Effects of chronic uncontrolled hyperglycemia on neuroglial population of CA3 region of hippocampus: A study by TEM

Original Article

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ABSTRACT

Introduction: Altered function of astrocytes has been noticed in diabetes mellitus type 1.

Aims: This study was carried out to determine the effects of chronic uncontrolled hyperglycemia on neuroglial elements of the CA3 region of hippocampus.

Materials and Methods: Diabetes was induced in adult male wistar rats by application of streptozotocin (60mg/kg). After 8 weeks, the hippocampi were removed and the number of astrocytes and degenerated neurons in CA3 were studied using transmission electron microscopy.

Results: The astrocytes exhibited structural changes, including reduced electron density of the nucleus, electro-lucent heterochromatin, and vacuolated cytoplasm. The mean number of astrocytes in the CA3 region of diabetic group (7±2) showed a significant increase compared to the control group ($P<0.05$). The number of degenerated neurons in the diabetic animals showed significant level of difference in comparison with control ($p<0.05$).

Conclusion: Our data revealed that chronic diabetes mellitus type 1is associated with increase in the mean number of astrocytes and ultrastructural astrocyte changes in the CA3 region of hippocampus.

Key Words: Hippocampus, hyperglycemia, neuroglial, TEM.

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INTRODUCTION

Astrocytes are heterogeneous and pleomorphic glial cell population in the central nervous system (CNS). They play a key role in the production and regulation of neurotransmitters, antioxidant production, glutamate and potassium uptake, energy metabolism, and vascular coupling in the CNS[1]. Altered function of astrocytes has been documented in neuroinflammatory and neurodegenerative disorders in which there is a dramatic increase in free radical generation[2,3]. Diabetes mellitus type1 (DM1) is one of the metabolic disorder characterized by insulin deficiency and hyperglycemia[4]. In recent decades, several studies have provided evidence indicating the adverse effects of DM1 on the CNS. For instance, DM1 is associated with increased risk of neurologic and psychiatric disorders, such as Alzheimer's disease, major depression, and schizophrenia[5,4]. It is believed the DM1-related pathologies are caused by disturbance in glucose metabolism and subsequent increase in oxidative stress[6,7]. Recent findings have confirmed that increased oxidative stress triggers neuronal death pathways in the brain[8-10]. One of the most sensitive regions of the CNS to the oxidative stress is the hippocampus. This structure is the core part of the limbic system and plays a pivotal role in memory and learning[11, 12].

Hippocampal astrocytes actively contribute to the regulation of neurotransmitters, antioxidant production, and glutamate uptake[13]. Growing bodies of data have shown that the function of the astrocytes alters in diabetic state, but the results of quantitative studies are contradictory[14-18]. Additionally, recent data have demonstrated that astrocytes could play controversial roles in neuronal viability, either by enhancing neuronal survival or by contributing to further injury[19,20]. Considering these observations in one hand and their critical role in neuronal homeostasis on the other hand, we aimed to study the effects of experimental DM1 on the astrocytes in the CA3 region of hippocampus of diabetic animals by transmission electron microscopic study.

MATERIALS AND METHODS

The present study was approved by ethics committee of North Khorasan University of Medical Sciences. Male wistar rats (8 weeks old, 240-260 gr) were randomly divided into 2 groups (n =5 per group). All the rats were maintained in animal house and allowed free access to drinking water and standard rodent diet. Experimental diabetes mellitus was induced by single intraperitoneal injection of streptozotocin (Sigma, USA) at a dose of 60 mg/kg. The control group received only saline[21]. Animals were considered diabetic

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when the fasting blood glucose level was above 250 mg/dl four days after application of streptozotocin. Eight weeks after application of streptozotocin, the animals were anesthetized with chloroform. After cardiac perfusion with 100 mL of saline followed by 200 ml of fixative containing 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), the hippocampi were removed and divided into small parts and prefixed in the buffered osmium tetroxide[22].

Transmission Electron Microscopy (TEM)

The hippocampus samples were processed as follow (21): washing in phosphate buffer 0.1 M (pH 7.4), fixation in 1% osmium tetroxide, dehydration by graded acetone solutions (at 50%, 70%, 80%, 90% for 20 minutes and at 100% three changes for 30 minutes), infiltration by resin/acetone (1/3 overnight, 1/1 for 8 hours and 3/1 for 8 hours), epoxy resin (overnight) and embedding, primary trimming, thick sectioning, thin sectioning (60–90 nm), staining with uranyl acetate and lead citrate. Finally, electron micrographs were taken by EM900 (Zeiss, Germany) equipped to TFPO camera. From each animal, 10 random fields of the hippocampus were selected (collectively 100 fields per group). The number of astrocytes and their ultrastructure were studied in each selected field CA3[21]. Statistical analysis was performed by t-test and statistical significance was set at $p<0.05$.

RESULTS

The blood glucose level in diabetic group (567.92 ±45.20 mg/dl) were significantly different from those of control animals (101± 6.31mg/dl; *P<0.001*). The number of astrocytes in CA3 (per 100µm2) in diabetic group (7±2) showed a significant increase compared to the control group (*P<0.05*). The counted degenerated dark neurons in CA3(per 100µm2) in diabetic animals (5± 1) showed meaningful difference in comparison to those of control groups (*P<0.05*). Diabetic group showed remarkable ultrastructural changes mainly in the astrocytes of the hippocampus after 8 weeks of hyperglycemia in comparison to the control group (Fig. 1). Cytoplasm of astrocytes in diabetic group exhibited marked changes, including large empty spaces of glycogen and translucent nuclei with marginalized heterochromatin (Figs. 2, 3). Other ultrastructural findings were swollen mitochondria and hydropic processes (Fig. 3). In some sections, astrocytes were observed in vicinity of degenerated dark neurons (Fig. 4).

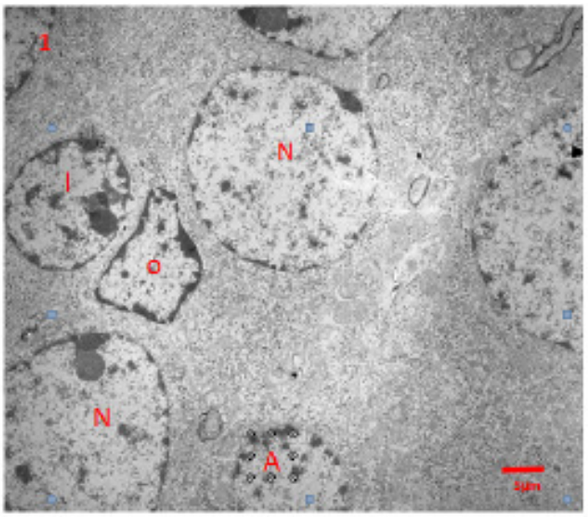

Fig. 1: Neurons (N), interneuron (I) , oligdendrocyte (O) and an astrocte(A) are seen in Pyramidal layer of CA3 in control group.

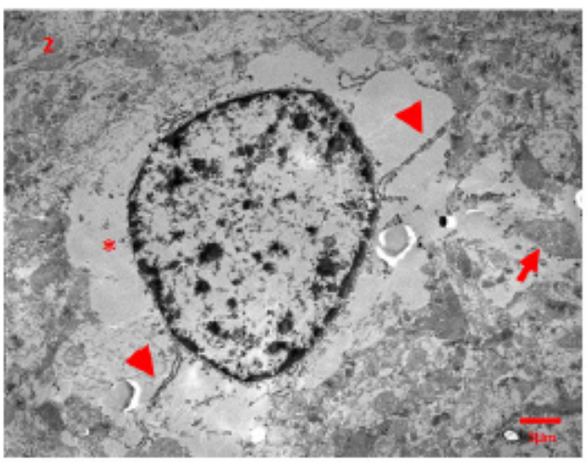

Fig. 2: an astrocyte (aster like) with depleted cytoplasm, stretched RER(arrowhead) and swelled mitochondria(arrow) in diabetic animals.

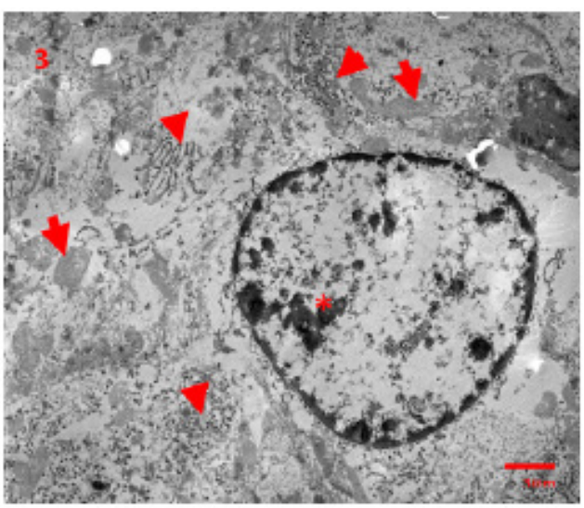

Fig. 3: an astrocyte (asterlike) with abnormal heterochromatin pattern (Chromatin clumping).swelled mitochondria (arrow) and dispersed ribosomes (arrow head) in diabetic animals.

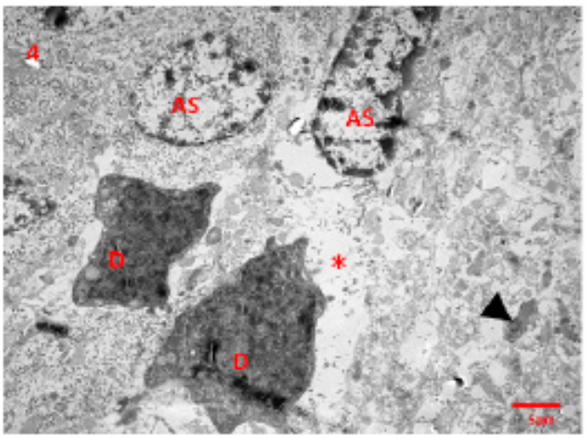

Fig. 4: Two astrocytes (AS) with round and elongated nuclei close to two dark neurons (D). Degenerated mitochondria (arrowhead) and hydropic astrocyte processes (aster like) are seen in diabetic animals**.**

DISCUSSION

Our findings showed that streptozotocin - induced diabetes results in qualitative and quantitative changes in the hippocampal astrocytes in CA3. In this study, we attempted to ascertain the entity of ultrastructural changes of astrocytes in response to uncontrolled chronic diabetes by TEM. Astrocytes in diabetic animals were characterized by abnormal patterns of chromatin, damage to mitochondria (swelling) and glycogen accumulation. The number of astrocytes showed an increase in diabetic animals in comparison to the control group. These results are compatible with Augé *et al.* (2018) report on astrocytes response in diabetic state[23].

Astrocytes and neurons, in physiological condition, are coupled by the glutamate-glutamine cycle. Extracellular glutamate is taken up and converted to glutamine by astrocytes. The released glutamine is taken up by neurons and used to synthesize glutamate to replenish the neurotransmitter pool[24].

Several studies have reported high levels of extracellular glutamate in the hippocampal tissue of diabetic animals, which in turn triggers a free radical generation[25-26]. Rivera-Aponte *et al*. (2016) showed that hyperglycemia reduces functional expression of astrocytic Kir4.1 and contributes to the pathophysiology of diabetes-induced CNS disorders[27]. It seems the altered function of astrocyte induces neurodegeneration in CAs of hippocampus in diabetic paradigm[28].

Ultrastracturally, astrocytes in streptozotocin-diabetic group exhibited morphological abnormalities in chromatin and organelles, particularly in mitochondria. Increased free radical generation in hippocampal tissue induces mitochondrial dysfunction (mitochondria swelling)[28]. The observed ultrastructural abnormalities in diabetic group may suggest some disturbances in energy production in glutamine-glutamate cycle[23,30]. High levels of glutamate can accelerate oxidative stress in the hippocampus, which in turn leads to excitotoxicity and dark neuron formation. Dark neuron formation is a spreadable phenomenon which is specified with remarkable shrinkage and volume reduction. During this phenomenon, a large amount of water is expelled to extracellular space and absorbed by surrounding astrocytes[31]. The presence of reactive astrocytes in the vicinity of dark neurons may suggest a protective mechanism to wall off the affected neurons and prevents the spreading of the damage to the adjacent areas. Although astrocytes are primarily involved in improving neurons viability, diabetic paradigm would compromise the functions of astrocytes, leading to an increased neuronal loss. Astrocytes are thought to be involved in extracellular matrix synthesis in the CNS and shaping the synaptic space[32]. Alteration in the synaptic geometry will influence the speed and level of neurotransmitter[33]. Therefore, it seems that efforts should be directed toward the preservation of astrocytes functions in order to maintain the neuronal integrity.

CONFLICT OF INTEREST

There are no conflicts of interest.

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