Comparative Evaluation of Neuropathological Lesions Progression in Different Brain Areas in A Rat Model of Chronic Hepatic Encephalopathy

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Abstract

Hepatic encephalopathy (HE) is considered as serious clinical complication of acute and chronic liver injury. Previous researches elucidated HE but characterization of neuropathology in different brain areas is limited .The present study is conducted to evaluate the progression of neuropathological lesions in different brain areas in rat model with liver fibrosis induced by thioacetamide (TAA). For this experiment 20 male rats was divided into two groups control and TAA treated group that was I.P injected twice a week with 100 mg/kg.b.w TAA dissolved in saline for 3 months. Three rats from each group were euthanized after 3 months and the liver specimens were collected for ensuring the development of rat model for liver fibrosis.The remained rats were kept without further treatment with TAA for 2 months, rats were carfeully monitored for abnormal signs. After the end of experimental period, rats were euthanized and the brains were collected for histopathological examination. Microscopic examination of brain revealed various neurpoathological lesions that varied in severity between different brain areas. The most severe lesions were recorded in midbrain and pons while mild lesions were detected in cerebral cortex and striatum. The brain lesions comprised varied degrees of neuronal degeneration with marked astroglial and microglial reactions in the different brain regions. The obvious inflammatory reaction indicates that the neuroinflammation play an important role in the mediation of HE.

Key words: Cirrhosis, hepatic encephalopathy, rats.

Introduction

Hepatic encephalopathy (HE) is a complex neuropsychiatric clinical condition developed in patients with liver diseases, which may develop to several neurological and psychiatric alterations that affect cognition and lead to motor impairment and can progress to coma and death ***(Weissenborn et al., 2004).*** HE developed as a complication of acute and chronic liver failure ***(Farjam et al, 2012)***

The neurological manifestations developed with acute and chronic liver injury were defined into: type A represents HE with acute hepatic failure (ALF), type B is rare and was defined to be the neuropsychiatric complication of portal-systemic by pass without any intrinsic hepatocellular pathology and type C is the involvement of the brain seen in cirrhotic patients ***(Weissenborn et al., 2004, Monto liu et al., 2010, and Butterworth, 2011).***

Thioacetamide (TAA) is used widely for the induction of experimental liver fibrosis ***(Wallace et al., 2015)*** ,on other hand TAA-induced hepatic encephalopathy model is one of the most popular one for acute hepatic disorders ***(Butterworth et al., 2009 and Bismuth et al., 2011).***

The pathogenesis of HE developed in liver disease is related to hyperammonemia that associated with acute and chronic liver injury ***(Cauli et al., 2014)***. In addition, neuroinflammation rather than brain edema are implicated in the pathogenesis of HE ***(Rodrigo et al., 2010, and Butterworth, 2011).***

The present work was conducted to perform intensive study on chronic hepatic encephalopathy associated with chronic liver disease through microscopic examination of neuropathological lesions progression in different brain areas in thioacetamid treated rats .

Material and methods

Animals and experimental design

Experiments were performed on adult male Wistar rats, weighting 120-150 g, that were purchased from VACSERA. Animals were kept in clean metal cages with pelleted food and tap water ad libitum. All experimental procedures were approved by the Ethical Committee of the Faculty of veterinary medicine, Cairo

University (Vet.CU.IACUC-Vet-CU03252019026-25/3/2019).

All animals (n=20) were divided into two groups 10 rats each, group A kept as control untreated group ,and group B was I.P injected with TAA (Lobachemei-India code no.0625900100) at dose of 100 mg/kg B.w two times weekly for 3 months ***(Dongmei Qin, et al.,2014)***. Three rats from each group were euthanized after 3 months from the beginning of experiment and liver specimens were collected to check the development of liver fibrosis model.

The remained rats were kept without further treatment for 2 months. Rats were euthanized at the end of the experimental period, and the brain tissues were collected for histopathology.

Histopathogical examination

Brain specimens were collected in 10 % buffered formalin, routinely processed, and stained according to ***Bancroft (2013).*** Tissue sections were examined using Olympus BX43 light microscope and captured using Olympus DP27 camera linked to Cellsens dimensions software (Olympus) with microscopic examination different brain areas including cerebral cortex, striatum, hippocampus, midbrain, cerebellum and pons.

Results

Clinical signs

The treated rats lost the normal behavior, like grooming and continuous movement in the cage. The rats were sluggish, showed incoordination and slowly moved in the cage compared with control one.

Histopathological alterations

The microscopic examination of different brain areas revealed variuos histopathological alterations that vary in nature and severity among different brain areas.

1-Cerebral cortex

The lesions in cerebral cortex were mild in severity and restricted to cerebral grey matter. It included endothelial capillary proliferation (Fig.1a) and hypertrophy , in addition to distenstion of perivascular area especially in the inner granular and pyramidal layers.

2-Striatum

The lesions in striatum were similar to that detected in cerebral cortex. It involved mostly the caudate ,putamen and the globus pallidus with mild microgliosis (rod cells) (Fig.1b) with no reactions were detected in internal capsule.

3-Hippocampus

There was cellular reduction of the pyramidal neuronal cells involving all layers of cornus ammonis (CA). The lesion characterized by loss of neurons with gliosis and hypertrophy of endothelium. The cellular reduction was more severe in

CA3 &CA4 (Fig.2a). The dentate hilus showed gliosis and neuronal degeneration of pyramidal neurons. In individual case, there was a focal atsrocytosis associated with neuronal loss. (Fig.2b).At the periphary of such lesion, there was reactive astrocytes appearred enlarged with abundant eosinophilic cytoplasm (Fig.2c).on the other hand individual Alzehiemer type II astrocytes were detcted in the hippocampus hilus.

4-Thalamus

It showed different degree of neuronal degeneration varied from central chromatolysis associated with gliosis (Fig.3a) to severe lesion charcterized by massive loss of neurons with vacuolation of neuropil associated with reactive astrocytosis and microgliosis (Fig.3b). There was also perivascular mononuclear cells aggreagtion(Fg.3c).

5-Cerebellum

Concerning cerebellum,the lesions were more severe comapred with cerebrum.The lesions included microgliosis of molecular layer,focal loss of purkinje cells (Fig.4a) .the cerebellar white matter showed microgliosis and endothelail capillary proliferation (Fig.4b). Vacuolation of neuropil with microgliosis and chromatolysis of large neuronal cells were also seen in the cerebellar dentate nucleus(Figs. 4c).

6-Midbrain and Pons

The lesion in mid brain and pons were more severe comapred with other brain areas. It involved the reticulate part of substantia nigra and characterized by neuronal necrosis and microgliosis (Figs.5a&b).

The lesions in pons charcterized by chromatolysis and necrosis of large neurons of pontine nuclei assocaited with intense glial reaction microgliosis and astrocytosis (Fig.5c).The glia reaction represented reactive microglia (rod cells) and reactive astrocytes(Gemistocytes) admixed with other histicytes (Fig.5d).The lesion was also associted with hypertrophy of capillary endothelium.

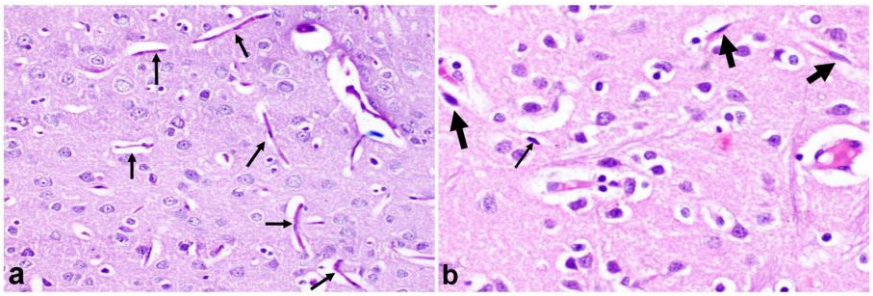

Fig.1: *a) Cerebrum of rat model showing endothelial capillary proliferation (arrow) X 200. b) Caudate showing Endothelial lining hypertrophy (thick arrow), note the presence of rod cell (thin arrow) X400.*

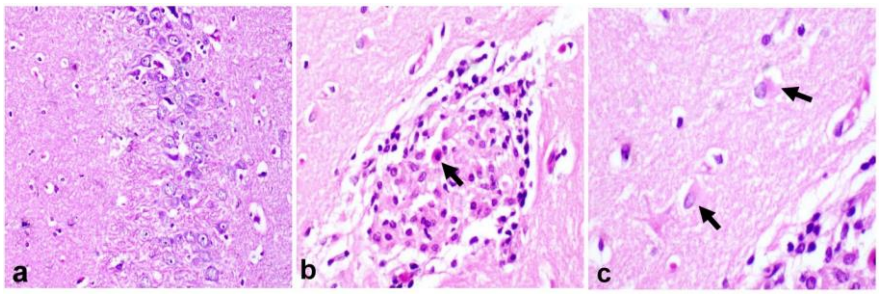

Fig.2: *Hippocampus of rat model showing: a) massive cellular reduction and loss of large pyramidal neurons in CA4 layer X200. b) focal necrosis of pyramidal neurons (arrow) in the dentate hilus invaded with microglia and astrocytes X400. c) Higher magnification of previous image, note the reactive astrocytes(arrow) X600.*

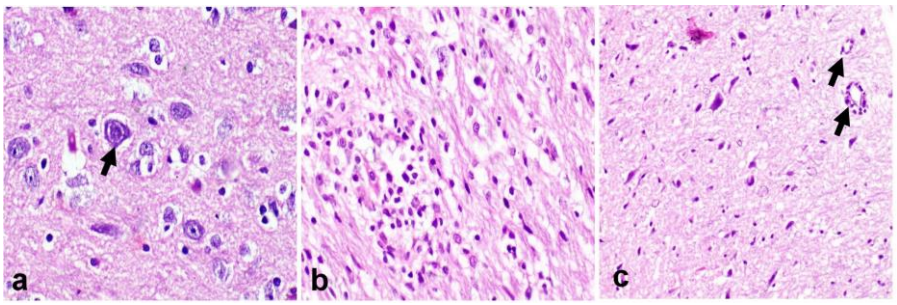

Fig.3: *Thalamus of rat model showing a) central chromatolysis of pyramidal neurons (arrow) with gliosis X400. b) Encephalomalcia with microgliosis and reactive astrocytosis X400. c) Perivascular mononuclear cell aggregation (arrow) X200.*

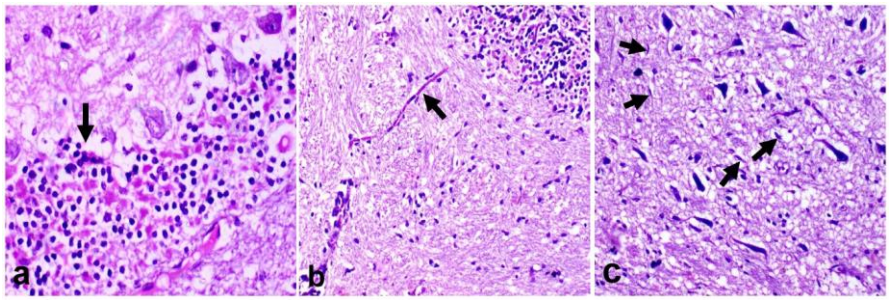

Fig.4: *Cerebellum of rat model showing: a) Focal loss of Purkinje cells (arrow) associated with microgliosis of molecular layer X400. b) Microgliosis of the cerebellar white matter with endothelial capillary proliferation (arrow) and perivascular mononuclear cells aggregation X200 c) cerebellar dentate nucleus showing diffuse gliosis mainly microglia (arrow) with vacuolization of neuropil X200.*

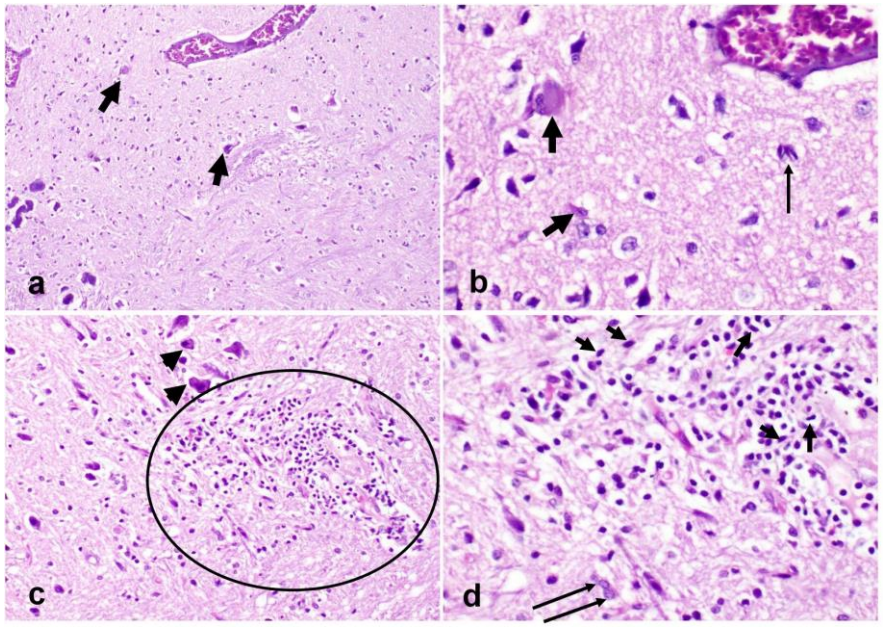

Fig.5: *Mid brain and pons of rat model showing a) necrosis of neurons (arrow) comprising the substatia nigra reticularis X100. b) Necrosis of large nerve cells (thick arrow) associated with microgliosis (thin arrow) X400. c) Pons with chromatolysis and necrosis of neurons comprising the pontine nuclei associated with intense glial reaction (circle) X200. d) Higher magnification of the previous image, note the microgliosis with typical rod cells (short arrow) admixed with other histiocytes and reactive astrocytes (long arrow) X400.*

Discussion

The present work revealed that HE is associated with progression of neuropathological lesions comprising neuronal injury and cellular reaction that vary in severity among different brain areas. Concerning the cellular reaction in the brain of rat model, astrocytosis were detected in areas of neuronal necrosis or associated with foci of microgliosis, while Alzheimer type 2 astrocytes were detected incidentally in dentate hilus of hippocampus. There was a correlation between the presence of Alzheimer type 2 astrocytes in HE and increase ammonia level in blood as discussed by ***Butterworth (2009)*** who reported that HE in rat model developed Alzheimer type 2 astrocytosis and he added that one of pathophysiology of HE is due to increased blood ammonia. On contrary ***Cauli et al. (2013)*** found low blood ammonia level in rats with chronic liver disease and develop HE. The microgliosis was extensively seen in areas of hippocampus, thalamus, midbrain, cerebellum and pons not in cerebral cortex. These results indicate the neuroinflammation mediated injury in these areas with subsequent development of clinical signs. ***Butterworth (2011)*** stated that microgliosis was developed in HE in cirrhosis and indicative of proinflammatory mechanism. Previous researches stated that the neuroinflammation and ammonia toxicity were considered as one of predominant factor in developing HE in acute and chronic liver diseases ***(Rodrigo et al., 2010, Butterworth, 2011, and Cauli et al., 2014).***

Neuronal injury, degeneration and loss were seen in the brain but the lesions were varying in severity according to brain areas. The most severe neuronal degeneration and loss were detected in pons and mid brain areas and associated with intense reactive astrogliosis and microgliosis. ***Butterworth (2009)*** stated that deep cerebral cortex, basal ganglia and cerebellum developed spongiform degeneration associated with hepatic cirrhosis, on contrary we found that the neuronal loss and degeneration were scarce in basal ganglia, cerebral cortex and cerebellum.

The neuronal loss and neuroinflammation reflected on behavior of rats that showing slow motion and loss of abnormal behavior of arts like grooming and exploring behavior in spite of normal feeding habits. Previous researches were conducted on progression of nervous signs

with HE into stages, mild associated with mild cognition deficits and cannot be detected by general examination ***(Huaussinger et al., 2006, and Romero-G et al., 2001)*** and moderate includes psychomotor slowing and mild cognitive impairment with attention deficit and alterations in visuo-motor coordination and working memory ***(Amodio et al., 2005, and Bajaj et al., 2009)*** and severe motor and cognitive impairment ***(Felipo et al., 2012).***

Distension of perivascular spaces was detected in cerebral cortex and striatum indicated mild cerebral edema in these areas. Previous studies showed that low grade cytotoxic cerebral edema was detected in HE and had been attributed to increased glutamine formation ***(Häussinger et al., 2000, and Häussinger, 2006).*** The magnetic resonance applied on patients with cirrhosis and HE confirmed presence of low-grade cerebral edema in cerebral cortex ***(lodi et al., 2004, and Kumar et al., 2008).*** In this respect, ***Cauli et al. (2013)*** found that weak role of cerebral edema in the neuropathological lesions in minimal or clinical chronic HE, due to low level of blood ammonia at this stage of chronicity.

Endothelial capillary proliferation with hypertrophied endothelium were detected in many brain areas but were more severe in cerebral cortex this indicative for hypoxia exerted on this part of brain, these results confirmed by ***Butterworth (2009)*** who stated that cerebral blood flow in patients with cirrhosis is changed in a region-selective manner.

We can conclude that HE developed in chronic liver disease rat model is a pecular selective distribution pattern of neuropathy among brain areas, in which neuronal loss was severe in mid brain and pons compared with other brain areas. Astroglial and microglial reactions were prominent in most of brain regions indicating that the neuroinflammation play an important role in the mediation of HE.

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التقييم المقارن لتطور العلل العصبية في مناطق الدماغ المختلفة في نموذج الجرذان لاعتلال الدماغ الكبدي المزمن

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الملخص العربى

صممت هذه التجربة لدراسة التأثير المزمن لتليف الكبد لأحداث الأعتلال العصبى لأنسجة المخ مع التركيز على مقارنة التغيرات الباثولوجية فى مناطق مختلفة للدماغ. تم احداث نموذج تليف الكبد بحقن عدد عشرة جرذان بمادة الثيواسيتاميد بجرعة 100 ملى جرام لكل كيلو جرام من وزن الجسم، تم الحقن فى الغشاء البريتونى يومين اسبوعيا لمدة ثلاث اشهر بالأضافة الى مجموعة ضابطة غير معالجة.
بعد التأكد من احداث نموذج تليف الكبد للجرذان من خلال الفحص المجهري لنسيج الكبد، تم إيقاف حقن المادة السمية المسببة لتليف الكبد وترك الجرذان لمدة شهرين بدون أى معالجة .تم ملاحظة الجرذان على مدار فترة التجربة وتسجيل اى اعراض عصبية تتطرأ عليهم.
بعد انتهاء مدة التجربة تم القتل الرحيم للحيوانات واخذ عينات لأجزاء الدماغ وتثبيتها فى محلول الفورمالين للفحص المجهري.
أظهرت النتائج ظهور أعراض عصبية على الجرذان من تغير فى النمط السلوكى الطبيعى للجرذان كما أظهر الفحص النسيجى للمجموعة المعالجة عدد من التغيرات الهستوباثولوجية فى انسجة الدماغ والتى كانت تختلف فى شدتها بين أجزاء الدماغ المختلفة. شملت التغيرات الهستوباثولوجية تنكس فى الخلايا العصبية، داء الدباق مع ظهور الخلايا البلعمية العصوية فى انسجة المخ بالأضافة الى زيادة عدد الخلايا النجمية مصاحبة لعملية البلعمة العصبية .