen, and most lipid-soluble substances such as alcohol and anesthetic agents [29].

Non-Barrier Areas in the Nervous System

The blood-brain barrier in the CNS (and in peripheral nerves) is not complete in any site where there are specific areas in the brain that provide sites for transfer of proteins and solutes irrespective of size and lipid solubility [27].

Although the blood-brain barrier spans the entire length of the CNS, its absence has been conspicuously noted in several areas. The choroid plexus, neurohypophysis, median eminence, subfornical organ, pineal gland, organum vasculosum of the lamina terminals, subcommissural organ, and area postrema are all devoid of a blood-brain barrier [30].

Proliferation Cell Nuclear Antigen (PCNA)

Proliferating cell nuclear antigen (PCNA) is a 36 kd DNA polymerase delta auxiliary protein that is composed of 40% cyclin-D and cyclin-dependent kinase, it is involved in the proliferation of neoplastic as well as non- neoplastic cells and it is specifically expressed [31]. PCNA also is strongly associated in the nuclear region where DNA synthesis occurs, the PCNA has a role in DNA synthesis and repair [32].

Two basic forms of PCNA protein:

- Soluble sensitive to organic fixation not involved in DNA replication
- Insoluble associated with DNA synthesis [32]

PCNA is also involved in the DNA damage tolerance pathway known as Post-replication repair (PRR) [33]. Some studies showed a positive correlation between the severity of chronic inflammation and the expression of proliferative marker PCNA [34]. The proliferative rate of the choroid plexus in the ventricle decrease in adult, while in the embryo the epithelial cells of the choroid plexus have a high mitotic activity which continues to proliferate and differentiate during 6-29 weeks of gestation [35], the 4th ventricle firstly appears then lateral ventricle and finally third ventricle [5].

Mean a number of positive PCNA in epithelial cells of choroid plexuses leaving G stage of mitotic activity and starting to differentiated have a lower number than that of proliferating cells. PCNA is important in DNA replication and damage repair, transcription [36,37].

Previous studies reported that the PCNA expression in the cytoplasm of cells due to the biological role of PCNA in the cytoplasm, Stanislav, 2004 reported that the M form of PCNA is found in the cytoplasm; there are 3 types of PCNA isoform, which could be a result of post-translational modifications which include acidic (A), main (M) and basic (B) [38-40].

A considerable amount of PCNA proteins are found in the cytoplasm and their function is related to many biological functions as:

- Regulation of glycolysis process in the cytoplasm [38]
- Has a role in the regulation of energy generating system [38]

- Regulation of cytoskeleton of cell [38]

Aim of the Study

The aim of the study is to compare the choroid plexuses of the lateral and the 4th ventricles in the expression of the activity of DNA polymerase factor like the Proliferating cell nuclear antigen (PCNA).

MATERIALS AND METHODS

A prospective study of a total number of 50 adult male New Zealand rabbits (*Oryctolagus cuniculus*), aged 1-105 year with body weights of 2.5-3.5 kg were bought from the animal shopping and were cared until scarification, from each 1 of 50 rabbits' brains sample of lateral and 4th ventricles was taken for preparing H and E and immunohistochemical stains for PCNA.

The choroid plexuses tissue sections were histologically prepared for paraffin sections according to Bancroft, et al., [41]: fixation in 100 ml of 10% formalin as a primary fixation, dehydration by ascending grades of alcohol, clearing by xylene, impregnation, and embedding in a labeled baths of a molten paraffin wax (E. Merch), serial sections of 6 μm thickness were cut by using the electrical microtome, dewaxing, hydration, staining and mounting.

Total 3 sets of slides were made from each block (lateral ventricle and 4th ventricle blocks); one for H and E., the other one for immunohistochemical for PCNA stain. Then after, staining with H and E according to Bancroft, et al., with modified staining method by Harris using aluminum as a mordant and chemically ripened with mercuric oxide was used regressively for nuclear staining. This controlled stain was used to show the morphological feature of choroid plexus of the lateral, 3rd and 4th ventricles [41].

Immunohistochemical staining by using the Abcam anti-PCNA antibody prediluted ab 912 (mouse monoclonal antibody to PCNA) as the instruction from the manufacturer. The sections of H and E and immunohistochemical of choroid plexuses of lateral and 4th ventricle were then examined and different fields of the section were captured by using a microscope digital camera, Genex and new software for imaging top view. Assessment of the immunohistochemical activity in the tissue sections was evaluated by using Aperio positive pixel count algorithms program (Aperio image scope software V.11.1.2.760, Aperio technology, USA).

Aperio image scope can be used to measure the amount of specific stain present in slide digital image, this program has a set of defaults input parameters when first selected, these parameters have been pre-configured for brown color quantification in 3 intensity ranges: weak: yellow, positive: orange, strong: red. The results were stored in the annotation layer attached to the image and can be viewed in the image scope.

Statistical analysis was computer-aided, using SPSS version 20 (most widely used program for statistics in social science). Statistical analysis of 100 section (50 of lateral choroid sections, 50 of 4th ventricle choroid sections) was performed.

Some of the outcomes of the quantitative variable were normally distributed. There was a statistically significant difference in mean between 2 groups. The T-test of significance used for such variables was a parametric test of 2 groups. The $p<0.05$ level of significance was considered statistically significant, which does not require the assumption of normality.

RESULTS

Choroid Plexus Morphology by H and E Stain

Choroid plexuses of lateral ventricle: The CP was seen as a cluster of the solitary layer of cuboidal to low cylindrical cells with rounded nuclei surrounding vascularized cores rested on a basement membrane, nuclei of endothelial cells of choroidal vessels are flattened also found connective tissue in between vascularized core of CPs. In the current study, these clusters of cells are observed with abundant cytoplasm, the nucleus is larger with obvious nucleolus in the lateral ventricle if compared with the 4th ventricle and more vessels were observed in the lateral ventricle than 4th ventricle and the cells suspended by ependyma in the lateral ventricle as shown in Figures 3 and 4.

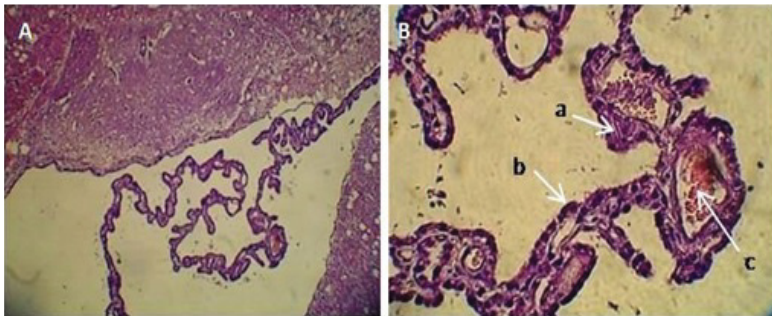

Figure 3 Coronal sections of the cerebral hemisphere with choroid plexuses inside of lateral ventricle by H and E show (a) large nucleus, (b) abundant cytoplasm, (c) larger blood vessels if compared with 4th ventricle (A-10 X, B-40 X)

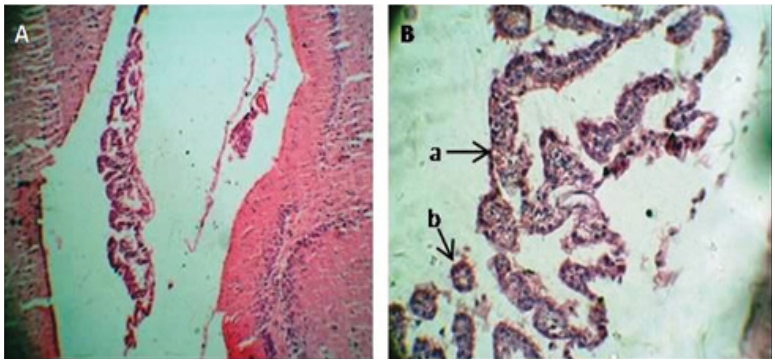

Figure 4 Coronal section of brain stem with choroid plexuses inside of 4th ventricle (B) by H and E stain show (a) round nucleus, (b) less cytoplasm and smaller blood vessels if compared with lateral ventricle (A-10 X, B-40X)

The immunohistochemical identification of the PCNA in choroid plexus of lateral and 4th ventricle: The mean intensity of PCNA was higher in choroid plexus of the lateral ventricle than in the 4th one (0.072 ± 0.049 Vs 0.041 ± 0.019) ($p \leq 0.002$). The difference between mean data was statistically highly significant (Figure 4). The immunohistochemical expression of PCNA was seen in the lateral ventricle as a nuclear stain in the choroidal cell and nuclei of the endothelial cells was very little when compared with cytoplasmic stained of the PCNA, so; intensity gradient of the PCNA was seen from dark brown (strong positive) to light brown (weak positive) while in the choroid plexus of the 4th ventricle although it was stained in general less than that of lateral ventricle (Figure 5).

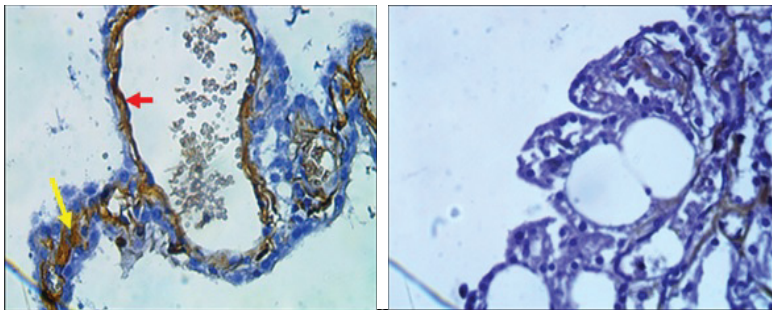

Figure 5 Immunohistochemical expression of PCNA in a coronal section of the cerebral hemisphere with choroid plexuses of (A) lateral ventricle, (B) 4th ventricle, Note that the basal site stained more than apical (red arrow) also the cytoplasm is granular and dark brown (strongly positive) as (yellow arrow), X40

DISCUSSION

Immunohistochemical Expression of PCNA

PCNA is a DNA clamp which acts as a progressive factor of DNA repair and synthesis during the cell cycle in the proliferation of cells [42]. Expression of PCNA in nucleus as a proliferating marker to work as a checkpoint in cell cycle to induce progression of cycle to its peak level during S and G phase of cell cycle and decrease in the G2 and

M phase without expressed in quiet cell, this protein has a role in DNA synthesis and repair, also can be expressed in the cytoplasm regarded for its role in biological activity [31,38].

In the current study, section from adult male rabbits brain was taken to show the differences between choroid plexuses of lateral and 4th ventricles in expression of PCNA, the expression of PCNA in both ventricles do not occur in all cells and this might be explained by the idea that the adult choroid cells are differentiated cells and have lower rate of proliferation less than 0.1% of total plexuses cell [43,44], although the post differentiation developed choroid plexuses cells that had been are processed more difficult to be investigated [45].

Also the expression of PCNA controlled by E2F transcription factors one factor is E2F5 which is high during development after that it will decrease in an adult of mice and human, and change location in adult to cytoplasm this may lead to a decrease of PCNA expression in adult cells [46,47].

The site of expression of PCNA in cell of choroid plexus of lateral and 4th ventricles was not restricted to stalk of choroid plexus especially in lateral ventricle of rabbit as shown in Figure 5 that was different from that occurred during embryological development as others authors studies about the PCNA intensity in comparison between lateral and 4th ventricle in mouse and human during development of choroid plexuses were reported that PCNA staining was largely restricted in stalk of choroid plexuses in 4th ventricle in contrast to the lateral ventricle distributed which occur throughout all choroid plexuses then in late pregnancy, the PCNA is expressed in stalk of choroid plexuses in both ventricles [47], this information means that there is a difference in proliferation of choroid plexuses between lateral and 4th ventricle during embryogenesis but in adult normal choroid plexuses there were no similar studies about this proliferation .

While Centeno BA said that normal choroid plexus was not stained by PCNA when used in the distinguishing between normal and papilloma of choroid plexus cell [48], other authors used the PCNA in distinguishing proliferation process between normal choroid plexus cell and choroid plexus neoplasm. Figen, et al., reported a highly significant difference between normal choroid plexuses and papilloma, also use it to determine the grading of papilloma [49].

In current study, the expression of PCNA was occurred in cytoplasm of choroid plexuses cells of lateral and 4th ventricle this agreed with Stanislav, N who reported the activity of PCNA in cytoplasm to regulate multiple biological pathways as glycolysis pathway in cytoplasm, especially in increase glucose metabolism due to increase glucose-membrane transportation and associated with flux of metabolites through the cascade 10 glycolytic enzymes, PCNA binding with 6 glycolytic enzymes (aldolase, isomerase, phosphate dehydrogenase, phosphoglyceromutase kinase, phosphoglyceromutase, enolase) of steps 4-9 in glycolysis process that suggests PCNA can be at least to coordinate part of glycolysis in cytoplasm, PCNA is like a vehicle that simultaneously and successively interacts with different enzymes in different stages of metabolic pathways and that facilitate signaling transfer to the next step [38].

The cytoplasmic PCNA expression is evidently more than nuclear PCNA which may give a clue to the role of choroid cells essential for mitochondrial energy production and regulation is needed for CSF production, transport of nutrients and material across the blood-brain barrier in ventricles [38].

Also, the expression of PCNA in the cytoplasm of choroidal cells was observed by Stanislav who reports that PCNA binds with malate dehydrogenase in mitochondria to regulate the energy generating system [38]. The choroid plexus cells since it is conserved with CSF production, so it contains a large number of mitochondria for the production of the energy required [50].

Another biological role for PCNA in the cytoplasm is the regulation of cytoskeleton integrity of cells by attaching with protein membrane like annexinA2, sarcolectin and others [38]. The cytoplasmic alteration of neuron-glial cells in the central nervous system rendering them in burden for aging or disease like Alzheimer disease which leads to increase PCNA expression, therefore it makes the cytoskeletal integrity alteration marker [51], PCNA might have a role in cytoplasm DNA.

The results obtained in this study shows that the PCNA intensity in choroid plexuses cells of lateral ventricle is statistically higher with mean of positivity (0.072) than 4th ventricle (0.041) as in Figure 6, and can demonstrate the finding of more CSF production in the lateral ventricle in addition to that, the rapid turnover process of the choroidal cells as well as the high activity of drug transporter and metabolic enzyme which are expressed more by the HNF4α immunohistochemical reactivity, therefore all the above-mentioned data can explain the higher PCNA express in lateral ventricle more than in the 4th ventricle .

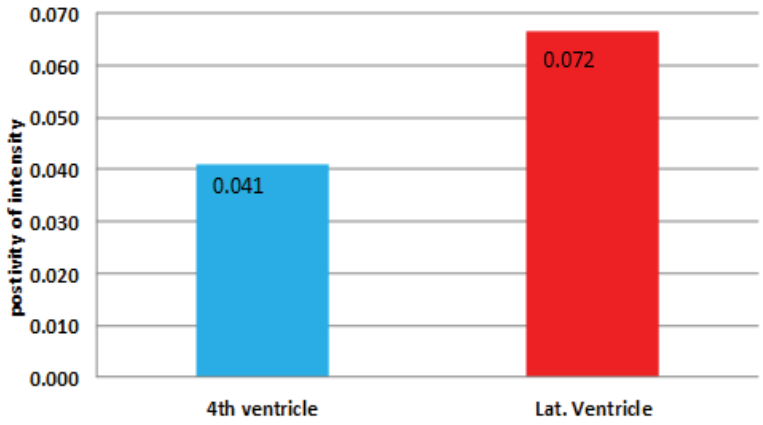

Figure 6 Histogram of mean intensity (positivity) of PCNA of choroid plexuses of the lateral and 4th ventricle

Clinically, previous studies found choroid plexus papilloma was more common in the lateral ventricle that might suggest the high proliferative of the choroidal cells in the lateral ventricle which render them more prone for proliferative diseases rather than that of the 4th ventricle [52,53]. From the above results, the choroid plexus in various ventricles was not regarded as one entity and this might affect clinically on the diseases which occur in these choroid cells and, the treatment.

CONCLUSION

- Choroid plexus is not a uniform entity, there are differences in the histochemical integrity between ventricles
- Expression of PCNA marker is more in the cytoplasm than in the nucleus
- PCNA expression is more in lateral ventricle than 4th one which indicates the higher activity and involvement in biological function like CSF production
- The high expression of PCNA in lateral ventricle may give a clue for the more vulnerability of choroid plexus for diseases in the lateral ventricle
- The higher expression of PCNA in the choroidal cells of the lateral ventricle suggests the clinical importance and pharmacological role in many neurological diseases like Alzheimer

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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