Original Article

The Possible Neurodegeneration of the CA3 and CA1 of Hippocampus in STZ - Induced Diabetes Mellitus Type1 on Male Wistar Rats

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ABSTRACT

Introduction: Hippocampus is one of the most sensitive region of central nervous system to metabolic disorders. Structurally it is composed of cornu ammonis and dentate gyrus. CA3 and CA1 play critical role in cognitive performance and psychosis.
Aim: The aim of this study was to study the effects of diabetes mellitus on bilateral CA3 and CA1.
Materials and Methods: Male adult wistar rats were randomly into two groups as diabetic and control. Diabetes mellitus was induced by a single intraperitoneal (IP) injection of STZ at a dose of 60 mg/kg. The control animals only received saline. Two months after uncontrolled diabetes, the animals were anesthetized. The harvested whole brains were stained with cresyl violet.
Results: The number of degenerated neurons in right side CA3 (600±25) showed significant level of difference with left CA3 (0) (*P<0.001*). The count of degenerated neurons in right side CA1 (90±5) showed significant difference with left side CA1 region (5±2) (*P<0.001*).
Conclusion: Our study showed that experimental diabetes mellitus type 1 leads to unilateral neurodegeneration in pyramidal layer of CA3 and CA1 regions of right hippocampus.

Key Words: Diabetes mellitus, hippocampus, Neurodegeneration.

Revised: 14 June 2021, **Accepted:** 27 July 2021.

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ISSN: 2536-9172, June 2021, Vol. 5, No. 1

INTRODUCTION

Since the early report in 1950s, when bilateral temporal lobe resection was carried to treat intractable seizure, many researchers around the world have been fascinated by a buried structure in the temporal lobe, which is known hippocampus[1]. Due to its critical role in cognitive and emotional behaviors it has been studied more than any other structures in brain[2]. Growing bodies of evidence have shown vulnerability of hippocampus in stressful and metabolic disorders[3 ,4]. One of the most known metabolic disorders is diabetes mellitus type 1, which is characterized with overt hyperglycemia and absolute or partial lack of insulin[5]. In recent decades central nervous complications (CNS) of diabetes mellitus have come into more focus[6,7]. Interestingly new data underlines the association between the developing psychiatric disorders and diabetes mellitus type 1[8-10]. Recent studies have documented evidence demonstrating the adverse effects of diabetes mellitus on hippocampus structure and function[11-12]. For instance, neuronal death in cornus ammonis and dentate gyrus, suppressed cell proliferation in dentate gyrus, electrophysiological changes, impaired cognitive performance have been reported in diabetic animals[13,14]. Structurally hippocampus is divided into two distinct regions including dentate gyrus and cornu ammonis (CA1-4). Moreover, functionally hippocampus is segmented into dorsal and ventral parts (anterior and posterior in human). Studies have revealed that certain regions of cornus ammonis are more susceptible to degeneration in hyperglycemia[15-16]. For instance, it has been shown that ventral segment is more vulnerable to uncontrolled diabetes mellitus type1[17]. Furthermore, functional asymmetry of hippocampus has been studied thoroughly and the results underline the laterality of hippocampus activities[18]. Among the different parts of cornus ammonis, CA1 and to more extent CA3 play critical role in cognitive performance including conjunctive memory, pattern separation and even in developing the psychosis[19,20]. Although a vast number of studies have dealt with the effects of hyperglycemia on hippocampus structures and functions, yet it has not been defined would diabetes mellitus type1 affect the cornus ammonis regions symmetrically? Therefore, this study aimed to study the effects of experimental diabetes type1 on pyramidal layer of CA1 and CA3 regions of hippocampus and assess lateralization between these regions of both side hippocami in response to chronic diabetes.

 DOI: 10.21608/jmh.2021.80629.1090

MATERIALS AND METHODS

This study was carried out on male Wistar rats (8 weeks old, body weight 240–260 g, N=10 per group). All rats were maintained in animal house and allowed free access to drinking water and standard rodent diet. Experiments performed during the light period of cycle and conducted in accordance with Regional Committee of Ethic complied with the regulations of the European Convention on Vertebrate Animals Protection (2005) and ethic committee for animal research of north Khorasan University of Medical Sciences. We considered fasting blood glucose (FBG) >250 mg/dL as a diabetic[17]. Diabetes mellitus type1 was induced by a single intraperitoneal (IP) injection of STZ (Sigma Chemicals. Louis, Mo) at a dose of 60 mg/kg dissolved in saline (control animals were injected with saline only)[17]. Four days after the STZ injection, FBG was determined in blood samples of tail veins by a digital glucometer (BIONIME, Swiss). Two months after uncontrolled diabetes, the animals were anesthetized and the harvested brains were post-fixed in formalin10% for a week. Additionally, the adrenal glands of animals were removed and weighed. Paraffin embedded sections (bregma -1.8 mm to -3.8mm) of 10 µm thickness were cut by microtome. Subsequently sections were sampled according to systematic random sampling (SSR) (100 µm interval) and stained with cresyl violet for demonstration of nerve cell bodies. 10 microscopic fields from each section were examined according to modified stereological method (collectively 100 fields for each animal)[17,21]. The images were captured by microscope equipped with camera. The boundary of each region was defined and quantitative analysis of hyperstained dark neurons in the pyramidal cell layer of CA1 and CA3 of bilateral hippocampus was performed. Hyperstained dark neurons were counted by an investigator who was blinded to the study.

Statistical analysis:

The obtained results were expressed as mean±SD. Statistical analyses were conducted by Statistical package for Social Sciences (SPSS version 17.0). The data were analyzed using t-test. Differences were considered to be significant at $P < 0.05$.

RESULTS

The blood glucose level in STZ-induced diabetes group (567.92 ± 45.20 mg/dl) showed significant level of difference with those of control animals (101± 6.31mg/dl) (*P<0.001*). Molecular(M), pyramidal (PY) and Polymorph (Po) layers of hippocampus proper were defined in both sides (Figures1, 2) were defined. Cytological examination of right CA3 and CA1 regions of diabetic animals revealed neuronal degeneration. The degenerated dark neurons were discernable with hyperstained dark appearance, shrinkage and vacuolated space around (Fig 1, 3, 5). In left side no dark neurons were observed in CA3 and in left CA1 only a few scattered dark neurons were noticed (Fig 2, 4, 6) The number of degenerated neurons in right side CA3 (600±25) showed significant level of difference with left CA3 (≈0) (*P<0.001*). The number of degenerated neurons in right side CA1 (90±5) showed statistically significant difference with left side CA1 region (5±2) (*P<0.001*). The comparison between CA3 and CA1 in right side showed significant level of difference (*P<0.001*). The number of degenerated neurons in both sides of the control animals was not statistically significant (≈0). The Weight of adrenal glands of hyperglycemic animals (90± 10 mg) showed statistically significant difference in comparison with those of control animals (18±2 mg) (*P<0.001*).

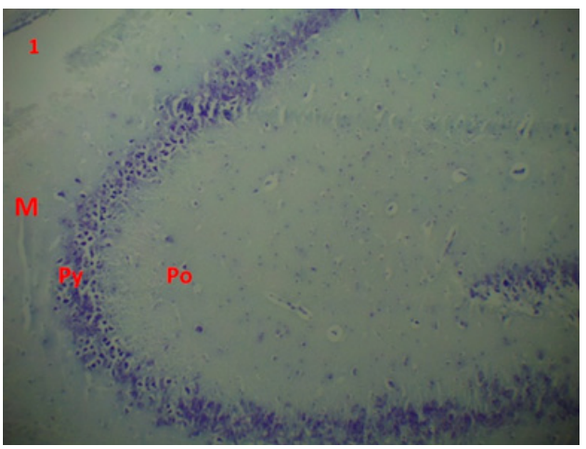

Fig. 1: right hippocampus. CA3 region. The hippocampus proper layers including Molecular (M), Pyramidal(PY) and Polymorph (po) were defined. Cresyl violet statining. X20.

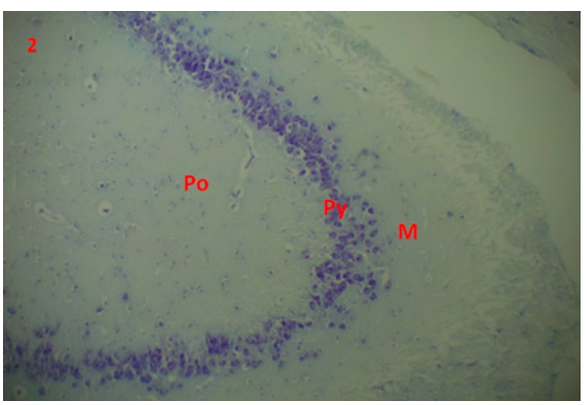

Fig. 2: left hippocampus. CA3 region. The hippocampus proper layers including Molecular (M), Pyramidal(PY) and Polymorph (po) were defined. Cresyl violet staining. X20.

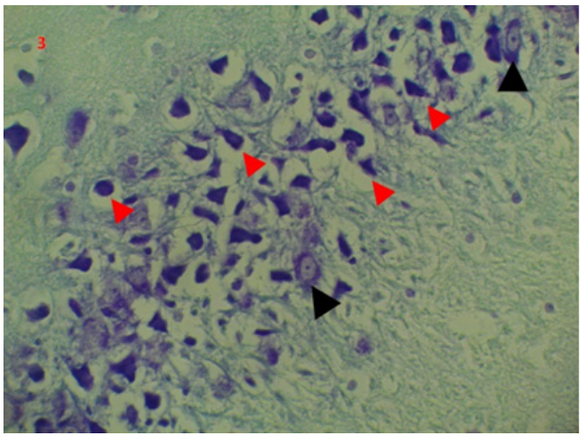

Fig. 3: right hippocampus. Numerous dark degenerated neurons in pyramidal layer of CA3 region (red arrow head. Some scattered healthy neurons are also visible (black arrow head). Cresyl violet staining X40.

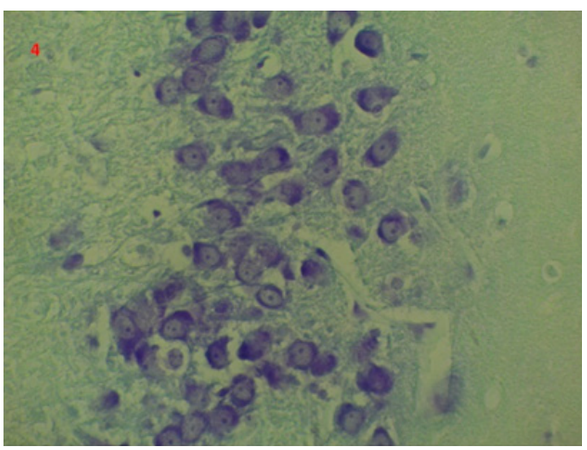

Fig. 4: left hippocampus. Pyramidal layer of CA3 region. Normal and healthy neurons with prominent nucleoli are seen(Arrowheads). Cresyl violet staining X40.

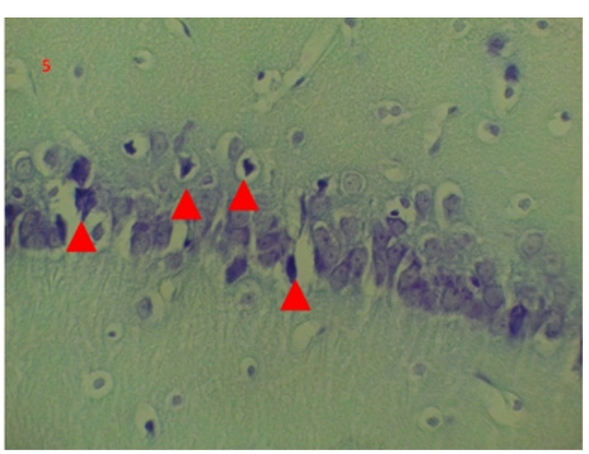

Fig. 5: right hippocampus. Some degenerated, dark neurons (red arrow head) among normal neurons are visible in pyramidal layer of CA1 region cresyl violet staining X40.

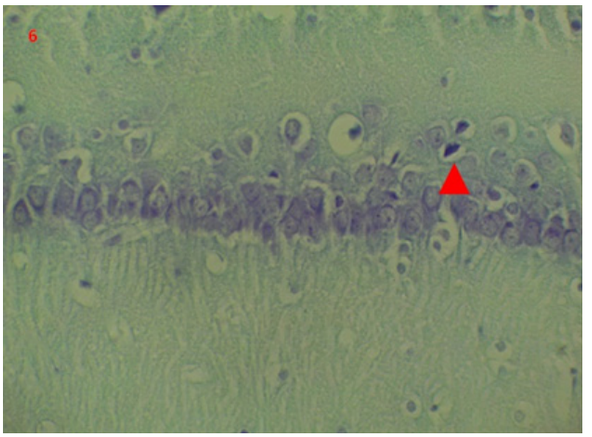

Fig. 6: left hippocampus. CA1 region. Few scattered dark neurons surrounded by a halo are seen (red arrow head) Cresyl violet staining X40.

DISCUSSION

The results of this study showed that experimental diabetes mellitus type 1 leads to unilateral neurodegeneration in pyramidal layer of right hippocampus. Likewise, our findings suggest that right CA3 pyramidal layer is more vulnerable to chronic diabetes than both ipsilateral and contralateral other cornus ammonis (CA) subregions. As yet, no direct evidence on selective vulnerability of right side hippocampus in diabetic state has been reported.

The results of this study can be discussed from two aspects; Firstly, neuronal death and secondly, lateralization of neurodegeneration. Diabetes mellitus related neuronal death has been documented in many studies[22-24]. Our present report on neuronal death is compatible with the previous reports, indicating the deleterious effects of chronic hyperglycemia on CA3 and CA1 region of the hippocampus. The second and most important aspect of our findings are the lateralized nature of degeneration in the hippocampus subfields which have been less noticed before. The lateralization of hippocampus functions has come to attention in recent years. Neuroimaging studies have revealed that cognitive functions are lateralized in hippocampus. For instance, a functional brain imaging study revealed significant correlations between the left-hippocampal volume and recall of verbal information and between the right-hippocampal volume and recall of spatial locations[25]. Developmental study could also provide evidence indicating the lateralization of hippocampus[26]. Asymmetry of hippocampus internal architecture has been suggested to be involved in unilateral seizure onset[26]. This feature of hippocampus is attributed to certain neurotransmitters, hormones like cortisol and their receptors density in the hippocampus[28-29]. Interestingly in one study reported that exposure to swimming stress is associated with unilateral degeneration in the cell bodies of CA2 and CA4 subregions of right hippocampus[30]. Medvedeva *et al* showed that CA3 region is more vulnerable to ischemia

which is attributed to rapid and long lasting accumulation of Ca2+ and Zn2+ in CA3 region[31]. A large number of studies have revealed that hippocampus is vulnerable to stressful conditions[32-34]. It has been documented that glucocorticoids like cortisol which are secreted during the stress are the main leading cause of the stress-related pathological consequences[35]. The release of the adrenal glucocorticoids is under the control of hypothalamo-pituitary-adrenal axis (HPA) which in turn is regulated by hippocampus[36-37]. Study showed that diabetes mellitus is associated with dysregulation of HPA[38]. Meanwhile diabetes mellitus type 1 is considered as an endogenous stressor[39]. Due to technical limitation we didn't perform Cortisol assay in this study, but adrenal hypertrophy in hyperglycemic animals could reflect a dysregulation in HPA. In this study we could provide evidence demonstrating selective degeneration in right side hippocampus of diabetic animals. The prevailing notion give emphasis to the role of free radicals resulted from hyperglycemia in triggering neuronal death[40], but according to the findings of this study vulnerability of a specific sub - region of hippocampus and asymmetric neurodegeneration root in internal structures and developmental factors[26-29]. A possible explanation could be that levels of glucocorticoids and glutamate receptors in both side hippocami may be different. So increased levels of glucocorticoids and glutamate may induce more sever abnormalities in one side[41-43]. Structurally CA3 and CA1 pyramidal neurons receive glutamergic mossy terminals of granular cells of dentate gyrus. These connections particularly CA3 and dentate gyrus is of functional importance in cognitive performances in human and as well as animals[44]. The more severity of neurodegeneration in CA3 pyramidal layer than CA1 region may also be explainable by the pattern and quantity of glutamergic mossy fibers, in sense that pyramidal neurons in CA3 region are exposed to altered levels of presynaptic glutamate[31]. Additionally, possible mechanisms such as hemodynamic factors should not be disregarded[45]. In conclusion, our findings suggest the right side hippocampus particularly CA3 region is more vulnerable to diabetes mellitus type 1. Further studies are recommended to reveal the involved mechanisms.

CONFLICT OF INTEREST

There are no conflicts of interest.

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