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Immunohistochemical Localization of HNF4α in the Choroid Plexus of the Rabbit Ventricles with Clinical Implication

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Abstract

Background The choroid plexuses are composed of highly differentiated epithelial cells in with connective tissue and permeable capillaries among them. These cells connected by tight junction to form the blood – CSF-barrier that plays important role in protection of CNS. HNF4α is transcription factor of many drugs transporter proteins and known as nuclear subfamily 2 group alpha encoded by HNF4 alpha gene can be found in the liver, pancreas, intestine, brain and recently in epithelial cell of choroid plexuses.

Objectives To evaluation the HNF4α intensity and localization of the choroid plexus in the lateral and forth ventricles of the rabbit.

Methods Choroid plexuses of lateral and forth ventricles of 30 adult male rabbits were studied by morphological and immunohistochemical evaluation of HNF4α in theses ventricles that play role in regulation of drugs transporters and drugs metabolism in B-CSF-B.

Results Histological method showed little different features by subjective examination between various localizations of the choroid plexuses. The immunohistochemical activity of HNF4α was different between lateral and forth ventricle where the IHC positivity is more in the lateral ventricle (0.067±0.029) than forth ventricle (0.032 ±0.018) and this results is statistically significant.

Conclusion The choroidal cells of lateral ventricle showed more IHC activity of HNF4α and might indicate targeting of many drug transporters proteins, metabolites and eliminate of toxic compounds from brain tissues.

Key word Choroid plexuses, HNF4α in lateral ventricle, drug transporter across the blood brain barrier.

List of abbreviation: CP = choroid plexus, CSF = cerebrospinal fluid, HNF4α = hepatic nuclear factor 4 α, DME = drug metabolism enzyme, H&E = hematoxyline and eosin, IHC = immunohistochemical, ABCB = ATP binding cassette transporter proteins, B-CSF-B = blood CSF barrier, HRP = horse radish peroxidase.

Introduction

The choroid plexus (CP) consists of highly differentiated vascularized epithelial tissue. It is found in four sites: in the roof of third, fourth ventricle and medial wall of each of lateral ventricle, combined is about 40 cm^2 [(1)].

Histological evidence of CPs differentiation in several species showed that metancephalic plexuses (forth ventricle) appear first then teloncephalic plexuses (lateral ventricle) and then third ventricle in rabbit and human [(2,3)].

The CP is multifunctional organ responsible for production of cerebrospinal fluid (CSF) in lateral, third and fourth ventricles by diffusion through the ependymal and pia vessels. In human, it is estimated that 95% of the fluid is formed in the lateral ventricles. Most of the remainder is formed in the third and fourth ventricles [(4)]. Then circulation to subarachnoid space and recycle 4 times per day in order to clean out metabolites and toxins like beta amyloidal.

The CP interface between the blood and CSF; it serves as a gateway for immune cell trafficking into the CSF and is in an excellent position to provide continuous immune surveillance by CD4+T cell [5].

The choroidal epithelial cells are closely connected to each other by tight junction and constitute the structural basis of the blood-CSF barrier [6].

Also they produce many substances like transthyrettine [7], transferrin [8], in studies on human and rat and growth hormone factor especially during development of CPs cell and in response to brain injury [9] in rodent species.

HNF4 α (hepatic nuclear factor 4 α) Is zinc-finger protein and known as nuclear subfamily 2 group alpha encoded by HNF4 alpha gene can be found in the liver, pancreas, intestine, brain and recently in epithelial cell of CP, that binds DNA as homodimer plays role in regulation of drug metabolism enzyme (DME) and drug transporters [10], it is required for development of liver and control of many enzymes in liver [11].

The immunohistochemistry study of HNF4 alpha in CP will determine HNF4 alpha –DNA binding activity search transcript expression of various ATP binding cassette (ABC) transporter like (ABCB1, ABCB4, ABCC1) in the CP; this provides evidence of HNF4 alpha to be an important regulator of ABCB drug transport in CPs [10].

HNF4 alpha playsa role in transcriptional control of drug transport [12]. Also HNF4 alpha act as a transcription factor of proteins released from CPs in cytoplasm and intracellular like transthyretin [13, 14].

Methods

A total of 30 adult male New Zealand rabbits (*Oryctolaguscuniculus*), animals were sacrificed by deep anesthesia and the skull open dorsally by strong pair of scissors starting from foramen magnum to the nasal bones. After the brain delivered fixated in 10% formalin for 24 hours then take the brain and divided by coronal section into two parts to obtain the CPs of lateral and forth ventricles, it is difficult to obtain the CPs of third ventricle.

Samples were processed for paraffin blocks, sectioned and then stained with hematoxyline and eosin (H&E) stain for general histological examination according to [15]. Paraffin sections on positively charged slides were further stained by anti-HNF4α antibody.

The anti –HNF4 alpha antibody ab 94748 (rabbit polyclonal to HNF 4 alpha)

- deparaffinize and rehydrate tissue section , then embedding in dry milk 0.5 in pbs solution.
- add enough drops of hydrogen peroxide block to cover the section. Incubate for 10 minutes. wash 2 times in phosphate buffer ph of it 7, then apply protein block and incubate 10 min. at room tempreture, then wash 2 times.
- apply primery antibody (anti –HNF4 alpha antibody) diluted as 1/200 by phosphate puffer incubate for 90 min., then wash 2 times.
- apply complement and incubate 10 min. wash 2 times .
- apply HRP conjugate, incubate for 15 min., rinse 4 times in buffer add 1 drope of DAB chromogen to 50 dropes of DAB substrate mixing by swing and apply to tissue incubate 10 min. rinse 4 times .
- apply counter staining (hematoxyllin) for 7 min., then wash in tabe water for 2 min.
- dehydration and cover slipe with use mounting.

The slides of CPs of lateral and forth ventricle were examined under light microscope and photographic picture was taken and use aperio scope image analysis softt ware positive pixel count (version 9) to measure the intensity of immunehistochemical (IHC) reaction and obtain data.

Result

Choroid plexus morphology by H&E stain

Choroid plexuses of lateral ventricle:

The CP was seen as cluster of solitary layer of cuboidal to low cylindrical cells with rounded

nuclei surrounding vascularised 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 current study, these clusters of cells, observed with abundant cytoplasm, the nucleus is larger with obvious nucleolus in the lateral ventricle if compared with forth ventricle and more vessels were observed in the lateral ventricle than forth ventricle and the cells suspended by ependyma in the lateral ventricle as shown in fig. 1 and 2.

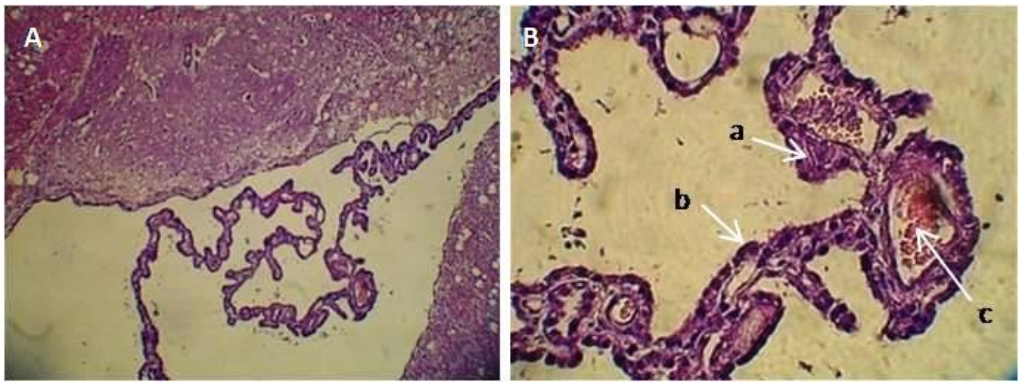

Fig. 1. Coronal sections of cerebral hemisphere with choroid plexuses inside of lateral ventricle by H.&E. show (a) large nucleus, (b) abundant cytoplasm, (c) larger blood vessels if compared with forth ventricle (A -10 X, B-40 X).

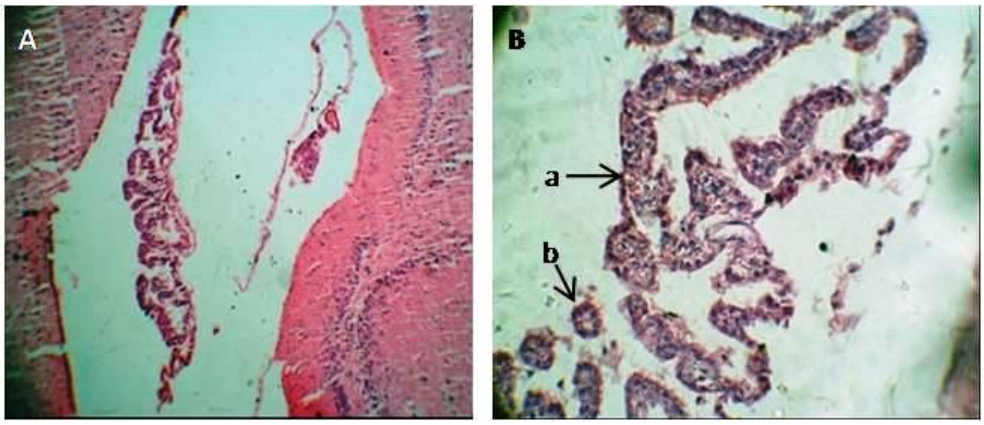

Fig. 2. Coronal section of brain stem with choroid plexuses inside of forth ventricle by H.&E. stain show (a) round nucleus, (b) less cytoplasm and smaller blood vessels if compared with lateral ventricle (A- 10 X, B- 40X).

Immunohistochemical identification of HNF 4 alpha in the lateral and forth ventricles:

The mean intensity of HNF4 alpha was highest in CPs of lateral ventricle and lowest in forth ventricle (0.032±0.018 Vs 0.067±0.029 ($p < 0.0001$). The IHC staining occurs in the basolateral side of choroidal cell near the lumen of blood vessels with a granular stain occur in this area due to founding of aggregation of transporters proteins across through the blood CSF barrier (B-CSF-B) as shown in fig. 3.

Discussion

Significance of Immunohistochemical reaction study:

Previous study on choroid plexuses of lateral, third and forth ventricle were done as a one entity but some authors reported a difference in activity of some enzymes of the lateral ventricle differ from that of forth ventricle [16].

This current study worked to establish the idea that CP of cerebral ventricle is not single entity by IHC reaction of proliferation process and activity in drug transporter in CSF-B-B.

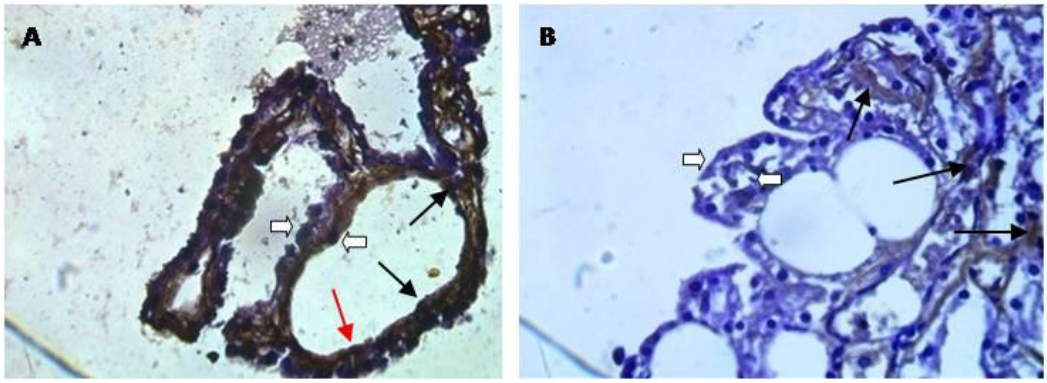

Fig. 3. Coronal section of cerebral hemisphere with choroid plexuses inside the (A) lateral ventricle (B) forth ventricle show expression of ani-HNF4 α in the basolateral site of epithelial cell of choroid plexuses (black arrow), Note that the basal site stained more than apical (white arrow) also the cytoplasm is granular and dark brown (strong positive) as (red arrow) (A & B 40 X).

The IHC reactivity of CPs of both lateral and forth ventricles were estimated by using the Aperio soft ware that can detect the cells stained by the marker stain and categorize them in to three areas negative (not show activity), positive and strong positive. The above is applicable for HNF4 α in the current study, the ependymal cells of CP, endothelial cells are stained by the anti- HNF4 α marker, in addition; others cells stained like RBC and some stromal cell which might give false positive reading and change the real estimation of what the study plane. So to overcome it, the weak positive reading by Aperoi should be omitted and excluded and only positive and strong positive reaction are counted in the field of tissue.

In following manner regarding HNF4α describe activity as:

The positive stained cells shown as dark and light brown colored which indicate strong and moderate positive reaction respectively and occur more in the basolateral side of choroidal cells near the lumen of blood vessels with a granular stain occur in this area due to presence of aggregation of transporter proteins across through the B-CSF-B like ABCC, ABCB1, ABCB4, transthyretein, as shown in fig. 3, also HNF 4α was observed in apical endothelium that means the drug back to blood and this agree with Monika who said that ABCC proteins help as the protective role of choroid epithelial cells and mediate basolateral efflux of conjugates resulting from CSF drugs metabolism in to the blood and apical distribution of ABCB1 in apical side of endothelium [10, 17].

Expression of HNF4α intensity of CP was significantly higher in lateral ventricle (mean of positivity (0.067)} than forth ventricle (0.032)) suggesting that the regulation of drug transporter is more in lateral ventricle, which is not agreed with Suzuki et al who said that CPs of lateral and forth ventricle similar in activity of drugs transporters [18].

HNF4α binding and expression of many proteins and metabolizing enzymes like multiple transporting factor like the ATP binding cassette ABCB4, ABCC1 in human and rat [10] and transthyretine which is one of proteins secreted by CP cells in cytoplasm [13,14] and this protein expressed in CSF-Blood barrier cell [19]. These proteins might be stained by anti HNF4α IHC stain and give cytoplasmic reaction which is detected by the Aperio software which indicates the excessive amount of these

binding proteins that might explain the probability of drugs metabolizing and transporting are more in the lateral ventricle than forth ventricle which is implicated clinically and pharmacologically.

Expression of HNF4α in the CPs of lateral ventricle was higher than forth ventricle this means higher role in defending programs to prevent entry of xenobiotic drugs into the brain because of this tissue express multiple transporter and drugs metabolizing enzymes [20], also regulates distribution and entry of various compounds between CSF and blood interface and is involved in numerous exchange processes therefore determining the supply of brain by nutrients and hormones [20].

Demonstration of cytoplasmic and intracellular reaction of HNF4α as shown in fig. 3 by binding with transthyretin in the choroidal cells cytoplasm to regulate the activity of this protein. The presence of numerous endoplasmic reticulum and Golgi apparatus in CPs made their ability to secret this protein high [7]. This protein secretion is specifically in the CP and not in other parts of brain and bind with HNF4α to control drug transporting, and this protein was the same contents in lateral and forth ventricle [13] also can be demonstrating by activity of HNF 4α in glycolysis process in cytoplasm [11].

Liddelow *et al*, said HNF4α is expressed in adult CP for regulation of many genes encoding junction –adhesion and tight junction and cytoplasmic regulatory adaptor in lateral ventricle like claudin 2, ocludin 6 [21] that may be demonstrate the positivity of HNF4 α in lateral ventricle more.

Relatively, The expression of HNF4α in the choroidal cells of the lateral higher than the forth one might indicate the abundant amount of the binding secreted proteins from the endoplasmic reticulum in the cytoplasm of choroid cells, this might suggest that the endoplasmic reticulum content of protein of the CP of the lateral ventricle is more if compared with that of the 4th ventricle.

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Author contributions

Dr. Farhan conceived and designed the study and preliminary analysis; Dr. Al-Kafagi collected, analyzed and interpreted the data and rewrote the manuscript. Both authors have read and approved the final version of manuscript.

Conflict of interest

The authors declare no conflict of interest.

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