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Blood Brain Barrier and Hepatic Encephalopathy

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1. Introduction:

Dyshomeostasis of the central nervous system (CNS) is a critical factor in HE physiology, homeostasis of nerve cells are maintained in a very limited range where in ions concentration such as Na+, K+, Ca+ along with different metabolites for proper neuronal functioning (Rolfe and Brown, 1997). CNS necessitates highly regulated extracellular environment, as is a critical and sensitive system in human body. The homeostasis is maintained by blood-brain barrier (BBB). A complex system of brain microvascular endothelial cells with connection made by a junctional complex with adherens and tight junction. The brain cells are characterised with slightest pinocytic ability, no fenestrations while great number of ATP-binding cassette (ABC) transporter. Brain stays in an extremely regulated environment with about 20% of blood supply of body. But, unlike all other organs of body, no direct exchange of molecules or cells occur between blood and brain. Thus, most molecules take up transcellular route to enter brain but majority of cells, small molecules, proteins and peptides can not cross the interface present between blood and CNS (Palmer, 2010). The composition of extracellular fluid of CNS differs greatly from non-neuronal cells and this compartmental isolation is brought by special adaptations of brain capillary walls. By this regulation, brain protects itself from several toxic and infective agents circulating in blood. All these functions are critical of BBB i.e. a dynamic ion balance regulator; nutrient transporter; barrier to toxic or potentially toxic molecules (Hawkins and Davis, 2005). ABC transporters function is critical as they prevent brain from toxin accumulation by pumping toxins out of brain. Evidences demonstrate the altered ABC transporters function affecting brain drug/toxin accumulation and CNS activity.

Liver failure and HE are associated because of excessive neurotoxin accumulation in brain affecting its functioning. Serving as an interface, BBB is crucial in limiting free diffusion across brain and blood. With the expression of multiple transporters, receptors and enzymes, BBB along with an anatomical barrier is a dynamic tissue. The endothelial cells are greatly polarised with apical (luminal) and basolateral (abluminal) compartments (Betz and Goldstein, 1978). Anatomical unit of BBB comprises primarily of brain capillaries where capillaries endothelial cells and closely apposed pericytes are unsheathed by overlapping astrocytic end feet, basement membrane, neuronal and microglia terminals together forming “neurovascular unit” (Redzic, 2011).

The significant role of BBB in neurological disorders is elucidated by Zlokovic, 2008. In HE, neurological dysfunctions requires BBB studies and its modulation has direct impact on severity progression of HE. The studies are majorly based on diffusion properties of dyes and their presence in brain is checked using tracers to indicate permeability alterations in HE patients. The molecular patterns of the brain and the signalling associated with BBB involves a number of factors.

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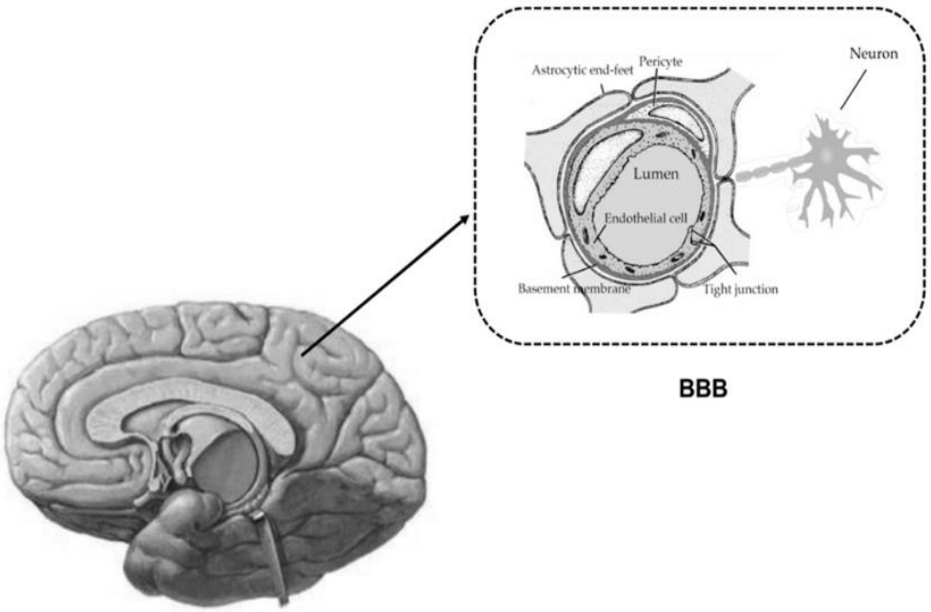

Fig. A represents "neurovascular unit", structure of BBB. The endothelial cells and closely associated pericytes are seen to be unsheathed by astrocytic end feet along with neuronal terminals (Yilin Fan and Xiaodong Liu, 2018). The phenomenon of barrier was observed by Ehrlich about a century ago by staining all organs by systemic administration of dye except brain and spinal cord, concluding the presence of BBB in vertebrates and showcased the evolution along with myelin as it also is absent in invertebrates.

2. The selectively permeable paracellular barrier:

The term barrier though implies a watertight system allowing no exchange but, BBB is more of a selectively permeable barrier or a gate allowing molecules up to a certain size and of specific nature. A molecule if has to cross BBB, the permeability is affected by its relative affinity towards plasma water; plasma protein like bilirubin; carrier protein in endothelial luminal membrane; toward lipid. Factors like experimental tracer, charge on the particle along with size plays crucial role in altering their permeability across the barrier (Cereijido et al., 1978; Lindemann and Solomon, 1962; Moreno and Diamond, 1975; Tang and Goodenough, 2003; Wright and Diamond, 1968). A healthy brain has this barrier at all places except certain regions referred as circumventricular organs. These regions contains the barrier similar as in non-neuronal cells and are the site for exchange between blood and brain for the impermeable substances like polar molecules; peptides and like others lipid insoluble molecules (Oldendorf WH, 1984). Maintenance of BBB is a task of astrocytic cells and any damage or lost of BBB holds astrocytic capability to keep it intact accountable.

The transmembrane protein occludin has been shown to be important for the regulation of tracer diffusion (Matter and Balda, 1999). Overexpression of occludin was shown to increase size-selective paracellular permeability and decrease ion conductance, suggesting not only that occludin regulates tracer diffusion but also that tracer and ion diffusion are likely to be mediated by different mechanisms (Balda et al., 1996b; McCarthy et al., 1996). Junctional ion

permeability seems to be primarily determined by claudins. Claudins are expressed in a tissue-specific manner and the claudin composition of a tight junction in cultured epithelial cell lines determines paracellular ion selectivity (Anderson et al., 2004; Tsukita and Furuse, 2002).

BBB is highly impermeable to systemic neurotransmitters like dopamine, serotonin because as they could undergo large fluctuations in systemic circulation but brain prevents their alterations in order to avoid cognitive and behaviour changes in CNS. Though, because of this property, several clinical problems as well arise like hepatic encephalopathy and also limits therapeutic effects of clinical agents like alpha methyl dopa, L-dopa etc.

BBB is subjected to maintain second order homeostatic functions of brain as well by keeping in check the pH, temperature, osmolarity, glucose concentration, oxygen concentration of neuronal cells in the narrow range and avoid fluctuations in order to avoid nerve cells degradation.The circumferential tight junction complexes at both apical and lateral positions of membrane highly restricts small-hydrophilic molecules (Brightman, 1969; Peppenheimer, 1951). The distinguishing feature between neuronal and non-neuronal cells are tight junctions which are composed of (Seigenthaler et al., 2013; Liu et al., 2012):

a. Occludin and claudin (Tight junction proteins).
b. E- cadherin and VE-cadherin (Adhesion junctions cadherins).
c. Junctional adhesion molecules (JAMs)

For the stability of these junctional transmembrane proteins, cytoplasmic adaptor proteins like zona occludins (ZO) stays bound to these adhesion proteins (Balda and Matter, 2009). Significant crosstalk between these junctional proteins keep the BBB put (Teitz and Engelhardt, 2015).

3. Substrate entry modulation via BBB:

Number of saturable protein systems are present in brain for the nutrient exchange yet at a relatively slow pace. The substrates are modulates as to keep the selective movement via barrier in check For eg. L-glucose permeability through BBB is nil and thus needs to be first modulated into its D form in order to pass through barrier. The pace varies as per clinical condition as in hypoglycaemia, the glucose flux to brain is more efficient than in hyper glycemia (Pardridge WM, 1983). Lactate movement is restricted by monocarboxylic acid carrier of BBB to protect brain after strenuous muscle exertion (Pardridge WM, 1983). Majorly seven substrates move across BBB includes glucose, lactate, phenylalanine, arginine, choline, adenosine, glutamate, adenine. Only glutamate amino acid carrier is an active efflux system rest all follows a bidirectional movement to sustain intermediary metabolism in brain.

Endothelial cell membrane is extremely rich in enzymes and during passage substances exposed to it also cause changes in these cells impacting barrier. The change could be mediated by decarboxylation of any amino acid or by oxidation enhancing BBB and further restricting the passage through it.

A diagrammatic representation by Paritge et al., 1986 showcases the permeability properties of the blood brain barrier, the anatomical unit along with physiological barrier properties of barrier briefly illustrates the functioning and importance of the BBB.

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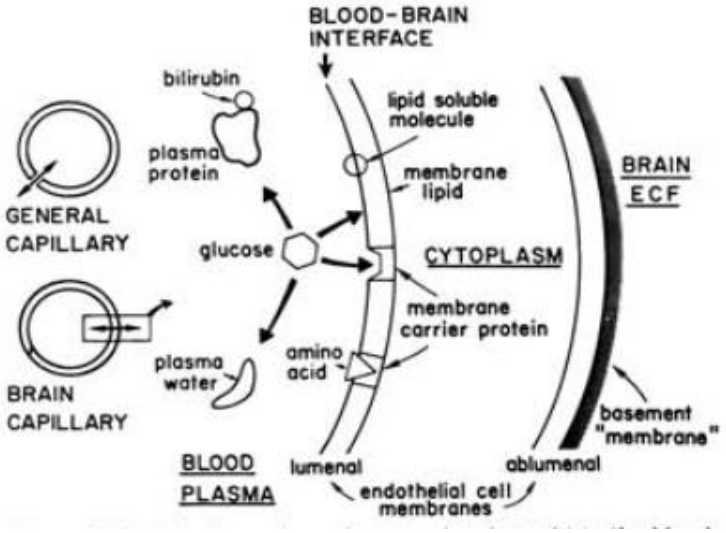

The movement of peptides via BBB holds a complicated position as because of the the specific transport systems for their movements like for Insulin in a study by Goodner and berrie, Pardrige et al., 1985; transferrin (Pardrige et al., 1986); Insulin like growth factors, IGF (Frank et al., 1986). The movement across BBB is directly proportional to their abundance in CSF to plasma. The CSF to plasma ratio is critical in nutritional movement across the brain and is high for developing brain and in large sized brains (Woods et al., 1983; Frank et al., 1985). Minimal vesicular trafficking across CNS (transcytosis) to limit transcellular cargo movement (Tuma and Hubbar, 2003). Selective transcytosis of peptides across BBB occurs for certain non-selective peptides like IGF-I, II which are found in brain in high abundance as well (Pardrige et al., 1986; Frank et al., 1986). The studies found its establishment by microvessels investigation. ABC transporters also plays a crucial role in restricting paracellular transport for influx of nutrients and efflux of toxins (Chow and Gu, 2015). Brain maintains a healthy immune privileged state by keeping immune cells at bay as the endothelial cells lack the expression of leukocyte adhesion molecules (Engelhardt and Ransoff, 2012; Muldoon et al., 2013).

4. Anatomy of neurovascular unit:

The neurovascular unit constituting BBB has astrocytes, microvascular epithelium, pericytes, neuronal cells along with ECM components (Wang et al., 2004). The permeability of BBB towards various components as well in drugs or during disease state, microvascular permeability is all regulated by neurovascular unit (Lo et al., 2004).

A brief schematic diagram by Mark and Davis, 2002 illustrates the components of tight junction with proteins like claudin and occludin; adhesion junctions; JAMs; accessory proteins.

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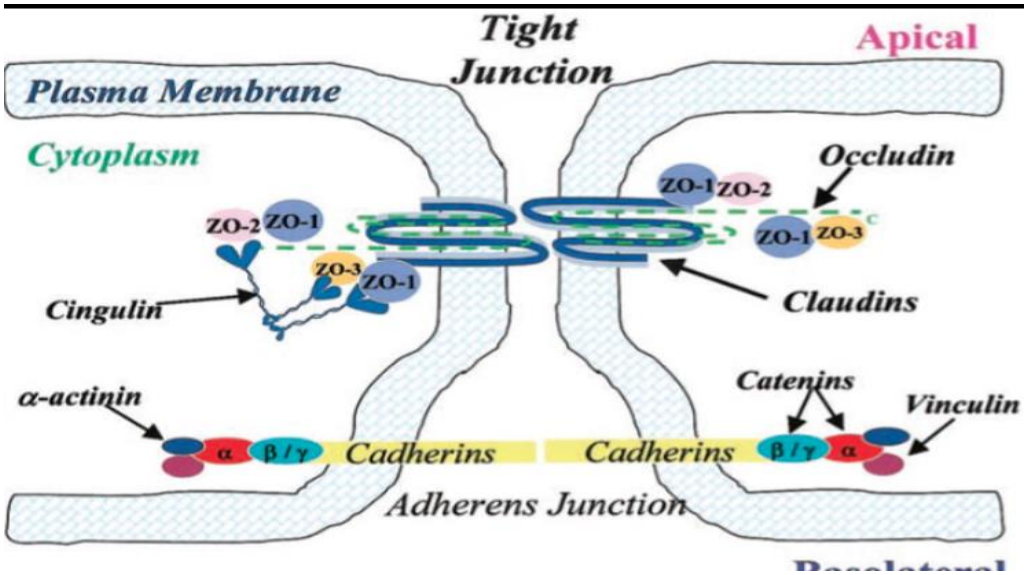

Tight junction complexes: Inter-endothelial space of BBB has several tight junction complexes having JAMs (Dejana et al., 2000); adhesion junctions (Schulze and Firth, 1993); tight junction proteins (Vorbrodt and Dobrogowska, 2003) and various accessory proteins.

These inter-cellular junctional complexes along with adhseion controls signalling for cell proliferation, differentiation (Aijaz, 2006). Tight junctions are to protect brain from substance entering with several transmembrane and cytoplasmic protein via intracellular signalling (Anny-claude Luissnt et al., 2012) while during a condition like ischemic stroke, BBB trans junction integrity compromises resulting permeability to paracellular components, edema situation, increased mortality (Karin E. Sandoval, Ken A. witt, 2008).

4.1 Junctional adhesion molecules (JAMs):

Transmembrane proteins in the tight junctions (TJ) present to maintain the integrity of BBB, trans-endothelial migration of leukocytes (Maschio et al., 1999). Junctional adhesion molecule-1 (JAM-1) is a 40 kDa protein belonging to IgG superfamily, used to facilitate attachment of adjacent endothelial cell membranes via homophilic interactions (Dejana et al., 2000). It is a single span protein with large extracellular domain (Pudra et al., 1998). These proteins with two Ig folds in extracellular domain of VH and C2 type (Bazzoni, 2003). Four JAMs are identified so far i.e. JAM-A,B,C,D with all having cell adhesion properties.

4.2 Adhesion Junctions:

Contains ubiquitous adhesion molecules, plays a significant role in vascular growth and remodelling; provides polarity to endothelial cells to mediate paracellular permeability (Bazzoni and Dejana, 2004). They usually are linked with actin cytoskeleton. Their distribution depends on cell type, could be located near tight junction and forms a morphologically distinct junction or could be distributed over entire lateral membrane (Bazzoni and Dejana, 2004; Vestweber, 2000; Wolburg and Lippoldt, 2002). The adhesion junctions majorly contains VE-cadherin, a ca+ regulated cell adhesion protein (Vincent et al., 2004). The stability of such junction depends on β catenin and cadherin binding and plakoglobin which further binds actin

cytoskeleton with vinculin, catenin and α-actinin Uchida et al., 1998; Knudsen et al., 1995; Lampugani at al., 1995)

4.3 Tight junctional proteins, Occludin and Claudin:

Occludin was the first discovered transmembrane protein at tight junction and was linked to various junctional processes (Feldman et al., 2005; Furuse et al., 1993; Matter and Balda, 1999). Occludin structurally is a four transmembrane protein complex with N- and C- terminals in cytosol forming 2 extracellular loop from where protein-protein interactions follow. Zona occluding 1(ZO-1)/ discs-large interacts with the protein there and creates specialized domain in endothelial cell membrane for the accessibility of non-permeablised cells (Furuse et al., 1994; Matter and Balda, 1998; Gonzalez-Mariscal et al., 2000; Nusrat et al., 2005; Van Itallie and Anderson, 1997). The main MAGUK identified in the field are ZO-1/2/3 (Fischer et al., 2002; Mark and Davis, 2002, Gottardi et al., 1996).

Claudins comprises another group of tight junction associated transmembrane proteins, they are purified in their pioneer forms claudin-1 and claudin-2 along with occluding from the chick hepatocytes (Furuse et al., 1998a). Anatomically have 4 transmembrane domains with both cytosolic N and C terminals like occluding. Their significance in tight junction is evidenced from the fact that even single claudin expression is sufficient for tight junction like intermembrane strands expression in fibroblasts (Furse et al., 1998b). Claudins have different extracellular loop amongst different family members and this suggests the role not just in barrier formation but in permeability of paracellular pathways (Tsukita et al., 2001; Van Itallie and Anderson, 2004; Van Itallie et al., 2001).

4.4 Accessory proteins:

Various proteins present in TJ functions to adhere, protect the junction and helps in signal transduction. The protein localization to TJ is not always straight forward, as because of proximity of tight and adherens junction, the movement is based on evidences gathered by confocal microscopy from tight junction markers colonization. These proteins mainly consists of members from Membrane-Associated Guanylate Kinase-like (MAGUK) family of homologs.

The cytoplasmic scaffold plaque at tight junction is a result of adaptor proteins like ZO-1/2/3, PATJ, PAR-3/6 and similar others as mentioned in table 1. This suggests significant amount of protein-protein interactions leading to the formation of an intricate protein network. Many such proteins have a role in interconnecting various cellular organelles like f-actin acts as a bridge between cytoskeleton components and adhesion proteins (Fanning, 2001; Schneeberger and Lynch, 2004).

Table 1 combines the number of accessory proteins along with their functions and the interactions made in order to keep the functioning in check

Protein	Characteristic, Functions	Interacting proteins

Occludin	4 Transmembrane protein involved in adhesion, permeability, G proteins signaling modulation.	ZO-1, ZO-2, ZO-3, TGF-β receptor type, E-3 ubiquitin ligase.
ZO-1	Cytoplasmic adaptor protein functions in regulation of gene expression, cell proliferation, junctional assembly	Occludin, Par-3, various virus like reovirus, adenovirus.
ZO-2	Cytoplasmic adaptor protein with function majorly in gene expression and inhibition of viral oncogenes.	Occludin, Claudins, ZO-1, oncogenic proteins like Fos, Jun, Pappilomavirus, Adenovirus.
ZO-3	Cytoplasmic adaptor protein	Occludin, claudins, ZO-1, PATJ, p120 catenin.
Claudins	Transmembrane protein functioning in cell adhesion, paracellular permeability, mediates cell migration and ion-selective migration.	ZO-1, Zo-2, ZO-3, MUPP-1, WNK4 kinase.
G proteins	Cytoplasmic trimeric GTP binding proteins, regulates assembly of junctions	ZO-1

5. Cells of neurovascular unit involved in BBB formation and maintenance:

Discussion above illustrates the unique and significant properties of BBB's endothelial cells, but these properties are not exclusive to endothelial cells (Stewart and Wiley, 1981) rater are induced by all components in CNS environment. BBB is indeed present in the endothelial cells but requires number of other cells for its functioning like pericytes; astrocytes; neurons along with the ECM components.

5.1 Pericytes:

The mural cells which enwrap capillary blood vessels on their abluminal side. The process of these cells extends from cell body and covers a number of endothelial cells. The role of pericytes in BBB needs elucidation however, the contractile proteins presence which enwraps the blood vessels suggests their functioning in blood flow regulation (Bandopadhyay et al., 2001). The ratio of "pericytes-to-endothelial" cells is higher in retina as compared to brain. The position of pericytes in BBB is crucial as, are present in basement membrane (BM) of endothelial cells of capillary and are thus positioned in between astrocytes; endothelial cells and neurons as showcased in Figure 1.

Pericytes and endothelial cell signals by PDGF-B pathway, showcasing the criticality in BBB formation (Armulik et al. 2010, Daneman et al. 2010). Absence of such signaling leads decreased pericyte coverage and a dysfunctional barrier (Bell et al. 2010) establishing the role of pericytes in barrier formation but its usage in adulthood is examined by (Park et al., 2017) by finding the pericytes ablated retinas in adult were more susceptible to leakage than normal

ones suggesting pericytes as an important factor in adult barrier maintenance though might not be required in formation of barrier.Studies are there on role of pericytes and their loss with BBB breakdown in Alzheimer's disease (Sagre et al., 2013), they have robust degradative and phagocytic abilities as have potent role in lysosomes, amyloid protein clearance as well.

5.2 Astrocytes:

Most of the abluminal part of neurovascular unit is formed by astrocytes. They play a critical role in BBB formation and maintenance (Davson and Oldendorf, 1967). They function by contacting outer basement membrane of brain vasculature and expresses aquaporin 4 (Aqp4). The close proximity of BBB and astrocytes indicates their role in BBB, but their postnatal appearance in brain ensures their significant role in BBB formation and maintenance (Yang et al., 2013).

A study performed by Janzer and Raff 1987 shows how a new barrier was induced just with an astrocytic environment. The transplantation of astrocytes in rat eyes after some days suggested the presence of a barrier by the evidence of new vessels formed there. The mechanism for such induction is unclear yet and requires more research.. Astrocytes along with combinational cells maintains microvascular permeability of brain by Ca++ signalling between CNS neuronal and astrocytes via purinergic transmission and gap junction (Braet et al., 2001; Zonta et al., 2003). The factors responsible for BBB maintenance are released from astrocytic feet via aquaporins. The current literature on astrocytic role in BBB implies a role of shh signalling (Alvarez et al., 2011). Though, many studies are present in contribution for astrocytes in BBB yet, the exact signalling pathway needs further elucidation.

5.3 Neurons:

The metabolic activity of neurons and the dynamic nature of neural activity has been in light for long for BBB. The extensive communication between vasculature and neurons is responsible for blood flow in BBB impacting its permeability. Attributing to conclusion that endothelial cells along with astrocytes; pericytes; non-adrenergic, serotonergic (Cohen et al., 1997); GABA-nergic (Vaucher et al., 2000); Cholinergic (Tong and Hammel, 1999) plays crucial role in BBB structuring, maintenance. The role of neurons in phenotyping BBB requires further elucidation.

5.4 Extracellular matrix:

Posing as an anchor to endothelial cells, Basement membrane (BM) is the ECM for neurovascular unit. It act as a hub for signalling pathways and intercellular communication amongst cells. BM has several structural proteins like laminins; glycoproteins; fibronectins; collagen. While two main family receptors are present in BM: integrins and dystroglycans. These receptors cause cell-ECM-cytoskeleton interactions and regulates various physiological processes in brain. These interactions and co-ordinations regulates the barrier function and interferes with the permeability in different conditions (George et al., 1993; Menezes et al., 2014).

6. Alterations in BBB during Hepatic encephalopathy:

BBB impairment is characteristically seen in patients with neurological defects suggesting critical role of BBB in CNS functioning. Literature analysis put light on to some sections in

case of liver cirrhosis and HE where BBB was altered and is associated with increased blood derived ammonia.

6.1 Change in permeability:

The permeability alterations were confirmed by dyes translocation in brain across the barrier like horseradish peroxidase (HRP) while several molecules presence is as well detected in brain across the barrier in hepatic encephalopathic patients like of ammonia, water indicating the disturbed barrier. (Laursen et al., 1975; Ott and Larsen, 2004; Dixit and chang, 1990). Though, limited evidences are present indicating BBB alterations in patients suffering acute and severe HE. Cauli et al., 2011 showcased how brain edema in usually seen in case of acute liver failure and cause progression to HE conditions. In an experiment with dyes, Evans blue dye was detected in brain after administered in systemic circulation indicates acute liver failure (Lv et al., 2010).

In a rat model after exposing endothelial cells with ammonia, an increased permeability was observed using tracer dye fluorescein isothiocyanate (FITC)-dextran (Skowronska et al., 2012). Quinn et al., 2014 observed increased BBB permeability in chronic liver patients following evans blue dye infusion. While specific studies in HE experimental and animal model is absent and requires further research in the domain.

6.2 Structural and Molecular changes:

BBB structural and molecular changes in the tight junctions are under research and getting out of haze. Shrunken endothelial cells; mitochondrial vacuolar degeneration; increased vesicles and vacuoles number along with decreased expression of TJ associated proteins like occluding-1 is observed in several HE models by Lv et al., 2010. In models galactosamine, TNF-α induced HE, several patients recorded to have reduced eNOS, epithelial nitric oxide synthase in tight junction proteins of occluding, in vonWillebrand factor, in brain of rats at severe stages like coma of HE with hepatic devascularization (Sawara et al., 2009).

In recent in vivo, in vitro study models of HE, permeabilizing effects in BDL induced HE was seen due to monomeric G protein, Ras-related botulinum toxic substrate 1 and as well decreased phosphorylation of TJ associated proteins like occluding causing disruption of TJ (Quinn et al., 2014). Pathways within the BM of the NVU coordinates readily to regulate the barrier functions. Breakdown of BM is usually seen in many disease and disorders related to CNS (Thomsen et al. 2017). This degradation is believed to be due to matrix metalloproteinases (MMPs), which increases/upregulates in neurological conditions. Concludingly, elevated MMP-2 and MMP-9 are associated with stroke, ischemia, and Alzheimer's and Parkinson's diseases (Rempe et al. 2016). MMPs and TJ dysfunctions are theorised as to have "Cause and effect" type relationship in BBB dysfunction.

6.3 Transport disruptions across barrier:

Metabolic disturbances caused due hyper ammonia causes cerebral energy disturbance and thus alter the transport of various substances and molecules across the barrier (Skowronska and Albrecht, 2012). Decreased brain glucose utilization, disruption in ratio of neutral amino acids in HE induced models affirms the above stated fact. In a research model, during acute liver failure, GLUT-1 which is the principal glucose transporter across the BBB increased in expression, indicating high glucose exchange, which is a consequence of energy impairment in brain and to maintain ATP concentration, more glucose is being imported. This indicates thr altered permeability (Belanger et al., 2006).

Creatinine, a key substrate in a number of metabolic pathways, acted partially against GABA-nergic neurotransmission (Cupello et al., 2008). Creatinine is a crucial compound in elongation of dendrites and axons. Hyperammonia leads to creatinine deficiency in CNS causing several neurological defects. Creatinine along with astrocytes has proven to be neuroprotective in case of HE (Braissant, 2002).

7. Conclusion:

Because each barrier operates in a distinct tissue environment, the site of the barrier is formed by different cell types in each tissue. For example, while endothelial cells form the BBB, epithelial cells form the gut barrier, and Sertoli cells form the testis barrier. However, these barriers and the BBB also exhibit many similarities. For example, these barriers utilize common mechanisms to regulate paracellular and transcellular passage, such as by the formation of TJs and control of the transcytotic pathway. Moreover, the cells that compose each of these barriers are polarized and rely on mechanisms of apical-basolateral sorting. Furthermore, the interaction of each barrier with the ECM and local cellular microenvironment dynamically regulates the function of each of these organ barriers. The discovery of novel BBB regulators and a deeper understanding of the mechanisms mediating barrier function will likely provide new targets to pharmacologically modulate the barrier in disease conditions. HE as is a complex, debilitating disorder thus, establishing the role of certain factors is of imminent importance. While, some factors established its foot base in the disease needs further research and enquiries. Literature lacks number of animal model studies performed on HE models top analyse BBB and its various physiological aspect is certainly a step that can be looked ahead with. After this initial identification, reliable and robust in vitro systems are needed to develop high-throughput screening of molecules that can change barrier properties. Finally, the heterogeneity of the BBB across various brain regions is an unexplored area of re- search that needs investigation. Over the next few decades, we anticipate all these research areas to reveal new aspects of barrier regulation that will provide a detailed understanding of the BBB in both health and disease.

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