Title: Diabetic Encephalopathy Affects Mitochondria and Axonal Transport Proteins

Running title: Eslami Gharaati .M Et Al, Diabetic Encephalopathy Affects Mitochondria & Axonal Proteins

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Highlight

- Short term memory impairment and retrieval deficit in combination with anxiety like behavior were observed following 8th week after the onset of diabetes.
- Biochemical changes (impaired glycosylated hemoglobin (HgbA1c), serum insulin level) were observed in diabetic rats.
- Cellular changes (decreased mitochondrial membrane potential (MMP) in hippocampus) were observed in diabetic rats.
- Molecular changes (increased kinesin (KIF5b) gene expression levels in hippocampus) were observed in diabetic rats.
- After 8 weeks of insulin injection, KIF5b gene expression levels, were reversed.

Plain language summary

Diabetes Mellitus (DM) is strongly associated with degenerative and functional disorders in Central Nervous System (CNS). One of the most chronic complications of DM in CNS is "diabetic encephalopathy". According to the definition, any cognitive impairment affected by diabetes called diabetic encephalopathy and/or type3 diabetes mellitus (T3DM). Several studies show the mitochondrial dysfunction in different regions of the brain. Also mitochondrial movement & transport disrupt in diabetes condition. The mechanisms underlying those impairments are still unknown. In this work, we evaluated effect of diabetic encephalopathy on mitochondrial function and transport by study the proteins that involved in axonal transport of mitochondria (KIF5b & dynein) in male rats. After 8 weeks of induction of diabetes (by single dose of STZ), animals monitored by behavioral test (elevated plus maze, Y-maze and passive avoidance learning),
biochemical parameters (HgbA1c & insulin) and mitochondrial function and transport using Rhodamin123 staining and real-time PCR. We showed that diabetic encephalopathy resulted in cognitive decline & mitochondrial dysfunction by decreased mitochondrial membrane potential (MMP) and kinesin gene over expression. We suggested that abnormal translocation of mitochondria and its malfunction induced by kinesin gene over expression, possibly resulted in cognitive and memory impairment in diabetic encephalopathy.
Abstract

**Introduction:** Diabetic encephalopathy is described as any cognitive and memory impairments and associated with hippocampal degenerative changes, include neurodegenerative process and decreased number of living cell. Mitochondrial Diabetes (MD) appears following activation of mutant mitochondrial DNA and is combination of diabetes and cognitive deficit. In this research we showed the correlation of diabetic encephalopathy, dysfunctional mitochondria and change in expression of axonal transport proteins (KIF5b, Dynein).

**Methods:** Twenty four male Wistar rats were divided into three groups: (n=8): 1_Control+saline 2_Diabetic, 3_Diabetic+Insulin. Before starting the experiments, animals with blood sugar lower than150mg/dl entered the study. Diabetes induction was carried out by STZ, IP administration. FBS and body weight was checked after 1week and at the end of the 8week. Then behavioral studies (elevated plus maze, Y-maze and passive avoidance learning) were performed. After behavioral studies, blood samples were taken to measure serum insulin level and HgbA1c. Then fresh hippocampal tissue was collected. Gene expression of motor proteins was assessed by R-T PCR and mitochondrial membrane potential was assessed by Rhodamine123.

**Results:** Our results showed impairment of HgbA1c, serum insulin, FBS and weight in diabetic group (p<0.05).Behavioral tests, revealed different degrees of impairment in diabetic rats (p<0.05).KIF5b mRNA expression was increased in hippocampus (p<0.05) with no change in dynein gene expression. These changes were associated with abnormal mitochondrial membrane potential (p<0.05).
**Conclusion:** KIF5b mRNA up-regulation in hippocampal neurons of STZ-diabetic rats is a factor which can be involved in abnormal axonal transport and decreased MMP, leading to impairment of mitochondrial function. These manifestations showed mitochondrial dysfunction on diabetes and resulted in abnormal behavioral tests and diabetic encephalopathy.

**Key words:** Diabetes mellitus type 1; Mitochondrial Encephalopathy; Axonal transport; mitochondria; KIF5b protein; Dyneins.
Introduction

Diabetic encephalopathy and/or type 3 diabetes mellitus is the chronic complication of diabetes mellitus (DM) (Steen et al., 2005, Rivera et al., 2005, de la Monte & Wands, 2008, Leszek, Trypka & Tarasov, 2017). Any cognitive disorder aligned with DM, in the absence of other predisposing factor is considered as diabetic encephalopathy or T3DM (Steen et al., 2005, Rivera et al., 2005). Diabetic encephalopathy include various pathologies and neurobehavioral changes in nervous system, such as impairment of memory, decision making and even mood disorders. In many experimental studies, cognitive impairments induced by diabetes or diabetic encephalopathy were observed in different behavioral tests. For example anxiety like behavior increased following T1DM in rodents (Miyata, Yamada & Hirano, 2007). Also learning and memory deficit were observed in behavioral test in diabetic animals (Mousavi, Eidi & Khalili, 2018, Roghani, Joghataie & Jalali, 2006). In this research, they found a significant cognitive decline in passive avoidance and Y maze tests diabetic rats.

Hyperglycemic conditions (Reske-Nielsen & Lundbaek, Rafaelsen, 1966), decreased serum insulin and c-peptide level, cerebrovascular change (Brands, Bell & Gibson, 2004, Sima & Li, 2005), neurotropic agent loss (Connor et al., 1997, Lupien, Bluhm, Ishii, 2003), NFκB pathway and inflammation (Cai & Liu, 2012) and oxidative stress (Beckman & Ames 1998), are all believed to be responsible for such neurodegenerative condition. In addition to hyperglycemic condition, low insulin concentration, decreased neurotropic factors and…., mitochondrial dysfunction is a well-known and confirmed factor to induce T3DM. Mitochondrial dysfunction on the other hand, is suggested to be the triggering factor for T3DM or Alzheimer disease (Belkacemi & Ramassamy,
Mitochondrial dysfunction is responsible for both acute and chronic hepatic encephalopathy (Bessman & Bessman, 1955, Hindfelt, Plum & Duffy, 1977). In MELAS (Mitochondrial, Encephalomyopathy, Lactic Acidosis and Stroke like episodes which is a genetic condition of mitochondrial genome), correlation of mitochondrial dysfunction and encephalopathy have been revealed (Finsterer, 2006). Neural microtubules are arranged polar, with negative pole in soma and positive in axon terminal. Mitochondrial traffic inside axon is carried out by two important motor proteins: Kinesin and dynein. Mitochondrial viability is dependent to its normal transport and movement along axon via transport proteins (Hollenbeck & Saxton, 2005). Compromised mitochondrial movement, following axonal transport defect, will impair normal mitochondrial membrane potential, ATP production and finally result in neurodegeneration (Zhao et al., 2001, Baloh, Schmidt & Pestronk, 2007, Guo et al., 2017). Among axonal transport proteins, kinesin and dynein play the key role. For example in fatal encephalopathy, a sever neurodevelopmental disorder, which is associated with hippocampal dysfunction, impaired mitochondrial function is present (Haas et al., 2007), in addition to trafficking kinesin proteins (TRAK) deficiency (Barel et al., 2017). Impaired kinesin and mitochondrial function have been reported in diabetes pathophysiology, as well (Lu et al., 2004, Wang, Bennett & Potter, 2016, Zilliox, Chadrasekaran & Kwan, 2016). In this study, we used different behavioral tests to prove the cognitive impairments in diabetes and we tried to evaluate the correlation of mitochondrial function, kinesin and dynein gene expression falling diabetic encephalopathy.
Materials and Methods

Animals

Twenty four male Wistar rats weighting 200-250g purchased from experimental and comparative studies Center of Iran University of medical science, Tehran, Iran. The rats were housed 1 week prior to study, at room temperature of 22 ± 2°C, under a 12h light/dark cycle and they had freely access to food and water ad libitum. Animals were housed in group of 4 rat per cage (4rats/cage) and were randomly divided in three groups (n: 8/ groups):


All of the procedures in use were under supervision of ethics committee of “Iran University of medical sciences”, (Ethic code: IR.IUMS.REC.1395.9221343204). All of the procedures are based on ethical guidelines for the care and use of laboratory animals, published by the National Institutes of Health (NIH Publication, revised 1996).

Diabetes induction

All animals were checked for body weight and plasma glucose levels first and rats with fasting blood sugar (FBS) below 150 mg/dl entered the exam. Following an overnight fasting, single dose of intraperitoneal Streptozotocin (STZ, Sigma Aldrich, USA), 60 mg/kg dissolved in cold normal saline, induced diabetes mellitus (Abebe, Harris & Macleod, 1990). The volume of the infused solution for each rat was 0.5 ml. Control+salin group received an equivalent volume of saline solution (0.9%) for 8 weeks. The rats were considered diabetic as blood sugar detected 150 mg/dl or higher. Rats with FBS over than 150mg/dl considered as diabetes. In diabetes group there was
no treatment at same time. Diabetic+Insulin group was treated with insulin (1.5U, NPH, 2times/Day) for 8 week. Body Weight and FBS were measured one week after STZ injection and at the end of the 8th weeks, before behavioral studies.

Behavioral study

Elevated plus maze (EPM)

After the end of 8th weeks, explorative activity and anxiety behavior were assessed using Elevated plus maze (EPM) task. The EPM included four arms (plus shaped), 60cm length and 10cm wide, elevated 50 cm above the ground. Two arms were enclosed by 30cm height walls and open arms with 0.5 cm edge. In this experiment, each rat was placed at the junction of the open and closed with head toward open arms and permitted to seek the arms for 5 min. The total time of presence in both open arms (OAT) and closed arm (CAT) were measured. Decreased presence time in OAT was considered as anxiety like behavior (Arfa-Fatollahkhani et al., 2017). Number of entry to open arm (%) and time spent in it (%) revealed anxiety indices (Hritcu, Cioanca & Hancianu, 2012). After each test, the instrument was carefully cleaned with wet tissue (Ethanol 75%).

Y-maze

48 h after elevated plus-maze task, animals subjected to working memory performance by recording spontaneous alternation behavior in Y-maze task. The maze was made of a Y-shaped Plexiglas holding consist of three arms (A, B, C). The arm converged in an equilateral triangular (120°) with 40cm length, 30cm height and 15cm width. Each rat was placed in the beginning of one arm “A” and permitted to discover the apparatus for 8 min. An entry occurred when all four limbs were inside the arm. Spontaneous alternation was defined as successive entries into the three arms on triplet sets. The spontaneous alternation percentage was calculated as the ratio of actual
to possible alternations (defined as the total number of arm entries minus two×100) (Roghani, Joghataie & Jalali, 2006, Mousavi, Eidi & Khalili, 2018). After each test, the instrument was carefully cleaned with wet tissue (Ethanol 75%).

**Passive avoidance learning (PAL)**

48 hours after Y-maze, passive avoidance learning (PAL) was performed in shuttle box. The shuttle box was used to evaluate passive avoidance behavior. The apparatus consisted of light and dark compartments with equal size (20×40×20) connected by a small central guillotine door. Electric shock was received by grid floor, in the dark compartment. This test was performed within four days. In the first and second days all rats were adapted to the apparatus for 5 min. On the third day, each rats settled in the light compartment for 2 min, then the guillotine door was opened then latency time to enter the dark compartment was recorded as initial latency or IL. When the rats entered the dark compartment, the guillotine door was closed and electric foot shock (1Sec, 1mA, 50HZ) was received. Rats with IL higher than 60sec were excluded. To evaluate memory retention, each rat was placed again in the light compartment next day. The interval between entrance to the light chamber and leaving into the dark chamber was measured as step through latency (STL). The STL was measured as the index for the passive avoidance behavior (cut-off time was 480s), (Roghani, Joghataie & Jalali, 2006). After each test, the apparatus was carefully cleaned with wet tissue (Ethanol 75%).

**Gene expression of KIF5b and Dynein by R-T PCR**

After behavioral tests, animals were deeply and irreversibly anaesthetized, then rats were scarified, skull was opened, brain was removed and washed with normal saline, hemispheres of the brain were separated by scalp blade and midbrain was removed then hippocampus was dissected and
tissue was stored at -80°C. KIF5b and Dynein gene expression was determined using real-time polymerase chain reactions (R-T PCR). The total RNA was extracted using RNX-plus solution (Sinaclon, Iran), 75% Ethanol, Chloroform, DEPC treated water and Isopropanol using the manufacturer's recommendations. The concentration of RNA was checked by UV spectrophotometer (Ultraspex2000, Pharmacia, Netherlands). By measuring optical density (OD) at a wavelength A260/A280nm, the quantity of the isolated RNA were determined. The 1st strand cDNA was generated from 1µg of total RNA by reverse transcriptions using Prim Script RT reagent Kit (Takara, Japan), based on the manufacturer's instructions and R-T PCR assays were down in 72-well plates in a Rotor-Gene 6000 device (Corbett Life Science, Australia).

The forward and reverse primers for Dynein gene:
Forward primer: TGCTTTGGAAGATGATTGTGC
Reverse primer: TCTTCTCCTCCTGGCTCAACTCA

The forward and reverse primers for KIF5b gene:
Forward primer: TGCCATTGTGAGCTTGG
Reverse primer: GCCGGTTTGCTGATTGGTAT

The mRNA expression of KIF5B and Dynein and ß-actin (as housekeeping) were determined. The PCR volume reaction including of 1µl cDNA, 5µl SYBER Premix EX TAQ (Takara, Japan), 0.5µl Forward primer, 0.5µl reverse primer was 10µl.

The analysis of Real-Time PCR performed by ∆ΔCT method. The fold change in expression was then obtained as $2^{-\Delta\Delta CT}$ (Prodanov & Feirabend 2007).

Measurement of mitochondrial membrane potential (MMP) by Rhodamin 123 probe
Mitochondrial membrane potential was assessed by monitoring the uptake level of cationic dye Rhodamin 123 (Mingatto et al., 2002). Hippocampus containing blocks, were prepared, washed with phosphate buffered saline (PBS) and homogenized in mitochondrial isolation buffer (0.01mol/liter Tris HCl, 0.0001mol/liter EDTA-2Na, 0.01 mol/liter sucrose, 0.8% NaCl, PH7.4) on ice (0°C) for about 2min. The homogenate was kept at 4°C and centrifuged at 1500 rpm for 10 min. The supernatant was collected and then centrifuged again at 10000rpm for 15 min at 4°C. The remaining was mitochondrial. Then 20 µl of Rhodamin 123 solution (Rhodamin 123 solution 1mg/10ml DMSO) and 180 microliter of PBS was added to it, stirred, transferred to 96 well microtiter plat and incubated at 37°C for 30 min, then the MMP was studied. Fluorescent signals of mitochondria were cleared at 488 nm and emission was monitored at 525 nm wavelength in a fluorescent plate reader (FLX 800, BioTek, USA), (Ding et al., 2013).

**Blood sampling and biochemical parameters**

After behavioral tests, rats were sacrificed and blood samples were taken. To measure serum level of insulin, blood samples stayed at room temperature for 2h to clot, then centrifuged for 20 min at 5000 rpm (Lulat, Yadav & Balaraman, 2016). The serum was stored at -20 C. Repeated freezing and defrosting were avoided. To measure HgbA1c, whole blood were collected. Serum level of insulin and glycosylated hemoglobin (HgbA1c) were measured using ELISA kits, according to the manufacturer instructions, for Insulin (Mercodia, Sweden), for HgbA1c (Roch, Germany).

**Statistical analysis**
Data analysis was performed by Graph Pad Prism 7 software. All data are represented as mean ± SEM. Data obtained from our study was assessed by one-way ANOVA followed by Tukey’s post-hoc test except body weight and fasting blood sugar (FBS). To compare results of body weight and FBS, Two way with Repeated Measures analysis of variance (ANOVA) followed by bonferroni post-hoc test were used. The value of P<0.05 was considered as statistically significant.

Results

Body weight, fasting blood sugar (FBS)

Before starting the experiments, all animals were checked for blood glucose levels and animals with levels less than 150 mg/dl entered the exam. Body weight and FBS was measured at first and 8th week after STZ injection. Mean±SEM of weight and FBS are given in table 1. The body weight of animals in all of groups was similar in first week. Significant impairment in weight gain occurred in diabetic rats comparing to control rats at 8th week after induction of diabetes \((F(2, 21)=151.06, p=0.0001)\). Also in diabetic group FBS significantly increased compared to control group \((F(2, 21)=305.57, p=0.0001)\). Insulin injection for 8 weeks improved weight gain in diabetic rat. Animals weight in Diabetic+Insulin was significantly higher than diabetic rats \((P<0.05)\). FBS significantly lower than diabetic rats \((P<0.05)\) (table 1).

Behavioral tests

Elevated plus maze (EPM)

Number of entry to open arm and time spent in open arms ware considered as anxiety indices and presented as percent. Time spent in open arms (%) was significantly decreased \([F(2,18)=4.88, p=0.02]\) (figure 1). This index decreased in diabetic rat compared to control group
(Control+Salin=61.88±20.14, Diabetic=21.18±18.49, p<0.05). Whereas the number of open arm entrance (%) was not changed in groups \( [F_{(2,18)}=2.26, p=0.1324] \), (Figure 2).

**Y-maze**

Y-maze test was performed 48 hours following EPM. The percentage of alternation behavior 8 week after induction of diabetes was significantly changed \( [F_{(2,18)}=3.60, p=0.048] \), (figure 3). This index decreased in diabetic rat compared to control group (Control+Salin=28.57±5.53, Diabetic=15.57±11.77, p<0.05).

**Passive avoidance learning (PAL)**

This test was performed 48h after Y-maze task. The initial latency was recorded when the rats entered the dark compartment. In all of three groups there was no significant change in IL \( [F_{(2,18)}=0.686, p=0.515] \), (figure 4). The step through latency (maximum480 s) was measured and recorded as the index for the passive avoidance learning. STL index was significantly changed \( [F_{(2,18)}=7.31, p=0.0047] \) (figure 5). STL in diabetic group was decreased compared to control group (Control+Salin=384±123.94, Diabetic=95.28±70.77, p<0.05).

**Gene expression of KIF5b and Dynein by R-T PCR**

R-T PCR, was carried out to reveal KIF5b and Dynein genes expression (image 1). Gene Expression of KIF5b dramatically changed after 8 weeks of diabetes induction \( [F_{(2,9)}=20.41, p=0.0005] \). Actually, KIF5b mRNA levels were significantly increased compared to Control group (Control+Salin=1±0.4, Diabetic=2.25±0.47, p<0.05). Insulin injection for 8 weeks significantly decreased expression of KIF5b compared with diabetic rats (Diabetic=2.25±0.4796,
Diabetic+Insulin=0.5±0.23, p<0.05), (Figure 6). No significant changes were observed in Dynein mRNA levels in different groups [F(2,9)=1.86, p =0.2096], (Figure 7).

**Mitochondrial membrane potential (MMP) by Rhodamin 123 probe**

Mitochondrial membrane potential of cells was assessed by monitoring the uptake of cationic dye Rhodamin 123. The results of MMP showed a significant change in different groups [F(2,18)=8.557, p=0.0024], (figure 8). In diabetic group, MMP was decreased 8week after induction of diabetes compared to control group (Control+Salin=100.1±12.14, Diabetic=68.05±20.64, p<0.05) and there was significant difference between the Control group and insulin injected groups (Control+Salin=100.1±12.14, Diabetic+Insulin=75.88±10.58, p<0.05).

**Biochemical parameters**

Serum level of insulin and percentage of glycosylated hemoglobin (HgbA1c %) were measured by using ELISA kits. Mean±SEM of HgbA1c (%) and serum insulin are given in table 2. Results of HgbA1c percentage showed a significant difference between the groups [F(2,18)=95.99, p=0.0001]. Diabetic animals revealed higher glycosylated hemoglobin compared to age-matched controls (p<0.05). Significant difference observed between diabetic and insulin treated group (p<0.05). Serum level of insulin was significantly different between the groups (F(2,12)=316.3, p=0.0001). Insulin levels in diabetic rat was significantly lower than control rats (p<0.05). This difference was cleared between control and insulin treated rats (p<0.05) as well.
Discussion

Diabetic encephalopathy is referred to any cognitive dysfunction following diabetes mellitus (DM). This is why this condition is called type 3 DM (T3DM) or Alzheimer disease (Steen et al., 2005, Rivera et al., 2005, de la Monte & Wands 2008, Leszek, Trypka & Tarasov, 2017). On the other hand, mitochondrial diabetes (MD) appears following activation of mutant mitochondrial DNA, which is age dependent. MD is the combination of diabetes and cognitive deficit (Maassen et al., 2004, Maassen, Janssen & Hart, 2005). In this research we showed the correlation between diabetic encephalopathy, dysfunctional mitochondria and change in expression of axonal transport protein kinesin (KIF5b).

We showed short term memory impairment and retrieval deficit in combination with anxiety like behavior following T3DM. To measure anxiety like behavior in rodents, Elevated plus maze, is an accepted test (Pellow, Chopin & File, 1985). Our findings on EPM, showed a decrease in time spent in open arm, in diabetic group, compared with control and /or insulin injected group. In 2007, Miyata, showed increase of anxiety like behavior following STZ induced T1DM in rodents (Miyata, Yamada & Hiran, 2007).

Another study has revealed that N_Acetyl_Cistein (NAC) is protective against anxiety like behavior in EPM test of diabetic rats (Kamboj, Chopra & Sandhir, 2008). Behavioral changes due to diabetic encephalopathy are not only dependent to insulin metabolism cascade impairment, but also because of decreased serotonin levels in central nervous system (Zalsman et al., 2006, Pittenger & Duman 2008). On the other hand hyperglycemia, induce neurodegeneration and pathologic behavior and cognition. In Diabetic+Insulin group, possibly insulin has prevented behavioral deficits. Our findings in passive avoidance learning task showed different degrees of learning and memory impairment in type 3 diabetes. Actually in shuttle box the significant
decrease in step through latency (STL) in T₁DM, referred to impairment of memory retention and consolidation. Scientist have reported the role of insulin on improvement of memory in DM, which is in agreement to our result, (Diabetic+Insulin group, showed increase in STL by insulin administration). Fallowing prolonged DM, impaired hippocampal plasticity, may damage learning and memory processing (Flood, Mooradian & Morley, 1990, Stewart & Liolitsa 1999, Stranahan et al., 2008). Scientists have found that anxiety level and cognitive dysfunction is directly correlated to diabetes duration in young rats (Rajashree, Kholkute, Goudar, 2011). Memory and learning deficit and even behavioral changes are among obvious manifestation of diabetic encephalopathy, as insulin and c-peptide are key factors for synaptic plasticity and cognition (Sima et al., 2009, McNay & Recknagel, 2011). Scientists have revealed the role of intracerebroventricular STZ in induction of cognitive dysfunction, which has been reversed after insulin infusion (Guo et al., 2017). Physiologic mitochondrial function is dependent to its intact transport process (Chada & Hollenbeck, 2004, Russo et al., 2009). Actually low insulin and c-peptide levels may disrupt normal cerebral metabolism and function sequentially (de la Monte & Wands, 2008, Sima et al., 2009).

Although our finding was on late course of T₁DM or T₃DM, but whether hypoglycemic course is related to cerebral malfunction is not clear. Although chronicity of T₁DM is correlated with encephalopathy (Garg, Bonanome & Grundy, 1988, Musen et al., 2006).

Natural flavonoids have been effective in reversal of anxiety-like behavior in diabetic mice has been detected in EPM test (Damian et al., 2014, Tang et al., 2015). Insulin plays a critical role in hippocampal memory processing and hyperglycemia is responsible for cognitive deficit and neuronal damage following DM (Abbatecola et al., 2006, Strachan et al., 2011).
In Rhodamin monitoring of hippocampal mitochondrial membrane potential, we found decreased level of mitochondrial staining. Mitochondrial function can be monitored by mitochondrial membrane potential or MMP. Normal detectable electrochemical gradient of mitochondrial membrane refer to ATP production (Hafner, Brown & Brand, 1990, Fontaine, Devin & Rigoulet, 1997). Rhodamin 123 transmembrane distribution reveals membrane potential strength (Emaus, Grunwald & Lemasters, 1986, Huang, Camara & Stowe, 2007). Following any mitochondrial and cellular damage, there will be abnormal or undetectable MMP and ATP production and neuronal apoptosis (Letai 2006, de la Monte, 2012). Even decreased MMP represent apoptosis (Ding, Han & Zhu. 2005). Electrophoretic mobility of mitochondria is directly related to its permeability and staining specifies (Emaus, Grunwald & Lemasters, 1986, Bunting, 1992). In 2017 scientists have shown that normal mitochondrial metabolism is potentially preventive against decreased age dependent neurogenesis in hippocampus (Beckervordersandforth, 2017). Besides mitochondrial count and morphology, mitochondrial metabolism is also dependent to the dynamic movement and distribution of these organelles, which is essential to maintain normal ATP synthesis (Lu et al., 2004).

Our study showed higher expression of KIF5b, a member of kinesin super family. KIF5b has a key role on both mitochondrial translocation and distribution. KIF5b depletion will stop normal mitochondrial transport. In 2011 for the first time scientists showed the vital role of kinesin protein in mitochondrial transport of hippocampal neurons (Brickley, 2011).

In 2013 scientists showed increased KIF5b gene expression in cultured hippocampal neurons of diabetic rat with no alterations in dynein gene expression (Baptista, Pinto & Elvas, 2013). Dynein is not only responsible for retrograde translocation of mitochondria, but responsible for movement
of other motor adaptors (King & Schroer, 2000, Pilling, Horiuchi & Lively, 2006). Although there are some kinesins also involved in retrograde transport (Hirokawa & Noda Tanaka, 2009).

In this study we found that diabetes alters KIF5b motor protein gene by increasing KIF5b gene expression levels in the hippocampus at 8th week after the induction of diabetes and the anterograde transport of mitochondria may be impaired in the hippocampus. As a consequence we suggested that, decrease in the number of synaptic vesicles and density may ultimately account for changes in synaptic transmission and mitochondrial transport in the hippocampus. In 2014 the effect of T1DM (duration of 2-8 weeks) on rat retinal distribution of dynein, KIF5b and KIF1a in retinal tissue was confirmed (Baptista, Pinto & Elvas, 2014). Which was considered as the possible factor for diabetic retinal neurodegeneration.

In this study we found no change in dynein heavy chain expression. Although to our knowledge there is still no report about encephalopathy and dynein gene expression but may be more chronic type of T3DM models (longer than 8 week) could have revealed different findings. Although IHC, as a qualitative method and western blotting as a quantitative one, show protein end product and complete study, but when mRNA production is impaired no protein will be found. In conclusion, this study revealed the correlation of diabetic encephalopathy, mitochondrial dysfunction and kinesin gene overexpression. We can suggest that possibly abnormal translocation of mitochondria and its malfunction induced by kinesin gene over expression, result in diabetic encephalopathy.

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**Conflict of interest**
The authors have no conflict of interest to the publication of this article.

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Tables & Figure legends

**Table 1.** Body weight and fasting blood sugar (FBS). Experimental groups: Control+Saline, Diabetic and Diabetic+Insulin. Values are represented as mean±SEM. *p<0.05, significant difference compare to Control+Saline. # p<0.05, significant difference compare to Diabetic.

**Table 2.** Serum level of insulin and percentage of glycosylated hemoglobin (HgbA1c %). Experimental groups: Control+Saline, Diabetic and Diabetic+Insulin. Values are represented as mean±SEM. * p<0.05, significant difference compare to Control+Saline. # p<0.05, significant difference compare to Diabetic.

**Figure 1.** Percentage of time spent in open arm in elevated plus maze (EPM) task. Experimental groups: Control+Saline, Diabetic and Diabetic+Insulin. Values are represented as mean±SEM. * p<0.05, significant difference compare to Control+Saline.

**Figure 2.** Percentage of number of entry in open arm in elevated plus maze (EPM) task. Experimental groups: Control+Saline, Diabetic and Diabetic+Insulin. Values are represented as mean±SEM. No significant change between groups.
Figure 3. Percentage of alternation behavior in Y-maze task. Experimental groups: Control+Saline, Diabetic and Diabetic+Insulin. Values are represented as mean±SEM. * p<0.05, significant difference compare to Control+Saline.

Figure 4. Initiation latency (IL) in passive avoidance learning (PAL) test. Experimental groups: Control+Saline, Diabetic and Diabetic+Insulin. Values are represented as mean±SEM. No significant change between the groups.

Figure 5. Step through latency (STL) in passive avoidance learning (PAL). Experimental groups: Control+Saline, Diabetic and Diabetic+Insulin. Values are represented as mean±SEM. * p<0.05, significant difference compare to Control+Saline.

Figure 6. Gene Expression of KIF5b in the Hippocampus. Experimental groups: Control+Saline, Diabetic and Diabetic+Insulin. Values are represented as mean±SEM. * p< 0.05, significant difference compare to Control+Saline. *p<0.05, significant difference compare to diabetic.

Figure 7. Gene Expression of Dynein in the Hippocampus. Experimental groups: Control+Saline, Diabetic and Diabetic+Insulin. Values are represented as mean±SEM. No significant change between groups.

Image 1. Melting curve based on temperature (horizontal axis) and df/dt (vertical axis). Part “A” for KIF5b and part “B” for Dynein.

Figure 8. Mitochondrial membrane potential using Rhodamin 123 probe in the Hippocampus. Experimental groups: Control+Saline, Diabetic and Diabetic+Insulin. Values are represented as mean±SEM. * p<0.05, significant difference compare to Control+Saline.

References


### Tables and Figures

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Table 2

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<td>2.01 ±0.17</td>
<td>0.23±0.10*</td>
<td>0.39±0.058*</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1
Figure 2
Figure 3
Figure 4
Step through latency time (second)

Figure 5
Figure 6

KIF5b gene expression (Fold change)

Control+Salin  Diabetic  Diabetic+Insulin

Fig 6
Dynex gene Expression (fold change)

Figure 7
Figure 8