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STUDY OF EFFECT OF INSULIN ON COGNITIVE FUNCTION IN DIABETES MELLITUS IN A TERTIARY CARE HOSPITAL

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ABSTRACT: Diabetes Mellitus is a major lifestyle metabolic disorder and doubtlessly a challenging public health problem of the 21st century. Diabetes is associated with reduced performance on various domains of cognitive function, this study was aimed to access the effect of insulin on cognitive function in patients of Diabetes Mellitus. It was a prospective, observational and non-interventional study. 60 confirmed cases of Diabetes mellitus, who were recently (within 7 days) started on insulin were enrolled as study subjects from medicine department in a random fashion. Cognitive function was assessed by Addenbrooke's Cognitive Examination (ACE III) at the time of enrolment, then 1 month and 3 months of insulin treatment. The results were analyzed using repeated measures analysis of variance (ANOVA) test. In results there was a significant increase in Attention, memory and visuospatial ability after one and three months, in language, there was substantial increase after one month but not after three months, and in verbal fluency, there was no significant increase after one and three months. Considering all the five domains, a total score of ACE III showed a significant increase after one and three months, suggesting some role of insulin in the improvement of cognitive function. Thus, it can be speculated that treatment with insulin improves cognitive function in patients of Diabetes Mellitus. As insulin therapy is generally lifelong, long term effects of insulin on cognitive function needs to be further evaluated.

INTRODUCTION: Diabetes mellitus is a major lifestyle and chronic metabolic disorder characterized by hyperglycemia, polyuria, polydipsia, *etc*. requiring lifelong treatment and care. The disease burden is rising briskly, and its prevalence is estimated to increase from 382 million people with diabetes in 2013 to 592 million by 2035 [1].

Ninety-two million persons are with DM in India, second to only China [2]. The etiopathogenesis is leading to the development of Diabetes Mellitus range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action, classifying it into type 1 and type 2 DM respectively. The deficiency of insulin on the target tissues is considered the ground base reason for causing abnormalities in carbohydrate, fat and protein metabolism in patients of diabetes [3].

Diabetes mellitus is considered to be one of the primary cause of morbidity and mortality, and it is a major risk factor for early onset of coronary heart disease. Its microvascular complications include

diabetic nephropathy, neuropathy, and retinopathy [4]. Cognition which is known as work of the brain is required for performing various day to day activities. It is defined as all mental activities that are involved in the acquisition, processing, storage and retrieval of information. It includes a variety of skills attention, learning, memory, verbal ability or language, visuospatial function, and a group of abilities, known as executive function, *i.e.* reasoning, abstraction and mental flexibility [5]. In simple terms, it is the thinking and understanding process of the brain.

Several studies have demonstrated an association between diabetes mellitus, cognitive impairment and dementia [6, 7, 8]. The risk of dementia is positively associated with the duration and insufficient treatment of diabetes [8]. Both type 1 and type 2 diabetes mellitus have been associated with reduced performance on numerous domains of cognitive function. The exact pathophysiology of cognitive dysfunction in diabetes is not completely understood, but it is likely that hyperglycemia, vascular disease, hypoglycemia, and insulin resistance play significant roles [9].

The profile of cognitive decrements in type 2 DM is generally similar to that found in type 1 DM [10]. Mental processing speed, flexibility, and attention are more affected in type 1 whereas memory and language are more affected in type 2 DM [11, 12].

Insulin is one of the commonly prescribed ant diabetic agents. Various studies have shown an improving effect of Insulin on cognitive function [13]. In the light of the increasing problem of cognitive functions associated with DM and dubious reports of the effect of insulin on cognitive function, it is considered worth to undertake a study to estimate the effect of insulin on cognitive function in patients of DM. If insulin shows a positive effect on cognitive functions in patients of DM, a hypothesis regarding the additional beneficial effect of insulin in these patients can be generated and can be tested by a further appropriate method.

MATERIALS AND METHODS:
Study Design: It was a prospective, observational and non-interventional study.

Ethical Approval: The study protocol, patient data sheet, participant information sheet and informed consent form (in English and vernacular languages) were submitted and approved by scientific review committee and human research and ethics committee of the Institution. IEC Approval No. MCS/STU/ETHICS/Approval/12641/15   Date 11/06/15.

Study Subjects: 60 confirmed cases of diabetes mellitus patients of both gender who were recently (within 7 days) started on Insulin were enrolled as study subjects from OPD's and IPD's of medicine department of New Civil Hospital Surat, randomly. Diabetes was confirmed by using WHO criteria 2010.

Confirmed DM patients were selected for the study according to the following inclusion and exclusion criteria:

Inclusion Criteria: Patients of Diabetes Mellitus who just started Insulin (*i.e.,* within 7 days).

Exclusion Criteria:

   a. Patients are not giving consent for enrolment in the study.

   b. Patients taking drugs that are known to affect cognition such as Anticonvulsants, Tricyclic Antidepressants, Anticholinergic, Barbiturates, Benzodiazepines, Opiates.

   c. Progressive neurological disorder.

   d. Head injury.

   e. Mental Retardation.

   f. Drug and alcohol abuse.

   g. Severe Psychiatric problem.

Study Procedure: Written informed consent was taken before cognitive function testing. Cognitive function testing was done by Addenbrooke's Cognitive Examination III (ACE-III). Cognitive function testing was done at 0 months, *i.e.* after starting Insulin treatment once its dose gets stabilized (within 7 days), then 1 month and 3months of insulin treatment.

The Addenbrooke's Cognitive Examination-III (ACE-III) is a brief cognitive test that assesses five cognitive domains: attention, memory, verbal fluency, language, and visuospatial abilities. ACE-III replaced the previous ACE and ACE-R versions

in November 2012. The test is widely used for determining mild cognitive impairment and dementia. It is the sum of each of the five domains, *i.e.* Attention/18, Memory/26, Fluency/14, Language/26, and visuospatial ability/16 to give a total score of ACE III out of 100. The total score is 100; higher scores indicate better cognitive function.

After taking the basic details, the patient is informed about the test and that he will be asked questions according to the Addenbrooke's scoring guide and cognitive function will be assessed.

The patient is asked questions from Addenbrooke's Cognitive Examination score sheet and scoring done accordingly as per the scoring guidelines then, next examination was done after one month, and last was after 3 months of the first examination. Routine investigations such as FBS, PPBS, and RBS were done during each examination. The Subjects were given participant information number which contained details of the subject, which were kept confidential.

Statistical Analysis: The data were analyzed using SPSS 16.0 software. The results were expressed as Mean ± Standard error of the mean. The result was analyzed using repeated measures analysis of variance (ANOVA) test. P value < 0.05 is taken as significant.

RESULTS: Mean age of 60 subjects recently started on Insulin is 45.88 ± 1.70. Out of these 60 subjects, 7 were of type 1 DM and 53 of type 2 DM out of 60 subjects, 34 were male, and 26 were female. Baseline mean Fasting blood sugar (FBS), Post Prandial blood sugar (PPBS) and random blood sugar (RBS) were 176.80 ± 8.58 mg/dl, 247.10 ± 11.44 mg/dl, 235.93 ± 10.90 mg/dl respectively.

TABLE 1: GENERAL CHARACTERISTICS OF STUDY POPULATION

Variable		Study group (n=60)
Age (years)		45.88 ± 1.70
Type 1 DM		7
Type 2 DM		53
Sex	Male	34
	Female	26
FBS		176.80 ± 8.58
PPBS		247.10 ± 11.44
RBS		235.93 ± 10.90

FBS, PPBS and RBS: A repeated measures ANOVA with a Greenhouse-Geisser correction determined that FBS, PPBS and RBS differed statistically significantly between time points (for FBS – $F_{(1.091, 64.386)} = 58.689$, [$p < 0.05$]; for PPBS – $F_{(1.07, 63.136)} = 92.769$, [$p < 0.05$] and for RBS – $F_{(1.132, 66.798)} = 47.83$. Post hoc tests using the Bonferroni correction revealed that FBS, PPBS and RBS are decreased at one month (115.72 ± 8.58 mg/dl, 151.67 ± 2.26 mg/dl and 174.23 ± 3.915 respectively), [$P < 0.05$] and at three months (108.13 ± 1.38 mg/dl, 144.02 ± 2.52 mg/dl and 163.43 ± 2.68 mg/dl respectively) [$p < 0.05$] significantly in comparison to baseline (176.80 ± 8.58 mg/dl, 247.10 ± 11.44 mg/dl and 235.93 ± 10.90). There is also a significant difference in FBS, PPBS, and RBS after three months of treatment in comparison to after one month of insulin treatment **Fig. 1**.
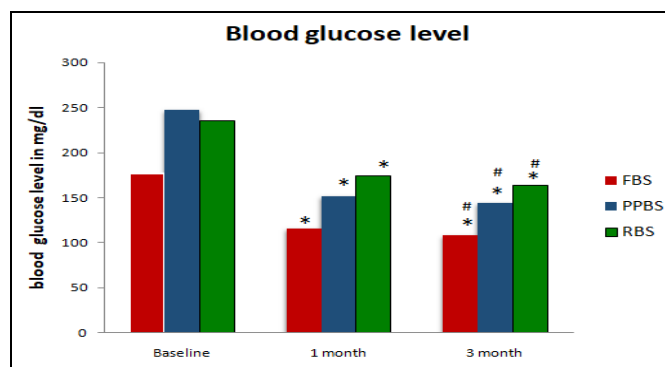

FIG. 1: FBS, PPBS, AND RBS IN PRE-TREATMENT ANFD POST TREATMENT GROUPS. *p<0.05 *vs*. Baseline group. #p<0.05 *vs*. after 1 month of insulin

Domains of Cognitive Function:
1. Attention: A repeated measures ANOVA with a Greenhouse-Geisser correction determined that attention differed statistically significantly between time points ($F_{(1.833, 108.135)} = 15.871$, $p < 0.05$). Post hoc tests using the Bonferroni correction revealed that attention is increased after 1 month (14.03 ± 0.26), [$p < 0.05$] and after 3 months (14.52 ± 0.22), [$p < 0.05$] significantly in comparison to baseline (13.58 ± 0.29). There is also a significant increase in attention after 3 months of treatment in comparison after 1 month, [$p < 0.05$] **Table 2**, **Fig. 2**.

2. Memory: A repeated measures ANOVA with a Greenhouse-Geisser correction determined that memory differed statistically significantly between time points ($F_{(1.589, 93.771)} = 16.602$, $p < 0.05$).

Post hoc tests using the Bonferroni correction revealed that memory is increased after 1 month (13.483 ± 0.40), [$p<0.05$] and after 3 months (14.05 ± 0.39), [$p<0.05$] significantly in comparison to baseline (12.88 ± 0.55). There is also a significant increase in attention after 3 months of treatment in comparison to after 1 month, [$p<0.05$] **Table 2**, **Fig. 2**.

TABLE 2: EFFECT OF INSULIN TREATMENT ON VARIOUS DOMAINS OF COGNITIVE FUNCTION

Variable	Baseline	After one month	After three months
Attention	13.58 ± 0.29	$14.03\pm0.26^{*}$	$14.52\pm0.22^{*\#}$
Memory	12.88 ± 0.55	$13.48\pm0.40^{*}$	$14.05\pm0.39^{*\#}$
Verbal Fluency	4.38 ± 0.28	4.40 ± 0.23	4.48 ± 0.23
Language	21.63 ± 0.37	$22.28\pm0.25^{*}$	$22.37\pm0.26^{*}$
Visuospatial ability	11.65 ± 0.34	$12.12\pm0.28^{*}$	$12.65\pm0.26^{*\#}$
Total score	64.13 ± 1.50	$66.32\pm1.09^{*}$	$68.07\pm0.96^{*\#}$

* $p<0.05$ *vs*. Baseline group. #$p<0.05$ *vs*. after 1 month of insulin treatment

3. Verbal Fluency: A repeated measures ANOVA with a Greenhouse-Geisser correction determined that verbal fluency did not differ statistically significantly between time points (F $(1.577, 93.04)$ =0.220, $p>0.05$). Post hoc tests using the Bonferroni correction revealed that verbal fluency is not increased after 1 month (4.40 ± 0.23), [$p>0.05$] and after 3 months (4.48 ± 0.23), [$p>0.05$] significantly in comparison to baseline (4.38 ± 0.28). There is no significant increase in attention after 3 months of treatment in comparison after 1 month, [$p>0.05$] **Table 2**, **Fig. 2**.

4. Language: A repeated measures ANOVA with a Greenhouse-Geisser correction determined that language differed statistically significantly between time points (F $(1.471, 86.817) = 5.707$, $p<0.05$). Post hoc tests using the Bonferroni correction revealed that language is increased after 1 month (22.28 ± 0.25), [$p<0.05$] and after 3 months (22.37 ± 0.26), [$p<0.05$] significantly in comparison to baseline (21.63 ± 0.37). There is no significant increase in language after 3 months of treatment in comparison after 1 month, [$p>0.05$] **Table 2**, **Fig. 2**.

5. Visuospatial Ability: A repeated measures ANOVA with a Greenhouse-Geisser correction determined that visuospatial ability differed statistically significantly between time points (F

$(1.770, 104.423) = 19.138$, $p<0.05$). Post hoc tests using the Bonferroni correction revealed that visuospatial ability is increased after 1 month (12.12 ± 0.28), [$p<0.05$] and after 3 months (12.65 ± 0.26), [$p<0.05$] significantly in comparison to baseline (11.65 ± 0.34). There is also a significant increase in visuospatial ability after 3 months of treatment in comparison after 1 month, [$p<0.05$] **Table 2**, **Fig. 2**.
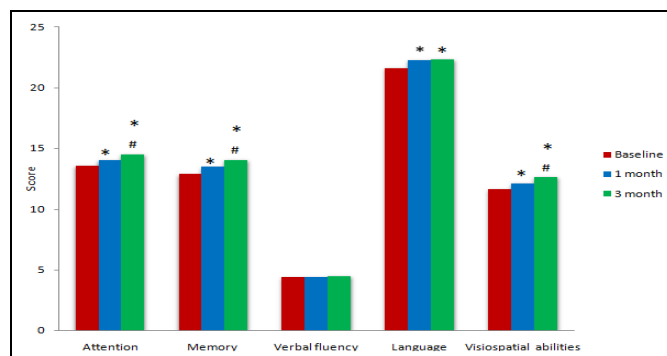

FIG. 2: EFFECT OF INSULIN ON VARIOUS DOMAINS OF COGNITIVE FUNCTION. (*$p<0.05$ *vs*. baseline). (#$p<0.05$ *vs*. after 1 month)

6. Total Score of ACE III: It is the sum of each of the five domains, *i.e.* Attention/18, Memory/26, Fluency/14, Language/26, and visuospatial ability/16 to give a total score of ACE III out of 100. A repeated measures ANOVA with a Greenhouse-Geisser correction determined that total score differed statistically significantly between time points (F $(1.306, 77.058) = 20.694$, $p<0.05$). Post hoc tests using the Bonferroni correction revealed that total score is increased after 1 month (66.32 ± 1.09), [$p<0.05$] and after 3 months (68.07 ± 0.96), [$p<0.05$] significantly in comparison to baseline (64.13 ± 1.50). There is also a significant increase in total score after 3 months of treatment in comparison after 1 month, [$p<0.05$] **Table 2**, **Fig. 3**.
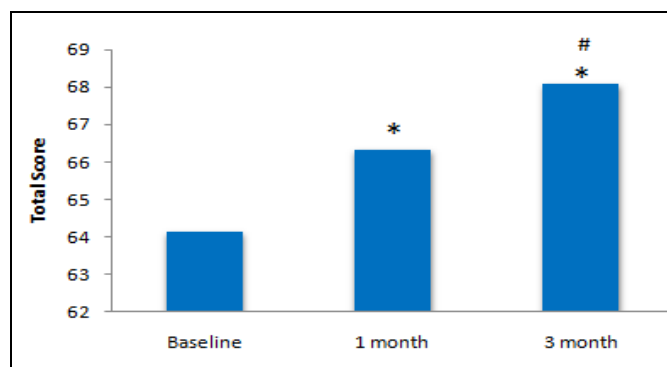

FIG. 3: TOTAL SCORE OF ACE III (*$p<0.05$ *vs*. baseline). (#$p<0.05$ *vs*. after 1 month)

DISCUSSION: Diabetes mellitus have gained the status of the potential epidemic and is considered a major cause of mortality and morbidity due to its various complications. Many organ systems are adversely affected by diabetes mellitus, including brain [14]. Diabetes mellitus is a major risk factor for cognitive impairment, vascular dementia and Alzheimer's disease [15]. Diabetic patients have 1.5-fold increased risk of decline in cognitive function [16]. Adequate functioning of cognition is required to perform daily activities of living. Impaired cognition can make a patient dependent and can reduce the quality of life. Even it can affect adherence to treatment. Studies have shown the brain to be the target of Insulin [16]. By keeping this in mind, this study was conducted to determine the effect of Insulin on cognitive function in Diabetes Mellitus patients. In this study, cognitive function testing of patients of Diabetes mellitus (both type 1 and type 2) was done by Addenbrooke's Cognitive Examination III (ACE-III). Cognitive function testing was done at 0 months, *i.e.* after starting Insulin treatment once its dose gets stabilized (within 7 days), then 1 month and 3 months of insulin treatment. Five domains of cognitive function were assessed by ACE III. Attention, memory, verbal fluency, language, and visuospatial ability. At last total scoring of these five domains was done.

In this study considering various domains of cognitive function, there was a significant increase in Attention, memory and visuospatial ability after one and three months. In language there was a significant increase after one month but not after three months. In verbal fluency, there was no significant increase after one and three months. Considering all these five domains total score of ACE III showed a significant increase after one and three months, suggesting some role of insulin in the improvement of cognitive function. Despite several studies conducted, the natural history and the mechanism behind the cognitive decline are still debatable, but hyperglycemia, vascular disease, hypoglycemia, inflammation, and insulin resistance are considered the possible pathophysiological mechanisms for the cognitive decline in Diabetes [9, 17]. Previous studies have shown improving the effect of insulin even in healthy adults [18]. Recent investigations have shown that Insulin affects the neurons and its growth controls the neurotransmitters and neuronal firing in the brain. Studies have also shown improving effects of intranasal insulin on cognitive function [16, 19].

Insulin injected peripherally can easily cross the blood-brain barrier. Intravenous insulin has also shown to improve cognitive function in patients with Alzheimer's dementia. Moreover, intravenous insulin increases concentrations of an extended form of beta-amyloid protein, Abeta42 [18]. The action of insulin in the brain and periphery are different. In brain, insulin acts as a neuromodulator in cognition, energy homeostasis, food intake, sympathetic activity, neuron–astrocyte signaling, synapse formation, and neuronal survival. Neuroprotective effects of Insulin has been demonstrated both *in-vitro* and *in-vivo* by activation of protein kinase and clearance of β-amyloid respectively [20].

Insulin receptors (IRs) are expressed in various regions of the brain, and insulin action through IR is considered responsible for the regulation of cortical blood flow in brain. Central IRs, however, is dependent upon insulin transport from the periphery through the blood-brain barrier and this transport are said to be affected by aging, obesity, diabetes, and AD. Type 2 DM decreases insulin sensitivity in the brain, insulin transport through the blood-brain barrier, and IRs receptor sensitivity, and it alters glucose metabolism [20]. Some studies have shown Apolipoprotein E, type epsilon 4 allele (APOE e4) to be responsible for Alzheimer's disease and also indicated that APOE e4-negative subjects exhibit greater insulin-mediated memory benefits [21]. In this study, there is a significant improvement in cognitive function in patients of Diabetes mellitus who are recently or newly started on insulin. In this study patients receiving drugs which affect cognitive function were excluded. Similarly, patients with a psychiatric disorder or any other systemic disorder were also excluded. Thus the changes observed in the study could be attributed to Insulin. Although, the exact mechanism by which insulin improves cognitive function is unknown, there are several possibilities. This improvement may be due to glycaemic control achieved by insulin or improved cerebral blood flow, regulation of brain glucose metabolism or may be due to improved delivery of insulin to the brain and its action on insulin receptors.

In this study, insulin treatment improved blood glucose level and improvement in cognitive function. Hence, Insulin might offer a useful therapeutic choice in either prevention or the treatment of cognitive disorders. Diabetic control from an early stage would be useful in preventing the onset of vascular events, as well as cognitive decline. Also, identification of the mechanisms through which hyperglycemia may impair cognitive function in patients with diabetes will stimulate new research into ways to prevent and treat all of the hyperglycemia-associated complications of diabetes.

CONCLUSION: This study concludes that insulin treatment improves cognitive function in patients of DM. However, in this study, we examined the effects of insulin on cognitive function for a period of three months only. As insulin therapy is generally lifelong, long term effects of insulin on cognitive function needs to be further evaluated.

Thus to generalize the results of this study, study parameters need to be evaluated in a larger population and for a longer duration. Also, understanding the natural history and identification of the mechanisms of cognitive decline in patients with DM will stimulate research to develop new and better ways to prevent and treat cognitive decline associated with DM.

ETHICAL APPROVAL: The study was approved by the Institutional Ethics Committee.

CONFLICT OF INTEREST: None

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