UNIVERSITY OF NAIROBI

COGNITIVE FUNCTION IN CHILDREN AGED 7 TO 16 YEARS WITH TYPE 1 DIABETES AT KENYATTA NATIONAL HOSPITAL

BY

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Declaration

I declare that this dissertation is my original work and has not been presented for the award of a degree in any other university. Where other people's work has been used, this has properly been acknowledged and referenced in accordance with University of Nairobi's requirements.

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DEDICATION

This work would not have been possible without the support of my family – I thank God for their support on every step of the journey. To my parents, who encouraged me to pursue my dreams, to my children, who rooted me on as we did our ‘homework’ together; to my siblings, especially my sister, for editorial support and a laugh, when things got tough; and last but not least, my husband. Without you, this would not have been possible. This one’s for you.

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I would like to thank my supervisors, for their guidance through this process, and the parents and children who participated selflessly in the study.

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ABBREVIATIONS

WHO - World Health Organization

KDHS – Kenya Demographic Health Survey

KNH – Kenyatta National Hospital

DM – Diabetes Mellitus

IDDM - Insulin Dependent Diabetes Mellitus

T1DM – Type 1 Diabetes Mellitus

NIDDM – Non Insulin Dependent Diabetes Mellitus

MMSE – Mini Mental State Exam

RQC – Reporting Questionnaire for Children

HBA1C – Glycosylated haemogloblin

GLUT – Glucose transporter

AGEs – Advanced Glycation Endproducts

DEFINITION OF TERMS

Diabetes – A chronic illness which occurs due to insulin resistance or insulin deficiency, leading to hyperglycaemia

Type 1 Diabetes Mellitus – Diabetes secondary to absolute insulin deficiency

Cognition - The function through which sensory input is processed. It includes attention, working memory, processing and comprehending language, learning, reasoning, problem solving and decision making.

Glycosylated Haemoglobin (HBA1C): Formed by non-enzymatic bonding of glucose molecules to haemoglobin. As the average plasma glucose rises, the fraction of glycosylated haemoglobin increases in a predictable way. Reflects blood sugar control over previous three months.

Glycaemic control: HBA1C < 6.5%

Hypoglycaemia: In diabetic children this is defined as blood glucose < 3.9 mmol/l

Neuroglycopenia: Symptoms of neurological impairment secondary to low blood glucose.

Hypoglycaemic unawareness: Condition in which there are no symptoms of hypoglycaemia despite low blood glucose

Chronic illness: WHO definition: Illness lasting longer than 3 months, causing periodic or continuous episodes of incapacity. Includes respiratory conditions such as asthma, cardiac disease, HIV and cancer.

Caregiver: A caregiver is someone, typically over age 18, who provides care for another.

Child: - The United Nations Convention on the Rights of the Child defines a child as "a human being below the age of 18 years unless under the law applicable to the child, majority is attained earlier". In Kenya, ‘a child is any human being below the age of eighteen years’ (Chapter 586 Laws of Kenya, 2001)

ABSTRACT

Background

Type 1 Diabetes Mellitus (T1DM) is one of the most common chronic diseases of childhood. The burden of diabetes in children in Kenya is unknown, but the Ministry of Health estimates that three million people have the condition. Diabetes is known to cause cognitive dysfunction secondary to both its acute and chronic complications. Early recognition of this is important to help these children maintain normal intellectual function and achieve their full potential.

Objectives

To compare the cognitive function in children with Type 1 diabetes with that of non-diabetic children at Kenyatta National Hospital; and within the population of diabetic children, determine the relationship between cognitive function and both the duration of diabetes and level of glycaemic control.

Study design

This was a hospital based cross-sectional comparative study with two arms recruiting diabetic and non-diabetic children, respectively.

Methods

Sixty-six children with T1DM aged 7 to 16 years were enrolled from the paediatric Endocrinology clinic. Sixty-seven children aged 7 to 16 years recruited from the paediatric out-patient clinic formed the comparative group. The Modified Mini-Mental State Exam (MMSE) was administered to all children meeting inclusion criteria and scores were categorized into either normal or impaired cognitive function using age-specific cut-offs.

Results

Median (lower - upper IQR) age of children with diabetes was 13 (10-15) years and the median age in non-diabetics was 12 (10-13) years. There was no significant difference in cognitive function assessed using MMSE scores between diabetic and non-diabetic children. Overall, 17 out of the 66 diabetics (25.8%) had low MMSE score compared to 14 (20.9%) non-diabetics (OR = 1.31, 95% CI 0.54-3.21). The cognitive function did not differ significantly for the subdomains of the MMSE with mean scores for diabetics and non-diabetics

of: 11.2 versus 11.3 (orientation); 6.0 versus 5.9 (attention and concentration); 3.0 versus 3.0 (registration); 2.3 versus 2.4 (recall) and 10.3 versus 10.4 (language). However, there was some evidence of higher scores for recall in non-diabetic children aged 12-14 years compared to diabetic children in the same age group ($p = 0.078$).

Conclusion

There was no significant difference in cognitive function in diabetic children compared to non-diabetic children as assessed using the Modified Mini Mental Status Examination.

Recommendations

Baseline and serial assessment may be more useful than a single assessment of the MMSE. A different tool may detect subtle differences in cognition.

1. BACKGROUND AND LITERATURE REVIEW

Diabetes is a chronic illness due to either absolute insulin deficiency or insulin resistance, leading to hyperglycaemia. While Type 2 diabetes is due to insulin resistance(1), Type 1 diabetes (previously known as insulin-dependent or childhood-onset diabetes) is characterized by absolute insulin deficiency. Studies indicate that autoimmunity is a major factor in pathogenesis for Type 1 diabetes in a genetically susceptible individual(2).

Global incidence of diabetes is estimated at 300M.(3) Incidence of type 1 diabetes is highest in Finland at 35/100,000. In Africa, incidence of Type 1 diabetes ranges from 4/100,000 in Mozambique to 12/100,000 in Zambia(4).

Kenya does not have accurate statistics on the number of diabetic patients, but the Ministry of Health estimates that three million Kenyans are living with diabetes. By extrapolation, 10% of these patients would have T1DM, and approximately 270,000 are expected to be children(5). However, only about 2,000 children with T1DM are being followed up in the main hospitals and clinics in the country; the majority die undiagnosed due to complications (2).

In the absence of endogenous insulin production, patients with T1DM require lifelong insulin treatment, which is administered by subcutaneous injections. Optimal control requires at least two or more daily injections and frequent self monitoring of blood glucose levels.

Diabetes can lead to both acute and chronic complications. While long-term vascular complications have been widely studied, effects of dysglycaemia and poor glycaemic control on intellect and cognition in diabetic children are less well known and documented. In recent studies, both hypo and hyperglycaemia have been recognized to cause cognitive impairment and reduced intellectual functioning (6,7).

Cognitive function

Cognition is the function through which sensory input is processed. It includes attention, working memory, processing and comprehending language, learning, reasoning, problem solving and decision making.

Brain development and metabolism

During intra-uterine development and in the first few years of life, there is rapid brain growth and maturation, with proliferation of neurons, formation and pruning of synapses. By age two, the brain's energy demand reaches adult levels and is nearly twice the adult rate by age ten, gradually reducing to adult levels in the next decade of life (8). This critical period of brain development is particularly vulnerable to metabolic disturbances, such as hypo and hyperglycaemia.

The brain utilizes about 10% of the body's glucose; a disproportionately high percentage of the body's metabolic needs. Because neurons cannot store glucose or glycogen; or derive glucose from gluconeogenesis, the brain requires a continuous supply of nutrients. While in a normal child cerebral blood supply increases with an increase in glucose demand, in diabetic children there is little increase in cerebral blood flow and limited ability to utilize other substrates such as lactate, alanine and ketones. There is also insufficient up-regulation of GLUT 1 transporters at the blood-brain barrier, which normally help in increasing glucose extraction from blood (7).

Endogenous insulin levels and serum glucose levels are tightly regulated in the non-diabetic child but exogenous insulin imperfectly mimics normal physiology, leading to poor glucose homeostasis. When normal homeostasis fails to maintain euglycaemia, neuronal function and viability are affected (8).

Cognitive function in diabetic children

Several hypothesis have been put forth to explain how dysglycaemia causes cognitive dysfunction. It is thought to occur due to oxidative stress and lipid peroxidation during glucose fluctuations (9), resulting in formation of Advanced Glycation End-products (AGEs). AGEs are formed through non-enzymatic

reaction of glucose with amino groups found in proteins, lipids and nucleic acids. Formation of AGEs is accelerated by hyperglycaemia and they are preferentially deposited in some tissues including collagen and myelin. A study done by Shah *et al* in New Orleans showed that many diabetic children have precociously high estimates of AGEs, comparable to what would naturally accumulate after 25 years of ordinary aging(10).

Advanced glycation end products are deposited in the basement membrane of the blood brain barrier and in the myelin sheath around neurons. This results in disruption of the blood brain barrier and axonal damage. AGEs also cause endothelial damage through stimulation of release of transforming growth factor β by pericytes and vascular endothelial growth factor and matrix metalloproteinase 2 by the endothelial cells(11,12). There is reduced synthesis of nitric oxide and increased production of endothelin resulting in vasoconstriction. Within the neurons, AGEs stimulate production of inflammatory cytokines increasing oxidative stress and tissue damage (13). These processes affect cerebral blood flow and cause neuronal damage.

Ferguson *et al* carried out a cross-sectional study in Scotland in 2005, examining the effects of early onset of T1DM on cerebral structure and cognitive function (14). The study also assessed the potential correlates of cognitive differences in the 2 groups, including age of onset of diabetes, duration of diabetes, preceding severe hypoglycaemia and presence of retinopathy.

A total of seventy-one children with T1DM were recruited and classified as having either Early Onset Diabetes (EOD; n = 26) developing before age seven years, or Late Onset Diabetes (LOD; n=45) developing between seven and seventeen years. Both groups were subjected to neuropsychological testing and MRI brain scans. Study results showed that children with EOD performed poorer than those with LOD in tests of intellectual ability. MRI scans also demonstrated that lateral ventricle volumes were 37% greater with higher prevalence of peri-ventricular atrophy (61 vs. 20% p=0.01) in EOD compared with later onset Type 1 diabetes. The factor most consistently associated with lower scores on cognitive tests were age of onset of T1DM, with children developing diabetes earlier than seven years of age more likely to have poorer scores, regardless of diabetes duration. The authors concluded that early age of onset of diabetes was associated with a greater decline in cognitive function, and that the changes in brain structure were a result of diabetes.

A similar study was carried out in Turkey by Tolu-Kendir *et al* looking at the relationship between metabolic control and neurocognitive function in children diagnosed with T1DM before and after 5 years of age(6). This was a case-control study with 60 children with T1DM further grouped as EOD < 5 years and those with onset at later than 5 years; and 40 age and gender matched controls. Diabetic children were found to have poorer scores in cognitive tests and specifically in visual perception, short term memory and selective attention. Those with early age of onset and poor glycaemic control had significantly poorer results.

These studies emphasize the importance of early diagnosis and good glycaemic control in delaying and minimizing damage to the developing brain and preserving cognition.

Dysglycaemia and cognition

Hypoglycaemia in diabetic children is defined as blood glucose < 3.9 mmol/l (10). It may occur due to ingesting insufficient calories, administration of inappropriately high levels of exogenous insulin or excessive exercise. A fall in blood glucose below this level stimulates production of counter-regulatory hormones including catecholamines, cortisol, glucagon and growth hormone.

Frequent hypoglycaemia lowers the threshold at which hypoglycaemic symptoms manifest, resulting in blunting of the hypoglycaemic response, termed 'hypoglycaemic unawareness.' This has been demonstrated to be influenced by the duration of diabetes(15), and leads to more frequent and more severe hypoglycaemic episodes.

Hypoglycaemia has been shown to affect memory and lead to structural changes in the brain white matter (16). The frontal and temporal regions especially in the left hemisphere, which are involved in language, memory and attention, are particularly affected by abnormally low blood glucose.

Perantie *et al* carried out a study in 2010 on 95 youth with T1DM and 49 sibling controls aged 7 to 17. Patients with T1DM were categorized as having 0, 1-2 or 3+ episodes of severe hypoglycaemia. MRI brain scans revealed that greater exposure to hypoglycaemia was associated with larger hippocampal volumes and those with 3+ hypoglycaemic episodes had the largest volumes after

controlling for age of diabetes onset (17). This enlargement may reflect damage to the developing brain such as reactive gliosis or disruption in pruning.

Hyperglycaemia is defined as blood glucose levels > 11.1 mmol/l. Insulin deficiency leads to impaired glucose utilization, impaired glycogenesis and uninhibited gluconeogenesis. When renal glucose threshold is exceeded, it results in glycosuria with osmotic diuresis and dehydration. Impaired glucose utilization with stimulation of counter-regulatory hormones leads to further stimulation of gluconeogenesis, ketonaemia and acidosis.

A study carried out at the University of Cambridge in 2012 examined the variation between countries in frequency of DKA as the first presentation in T1DM. (18). This was a systematic review of 65 study cohorts comprising >29,000 children in 31 countries. Frequency of DKA as the diagnosis at 1st presentation ranged from 12.8% - 80%; and was highest in developing countries, and lowest in Sweden and Canada.

In our set-up, due to a low index of suspicion, many diabetic children are misdiagnosed as having gastroenteritis, malaria or pneumonia. The resulting delay in diagnosis exposes the children to prolonged unmanaged episodes of hyperglycaemia and impaired cognition.

In a study done in the U.S. by Gonder-Fredrick *et al*, 2009, both hypo and hyperglycaemia were shown to affect cognition. This was a field study where 61 children aged 6-11 years with T1DM were subjected to two brief cognition tests just before home blood glucose readings. Time to completion of mental math problems and reaction time was slower in hypo compared to euglycaemia. In addition, blood glucose levels <3.0 mmol/l or >22.2 mmol/l caused equivalent decline in performance in mental math (19).

Management of children with diabetes requires good parental education, with emphasis on recognition of symptoms of hypo and hyperglycaemia. This will prevent or delay onset of cognitive decline.

Findings in other studies are summarized below:

Studies on Cognitive function in children with Type 1 Diabetes

STUDY	COUNTRY	TYPE OF STUDY	SAMPLE SIZE	TITLE	OUTCOME
Kaufmann et al, 1999 (20)	USA	Case control	52 T1DM 15 age matched siblings	Neurocognitive functioning in children diagnosed with diabetes before age 10 years.	Neurocognitive function comparable to controls, but affected by occurrence of hypoglycaemic seizures
Hannonen, et al 2003 (21)	Finland	Case control	11 T1DM with h/o severe hypoglycaemia, 10 T1DM without h/o severe hypoglycaemia, 10 healthy controls	Neurocognitive functioning in children with type-1 diabetes with and without episodes of severe hypoglycaemia	Children with reported episodes of severe hypoglycaemia had more neuropsychological impairments
Perantie, D. C et al, 2008 (22)	USA	Case control	117 T1DM aged 5-16 ; 58 non-diabetic sibling controls	Effects of prior hypoglycemia and hyperglycemia on cognition in children with type 1 diabetes mellitus.	T1DM had lower verbal intelligence. Within T1DM, verbal intelligence was reduced in those with hyper, not hypoglycaemia
Naguib, J M et al 2008 (23)	UK	Meta-analysis	A meta-analysis of 24 studies published between 1980 and 2005	Neuro-cognitive performance in children with type 1 diabetes--a meta-analysis.	Children with T1DM have mild cognitive impairment
Asvold, BO et al, 2010 (24)	Norway	Case control prospective study	28 diabetic children 28 age matched controls followed up 16 yrs	Cognitive function in type 1 diabetic adults with early exposure to severe hypoglycaemia: a 16-year follow-up study	Early exposure to severe hypoglycaemia has lasting effects on cognition

Anderson, M. et al, 2011 (25)	Australia	Prospective follow-up study	33 T1DM 32 controls	Neurocognitive outcomes in young adults with early-onset type 1 diabetes: a prospective follow-up study.	There was no difference in IQ scores in the two groups; differences noted in memory subtests
Zhongguo, et al, 2012 (26)	China	Case control	32 T1DM 32 healthy gender matched controls	Cognitive function in children with type 1 diabetes	T1DM may reduce verbal intelligence quotient and overall IQ. HBa1C is an independent risk factor

Cognitive development is affected by a wide range of factors including chronic illnesses like diabetes; and environmental factors such as socio-economic status, level of education of the parents and provision of a stimulating and emotionally safe home environment.

A study carried out in France in 1998 examined factors associated with glycaemic control in 2,579 French children with T1DM. The study identified the quality of family support and dietary compliance as contributory factors (27). In study done by Puri, *et al* in India in 2013, significantly lower cognitive scores were associated with a recent diagnosis, low socio-economic status, and higher levels of HBA1C.(28)

Al-Odayani *et al* carried out a study in Saudi Arabia in 2013 that assessed the relationship between glycaemic control and the mothers' level of education and knowledge. There was a significant association between mothers' level of education and glycaemic control (29), suggesting that higher levels of knowledge led to better glycaemic control. In a study carried out in Turkey, younger maternal age, higher paternal level of education and fewer siblings were found to be negatively associated with poor metabolic control (30).

Chronic complications of diabetes have been studied extensively. There is now evidence to show that Type 1 diabetes has significant effects on the developing brain and may lead to impaired cognition, lower performance on tests of cognition and may prevent the child with T1DM from achieving their full intellectual potential. This study intends to assess and highlight the effects of diabetes on cognitive function, and examine possible associations between

cognition in diabetic children and other factors such as age at diagnosis, duration of illness, socio-demographics and glycaemic control.

The Modified Mini Mental State examination will be used to assess cognitive function in all study subjects. It has been widely used as both a clinical and research tool in developed countries including the USA and UK, as well as developing nations like India, Kenya, Ecuador and South Africa. Several studies have been carried out on the MMSE to investigate its use as an instrument for testing cognitive function.

A study was carried out in Australia in 1993 by Ouvrier *et al*, (31). The MMSE was administered to 115 patients aged 4 to 15 years in a paediatric out-patient setting. The test took 5 – 20 minutes to administer. Highly significant correlations were found between the MMSE and chronological age, reading age and mental age and the authors concluded that the MMSE is a suitable tool for screening higher mental function in children at the age of 4 years and above, and can readily be incorporated into the neurological examination of children.

Rubial-Alvarez S., Machado M.C *et al* carried out a study in a population of Spanish children in 2007, to analyse results of the MMSE and assess the usefulness of the instrument as a cognitive screening tool for children's development. The authors also aimed to assess the relationship between MMSE scores and the intelligence quotient of the children. One hundred and eighty-one children were studied, and both tests of Intelligence Quotient (IQ) and the MMSE were administered. Scores on the MMSE were found to correlate significantly with their chronological and mental ages (32). The sensitivity and specificity of the MMSE was also assessed in Indian children in 2005 by Jain M. and Passi G. The MMSE was administered to 50 subjects without neurological illness and 50 subjects with neurological illness due to varied aetiologies. The test was administered by 2 observers at admission and repeated four days later. The test took an average of eight minutes and no inter-observer variability was found. The MMSE was found to identify children with poor outcome with a sensitivity and specificity of 68% and 100% respectively(33).

The MMSE was evaluated by Lancu I. and Olmer A. at the University of Tel Aviv in 2006. The authors reviewed seven studies previously done on the MMSE. They concluded that the validity construct of the test is considered good. The main disadvantage was found to be difficulty in identifying mild cognitive impairment and specificity was found to be between 80 and

100%(34). The MMSE has been used in Kenya, (35) by Khasakhala *et al*, to assess cognitive function in youths aged 13 to 22 years suffering from depression and was able to effectively identify those with moderate to severe cognitive dysfunction.

2. STUDY JUSTIFICATION

Among the children who are diagnosed to have diabetes, blood sugar control remains suboptimal, partly due to poor patient self management skills and lack of self-care equipment to monitor blood sugar. Cognitive dysfunction is a recognized complication of diabetes that is often overlooked, both while giving the patient diabetes education, and in mainstream learning.

While long-term vascular complications have been studied both internationally and locally, the effects of dysglycaemia and poor glycaemic control on intellect and cognition in diabetic children has not been studied in Kenya. Quantifying and increasing awareness of the cognitive dysfunction suffered by diabetic children will help the health care professional, patients and their care givers to better manage the condition.

Locally, children diagnosed to have conditions that negatively affect cognition such as attention deficit and hyperactivity disorder are given extra time during national examinations. This study sought to compare the cognitive function of diabetic children with their non-diabetic counterparts and explore the association between cognitive function, duration of diabetes and glycaemic control. Findings from the study would be useful to help diabetic children optimize their learning potential.

3. OBJECTIVES

3.1. Primary Objective

- To compare the cognitive function of children with Type 1 Diabetes mellitus at Kenyatta National Hospital with non-diabetic children using the Modified Mini Mental State Examination.

3.2. Secondary Objective

Within the population of children with Type 1 diabetes mellitus:

- To determine the association between cognitive function and glycaemic control (HbA1C) in diabetic children

- To determine the association between duration of diabetes and cognitive function

4. METHODOLOGY

4.1. Study Design

This was a hospital based cross sectional study with a comparative arm, evaluating the cognitive function of diabetic children compared to non-diabetic children.

4.2. Study Site

This study was conducted in Kenyatta National Hospital (KNH), which is the National Referral Hospital and Teaching hospital for the University of Nairobi Medical School. It is located in the Kenyan capital city Nairobi. KNH serves as one of the two national (tertiary) referral hospitals in Kenya and receives patients from all other hospitals in the country and neighboring East African countries. It also serves non-referral patients mainly from the capital city and the surrounding counties. There are four paediatric wards with a capacity of 240 beds with the occupancy often over 100%. KNH records over 11000 paediatric admissions in a year. The paediatric endocrinology clinic was started in 2008. Approximately 500 patients with T1DM are followed up in the clinic, drawn from Nairobi and the surrounding environs. Diabetic patients aged 23 years and below are followed up in this clinic where free insulin is provided. Patient records are kept in manual paper files, including clinical notes and laboratory results.

4.3. Study Population

The study included diabetic children aged 7 to 16 years recruited from the out-patient endocrinology clinic and children aged 7 to 16 years recruited from the paediatric out-patient clinic. The paediatric endocrinology clinic runs every Tuesday, with about 15 children followed up every clinic day.

4.4. Inclusion criteria

For diabetic children:

- Children with diabetes aged 7 to 16 years, currently managed as out-patients

For the comparative group:

- Children aged 7 to 16 years followed up in the Kenyatta National Hospital out-patient clinics

4.5. Exclusion criteria

Participants meeting the following criteria were excluded from the study:

For diabetic children:

- Hypoglycaemia – Random blood sugar at that clinic visit < 2.2 mmol/l - were unable to be assessed due to symptoms of neuroglycopenia
- Hyperglycaemia – Random blood sugar at that clinic visit > 15 mmol/l – due to need for further tests to diagnose and manage DKA
- Impaired conscious level
- Other chronic illness – as identified from medical records, history obtained from the patient, parents/caregivers, and clinical examination
- History of developmental disorder as defined by the WHO Reporting Questionnaire for Children **(Appendix II)**
- History of head trauma
- Failure to give consent

For the comparative group

- Diabetes – Random blood sugar at that clinic visit > 11.1. mmol/l
- Hypoglycaemia – Random blood sugar at that clinic visit < 2.2 mmol/l
- Any chronic illness - as identified from medical records, history obtained from the patient, parents/caregivers, and clinical examination
- Neurosurgical conditions
- History of developmental disorder as defined by the WHO Reporting Questionnaire for Children **(Appendix II)**
- History of head trauma
- Failure to give consent

4.6. Study Period

The study was carried out over 5 months, from August to December, 2014.

4.7. Study Tools

Mini Mental Status Examination (Appendix I)

The cognition of patients meeting inclusion criteria was assessed using the modified Mini Mental Status examination. The mini mental status examination was developed by Folstein in 1975. It is a brief 30 point questionnaire test that is used to screen for cognitive impairment. It can also be used to estimate

severity of cognitive impairment and follow the course of changes in cognition over time. The test, which takes 5-10 minutes to administer, examines 5 domains: orientation, immediate and short term memory, attention and calculation, language and praxis (31).

The tool is scored as follows:

Domain: Orientation	**Score**
Orientation in time	4
Orientation in place	4
Orientation in person	4
Total score for orientation	12
Domain: Attention and Concentration	7
Domain: Registration and Sensory Perception	3
Domain: Recall	3
Domain: Language and Praxis	12
TOTAL SCORE	37

The principal investigator administered the test and recorded the subtotal and overall score for each study subject.

The WHO Reporting Questionnaire for Children (RQC) (Appendix II)

The Reporting Questionnaire for Children was developed by the WHO as part of a collaborative study involving 7 countries, including Kenya, Algeria and South Africa (36). It is a 10 item instrument tailored to identify developmental disability, and significant degrees of emotional, behavioural and psychotic disorders. The instrument is designed to be used in children aged 5 to 16 years.

It was used to exclude children with the above disorders which would be associated with impaired cognition. Children found to have developmental disability; emotional behavioural or psychotic disorders were referred to the Child Psychiatrists in the Patient Support Center.

4.8. Sample Size Estimation

Using the formula for comparison of two proportions

$$n = \frac{\{Z_\beta \sqrt{[\pi_1(1-\pi_1) + \pi_0(1-\pi_0)]} + Z_{\alpha/2}\sqrt{[2\bar{\pi}(1-\bar{\pi}]}\}^2}{(\pi_0 - \pi_1)^2}$$

Where $\bar{\pi} = \frac{\pi_1 + \pi_0}{2}$

n = required minimum sample size

π_1 = the proportion of diabetic children with cognitive dysfunction

π_0 = the proportion of non-diabetic children in the comparative group with a cognitive dysfunction

Using a study by Imam, I et al, 2003(37), and assuming the difference in the two groups to be 20%; π_1 = 88.6%; π_0 = 68.6%

Z_β = one-sided percentage point of the normal distribution corresponding to 100%-the power. For this study power was set at 80%, (100%-80%) = 20% and Z_β = 0.84

$Z_{\alpha/2}$ = percentage of the normal distribution corresponding to the required (two-sided) significance level set at 5% in this study, therefore $Z_{\alpha/2}$ = 1.96

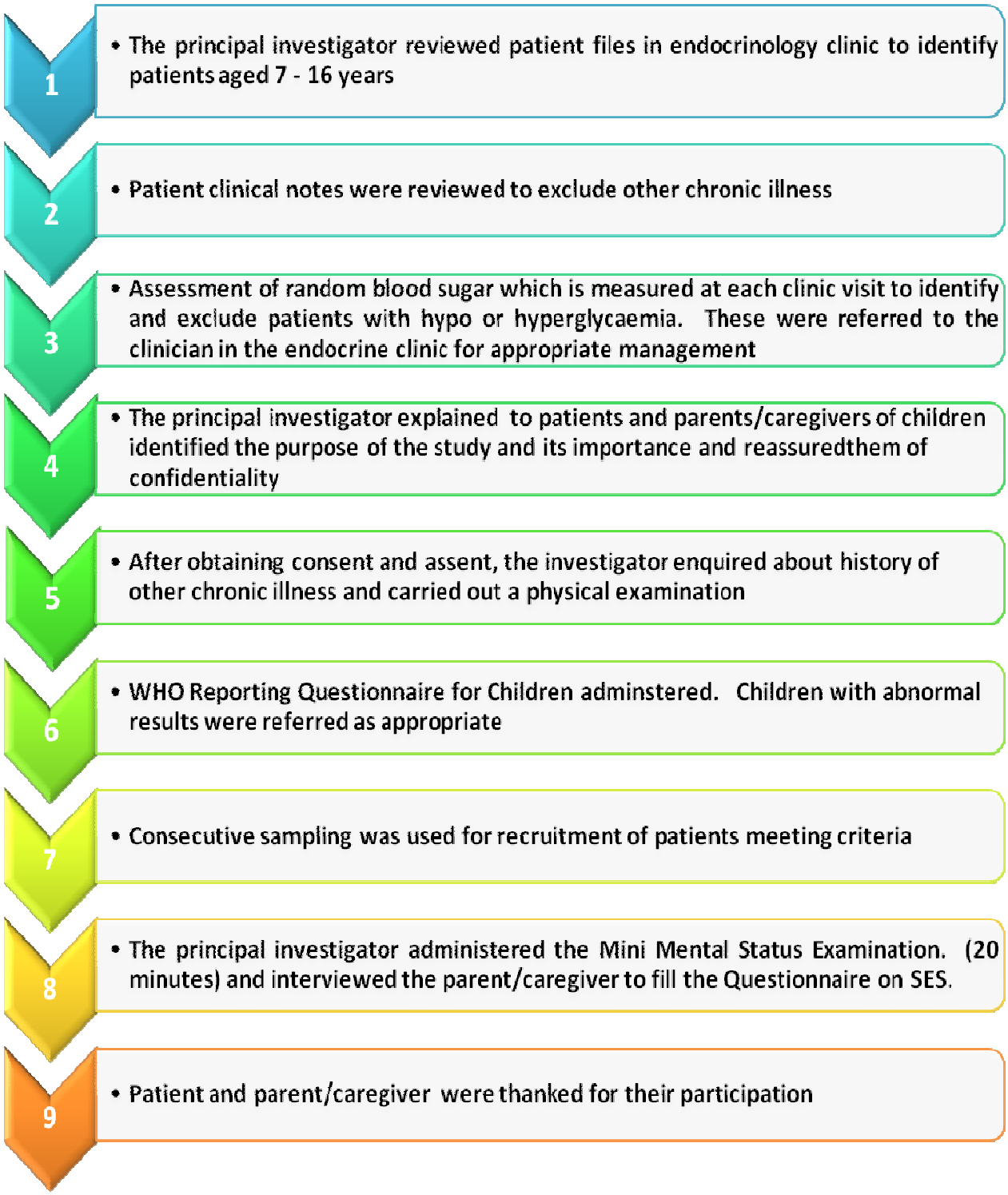
$$n = \frac{\{0.84\sqrt{[0.886(1-0.886) + 0.686(1-0.686)]} + 1.96\sqrt{[2\times 0.786(1-0.786]}\}^2}{(0.686 - 0.868)^2}$$

n = 66 children per group

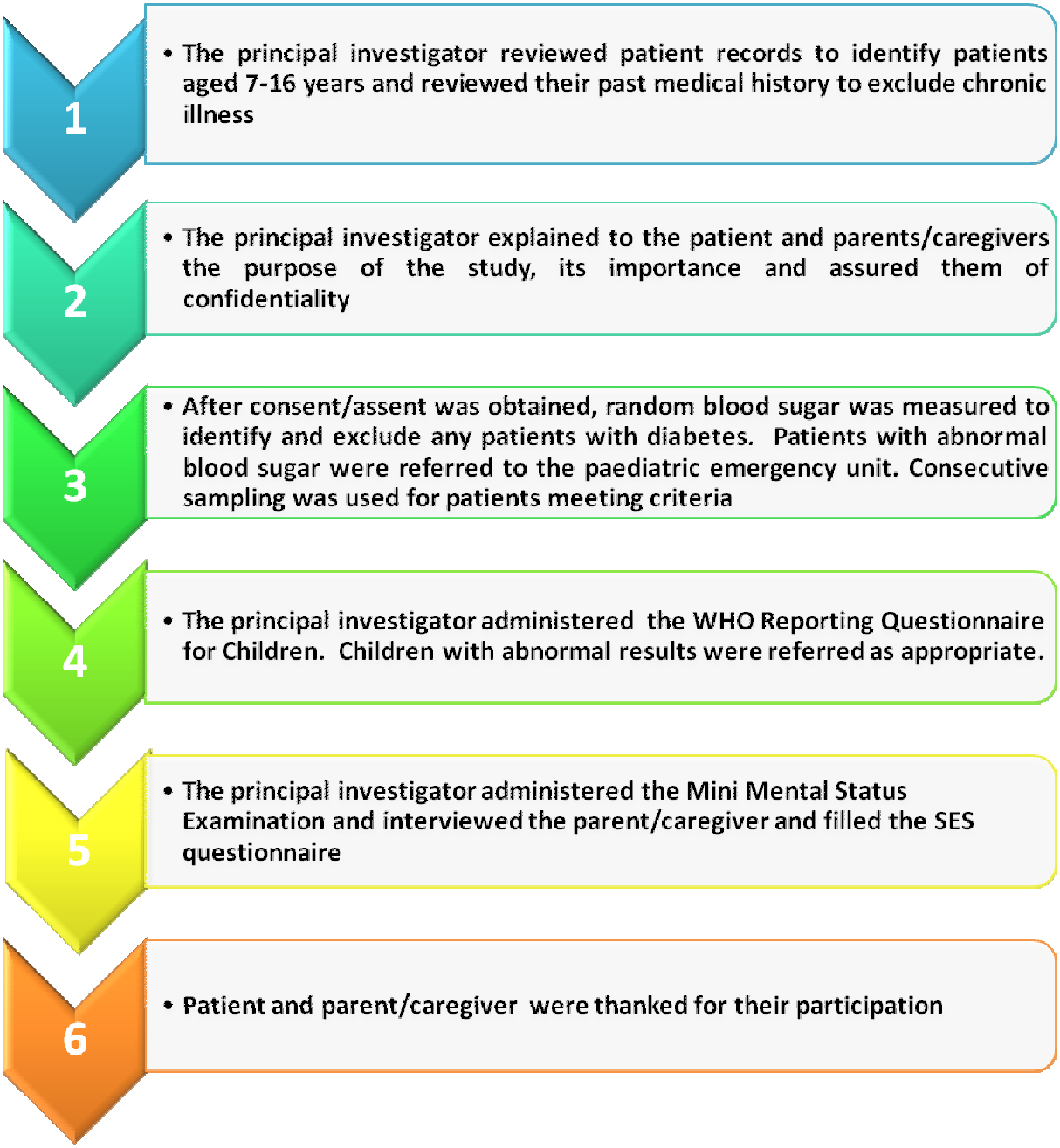
5. DESCRIPTION OF THE FLOW OF RESEARCH

5.1. STUDY DIAGRAM

DIABETIC CHILDREN



COMPARATIVE GROUP



5.2. Recruitment

Study subjects were recruited from the paediatric endocrinology clinic. Patients with Type 1 Diabetes Mellitus were identified from the patient records. The patient files were reviewed to identify children aged seven to sixteen years with no history of other chronic illness, including cardiac disease, renal disease and asthma. Blood sugar was measured by the nurse during each visit and any children with hypoglycaemia (Random blood sugar < 2.2 mmol/l) or hyperglycaemia (Random blood sugar > 15 mmol/l) were excluded from the study. These children were referred to the clinician for immediate intervention.

The principal investigator identified those meeting the age and blood sugar criteria, and explained the purpose of the study to the patient and their parents/guardians. The 10 point WHO Reporting Questionnaire for Children (RQC) **(Appendix II)** was administered to identify any children with developmental disorders. These were excluded from the study and referred to the child psychiatrist in the Patient Support Center. There was consecutive recruitment of study subjects meeting the inclusion criteria.

The comparative group was recruited from the paediatric out-patient clinics. Patients aged 7 to 16 were selected, and there was no matching for age or gender. Patient files were reviewed to exclude chronic illness – head injury, cardiac disease, renal disease or asthma. The principal investigator explained the purpose of the study to the patient and parents/caregivers. After explaining the procedure and obtaining consent, random blood sugar was measured using finger-prick samples, glucostix and a One-touch Glucometer. Patients with abnormal blood glucose were referred to the Paediatric Emergency Unit.

The 10 point WHO Reporting Questionnaire for Children was administered to identify any children with developmental disorders. These were excluded from the study and referred to the patient support centre as appropriate.

The investigator ensured privacy and non-disruption of services by using one of the consultation rooms in the clinics while interacting with the patient and their parents/caregivers.

5.3. Consent administration

The patients and their parents/caregivers were given detailed information by the principal investigator about the study and why it is important. They were informed that there were no direct benefits and the study is risk free except for their time and pin prick pain for sample collection (**Appendix V**).

Informed consent (and assent for children older than 7 years) was be obtained from parents/caregivers of children who met the inclusion criteria (**Appendix IV**)

Consent was voluntary and free from coercion.

5.4. HBA1C

A sample was collected for current $HbA1_C$ (if not available) to measure glycaemic control over a period of 3 months. Procedure for measuring HbA1c levels (**Appendix V**)

5.5. Socio-demographic questionnaire:

The principal investigator interviewed parents/care givers and fill the socio-demographic data section (**Appendix III**)

5.6. Mini- Mental State Examination

After identifying those meeting inclusion criteria, the principal investigator administered the MMSE. **(Appendix I)**

All patients were offered standard medical care whether they agreed to participate in the study or not.

5.7. Primary and Secondary outcomes

The primary outcome of this study that was assessed was the score on the mini Mental State Exam (MMSE) for diabetic children compared to the non-diabetic comparative group.

The secondary outcomes investigated within the group of children with type 1 diabetes were the relationship between scores on the MMSE; and duration of diabetes and glycaemic control as assessed by HBA1C.

6. DATA MANAGEMENT

6.1. Data collection

Diabetic children aged 7-16 followed up in the paediatric endocrinology clinic were assessed by the principal investigator. After seeking consent, the parents/guardians of eligible children were interviewed. A questionnaire was administered to determine the socio-demographics as outlined in Appendix III.

The random blood sugar was measured using finger-prick samples, glucostix and a One-touch Glucometer. Glycated haemoglobin was measured. Finger-prick samples were collected using sterile technique through collection of finger-prick samples and analysed using a Bayer DCA 2000+ machine for measuring glycated haemoglobin. All participants were screened for developmental disorder using the WHO criteria (Appendix II). The Mini Mental State Exam (Appendix I) was administered to each child and the results recorded.

The comparative group was recruited from the paediatric out-patient clinics and enrolled if they fulfil all inclusion and exclusion criteria. Consent was sought and the parents/guardians interviewed to determine their socio-demographic characteristics as outlined in Appendix III.

After measurement of blood glucose, the Mini Mental State Exam was administered to each child and the results recorded.

6.2. Data storage

All documentation was kept in the custody of the principal investigator. Questionnaires were stored in a locked cabinet. Electronic data were stored in the principal investigator's password protected lap-top, which was stored in a secure area when not in use. Paper based data did not have any patient names or information that would enable identification of the patient and was stored in a locked cabinet after collection, accessible only to the principal investigator.

6.3. Data Analysis

Data were entered into an SPSS database, cleaned and stored in a password protected PC. Data analysis was done using SPSS version 18. Descriptive analysis using frequencies for categorical data was carried out. Cognitive

function was defined using age specific cut-offs of MMSE scores. Association between cognitive function and diabetes was measured based on chi squared test. Logistic regression was used to obtain adjusted odds ratios of association between cognitive function and patient factors including diabetes status, duration of diabetes and level of glycaemic control.

7. ETHICAL CONSIDERATIONS

7.1. Confidentiality

This research was conducted in accordance with all the Kenyan laws and regulations that protect rights of human research subjects. All records and other information obtained were kept strictly confidential. All data collection tools were identified by number or otherwise coded to protect any information that could be used to identify the study subjects.

7.2. Ethics and Research Committee approval

This protocol was reviewed and approved by the Ethics and Research Committee. A letter of protocol approval by Ethics and Research Committee was obtained prior to the commencement of the study.

7.3. Protection of Health Records

Health records were only accessed by the principal investigator. Questionnaires used did not have the patient's name or file number but were serialized using a participant identification number. All written study materials were kept in a locked secure cabinet. Electronic records were stored on a password protected lap top.

7.4. Children with conditions identified during screening

Children found to have chronic conditions during review of their patient records and physical examinations were referred to the appropriate clinics. Patients found to have hypo or hyperglycaemia in the diabetic clinic were referred to the doctor in the endocrine clinic for management. Patients identified to have developmental delay using the WHO Reporting Questionnaire for Children were referred to Patient support services/Child Psychiatrist for further evaluation. Children with hypo or hyperglycaemia in the out-patient clinic were referred to the Paediatric Emergency Unit for management.

8. RESULTS

During the study period between September and December 2014, a total of 66 children with Type 1 diabetes were recruited from the Paediatric Endocrine clinic. Sixty-seven children without diabetes were enrolled from paediatric out-patient clinics to form the comparative group.

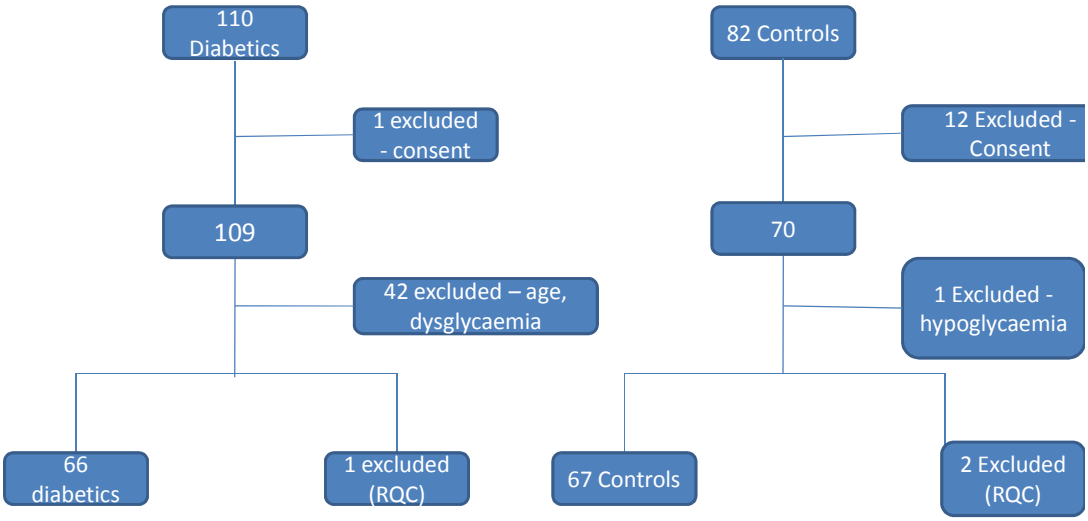


Figure 1: Flow Chart of participant recruitment

One-hundred and ten diabetic children were reviewed. Of these, 44 were excluded. One was excluded due to refusal to consent, four due to blood sugar below 3.0 mmol/l requiring immediate treatment due to symptoms of neuroglycopenia and two due to high blood glucose that could not be recorded; and needed evaluation for DKA. Thirty-six were excluded due to age less than seven or older than sixteen. One child was referred to the Patient Support Center after screening using the WHO Reporting Questionnaire for Children. After exclusion of these ineligible children, 66 diabetic children were enrolled into the study.

A total of 82 children were reviewed in the paediatric out-patient clinic. Twelve caregivers refused to give written consent, and one patient was excluded due to hypoglycaemia. Two patients were referred to the Patient Support Center after screening using the WHO Reporting Questionnaire for Children. Out of the 82

children screened for eligibility in the comparative group, 67 children were recruited into the study.

Table 1 shows the demographic characteristics of the diabetic children and non-diabetic comparative group studied.

Table 1: Characteristics of children with Type 1 Diabetes and Non-diabetic comparative group

	Diabetic n (%)	**Non-diabetic n (%)**	**P value***
Child's gender			
Male	33(50.0)	28(41.8)	0.391
Female	33(50.0)	39(58.2)	
Child's age			
7 to 11 years	26(40.0)	31(46.3)	0.467
12 to 16 years	39(60.0)	36(53.7)	
Parental marital status			
Married	53(80.3)	61(91)	0.001
Unmarried	13(19.7)	6(8.9)	
Maternal age			
20 to 40 years	45(68.2)	49(73.1)	0.530
Above 40 years	21(31.8)	18(26.9)	
Maternal education level			
Primary school	20(15.2)	13(18.8)	0.008
Secondary school	26(42.4)	47(68.1)	
Tertiary	20(30.3)	7(11.6)	
Paternal age			
20 to 40 years	28(50.9)	28(45.2)	0.534
Above 40 years	27(49.1)	34(54.8)	
Paternal education level			
Primary school	3(4.5)	11(15.9)	0.008
Secondary school	25(37.9)	35(50.7)	
Tertiary	24(36.4)	18(26.1)	
Household income			
< Ksh 20000	29(43)	46(68.6)	0.005
>Ksh 20000	37(56)	21(31.3)	

* P value obtained from Chi square test

Median (lower - upper IQR) age of children with diabetes was 13 (10-15) years and the median age in non-diabetics was 12 (10-13) years. The majority of children in both groups were age between 12 and 16 years (60% of diabetics and 53.7% of non-diabetics). A total of 33 (50%) female diabetic children were recruited, while 39 (58.2%) females were recruited in the comparative group. There were no significant differences between the groups in terms of age (p=0.467) and gender distribution (p=0.391) of participants.

Parental age was similarly distributed for the groups of children with and without diabetes. Mothers of diabetics were mostly aged between 20 and 40 years n=45 (68.2%) as were most mothers of children without diabetes n=49 (73.1%), p value=0.530. Fifty percent of fathers of children with diabetes were aged 20 to 40 years while 45.2% fathers of the non-diabetic children were aged between 20 and 40 years, p=0.534.

The two groups differed in parental marital status, with eighty percent of the parents of diabetic children reporting that they were married compared to 91% of parents of non-diabetic children, p value = 0.001.

Most mothers in both groups had attained post-primary education; n= 46 (72%) for mothers of non-diabetic children compared with 54 mothers of non-diabetic children. (n=79%). However, more mothers of diabetic children had achieved tertiary education n=20 (30.3%), compared to mothers of non-diabetic children n=7 (11.6%).

Higher levels of paternal education were also reported among diabetic compared to non-diabetic children; 36% vs 26%; p = 0.008. Fifty six percent of diabetic children came from households with monthly incomes above Ksh 20,000 compared to 31.3% of children without diabetes who came from families with incomes above Ksh 20,000.

Analysis of the overall Mini Mental Status Examination Score

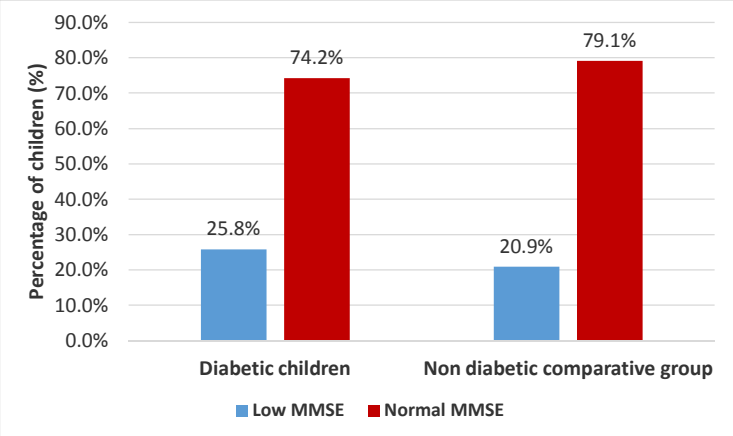
The primary objective of the study was comparison of scores on the MMSE in children with T1DM compared to non-diabetic children. An overall MMSE score was computed using cognitive performance data from the five sub-domains and applying age-specific cut-offs to classify overall cognitive function as normal or low MMSE scores. Seventeen out of the 66 diabetics (25.8%) had low MMSE score compared to 14 (20.9%) non-diabetics (OR = 1.31, 95% CI 0.54-3.21). These are tabulated in Table 2 below.

Table 2: Mini Mental Status Examination Scores in Diabetic children compared to non-diabetic comparative group

	Diabetic children n (%)	Non-diabetic children n (%)	OR(95% CI)	P value*
Low MMSE	17(25.8)	14(20.9)	1.0	0.45
Normal MMSE	49(74.2)	53(79.1)	1.31(0.54-3.21)	

*P values obtained from Chi square test

Figure 2: MMSE scores for diabetic compared to non-diabetic children



There was no difference in the mean scores for the two groups with mean scores of 33 and 33.1 out of a possible 37 for diabetic and non-diabetic children respectively. Using the age specific cut-off for cognitive function, 53 (79.1%) non-diabetic children had normal cognitive functioning compared to 49 (74.2%) of the diabetic children (OR = 1.31, 95% CI 0.54 – 3.21).

Analysis of scores in sub-domains of MMSE in diabetic and non-diabetic children

Cognitive function was assessed within five separate areas of functioning for each participant and the resulting age specific scores were compared for diabetics and non-diabetic children. The findings of the analyses are presented below:

Orientation

The performance of diabetic and non-diabetic children on the orientation sub-domain (maximum score 12 points) of the Mini Mental Status examination that assesses orientation to time (4 points), place (4 points) and person (4 points) is presented in Table 3.

Table 3: Orientation scores of diabetic and non-diabetic children in KNH

Orientation score	Diabetic children			Non-diabetic children			P value*
	N	Mean	SD	N	Mean	SD	
6-8 years	7	10.8	1.2	9	10.4	1.5	0.604
9-11 years	20	10.7	1.1	22	11.1	0.9	0.224
12-14 years	39	11.6	0.5	36	11.6	0.6	0.877
All children	66	11.2	0.9	67	11.3	1.0	0.857

*P values obtained from T-test

The mean score for orientation was 11.2 (SD 0.9) out of a total score of 12 for the diabetic children compared to a score of 11.3 (SD = 1.0) in the non-diabetic comparative group. Older children aged 12-14 years had higher scores than the younger age groups, before correcting for the effect of age. There were no significant difference in orientation scores according to diabetic status for all children ($p = 0.857$) and within the three age groups (6-8 years, $p = 0.604$; 9-11 years, $p = 0.224$; and 12-14 years, $p = 0.877$).

Attention and concentration

Findings of analysis of attention and concentration sub-domain which had a maximum score of 7 points are presented in Table 4.

Table 4: Attention and concentration scores of diabetic and non-diabetic children at KNH

Attention and concentration score	Diabetic children			Non-diabetic children			P value*
	N	Mean	SD	n	Mean	SD	
6-8 years	7	5.7	1.0	9	5.3	1.2	0.593
9-11 years	20	5.5	1.3	22	5.6	1.2	0.725
12-14 years	39	6.2	0.8	36	6.3	0.7	0.785
All children	66	6.0	1.0	67	5.9	1.1	0.761

*P values obtained from T-test

Out of the possible maximum score of 7, the diabetic children in the different age groups had scores ranging from 5.5 to 6.2, while the scores for the non-diabetics ranged from 5.3 to 6.3. There were no significant differences in attention and concentration scores overall ($p = 0.761$), and by age group (p values > 0.05).

Registration and sensory perception

The cognitive performance of diabetic and non-diabetic children assessed by the ability to identify and name objects is summarized in table 5 (maximum score of 3 points).

Table 5: Registration and sensory perception scores of diabetic and non-diabetic children at KNH

Registration and sensory perception score	Diabetic children			Non-diabetic children			P value
	N	Mean	SD	n	Mean	SD	
6-8 years	7	3	NA	9	3	NA	NA
9-11 years	20	3	NA	22	3	NA	NA
12-14 years	39	3	NA	36	3	NA	NA
All children	66	3	NA	67	3	NA	0.966

*P values obtained from T-test

The mean score in both diabetic and non-diabetics was 3 (SD = 0.1) out of the maximum score of 3. Most children were able to register and perceive the information provided during the assessment and there was very limited variability in performance overall, within the different age groups and within the diabetic and non-diabetic group.

Recall

In the recall sub-domain of the MMSE a maximum score of 3 points was given based on the ability of children to recall three previously presented objects. The results from the assessment of this domain are shown in Table 6.

Table 6: Recall scores of diabetic and non-diabetic children at KNH

	Diabetic children			**Non-diabetic children**			
Recall score	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**P value**
6-8 years	7	2.7	0.5	9	2.1	1.3	0.332
9-11 years	20	2.1	1.1	22	2.2	1.2	0.705
12-14 years	39	2.5	1.0	36	2.8	0.5	0.078
All children	66	2.4	1.0	67	2.5	0.9	0.401

*P values obtained from T-test

The average scores for recall in the various age groups ranged from 2.1 to 2.7 in the diabetic group and 2.1-2.8 in the non-diabetic child age groups. Overall, there were no significant differences in recall between diabetic and non-diabetic children ($p > 0.05$). However, there was some evidence of higher scores for recall in non-diabetic children aged 12-14 years compared to diabetic children in the same age group ($p = 0.078$).

Language

The language sub-domain had a maximum score of 15, and the performance on the language domain was lower than that in the remaining domains. The findings of the analysis of the language domain are presented in table 7.

Table 7: Language scores of diabetic and non-diabetic children at KNH

Language score	Diabetic children			Non-diabetic children			P value
	N	Mean	SD	N	Mean	SD	
6-8 years	7	9.7	1.6	9	10.1	0.9	0.511
9-11 years	20	9.8	1.1	22	10.2	1.2	0.287
12-14 years	39	10.7	0.8	36	10.6	0.9	0.587
All children	66	10.3	1.1	67	10.4	1.0	0.835

*P values obtained from T-test

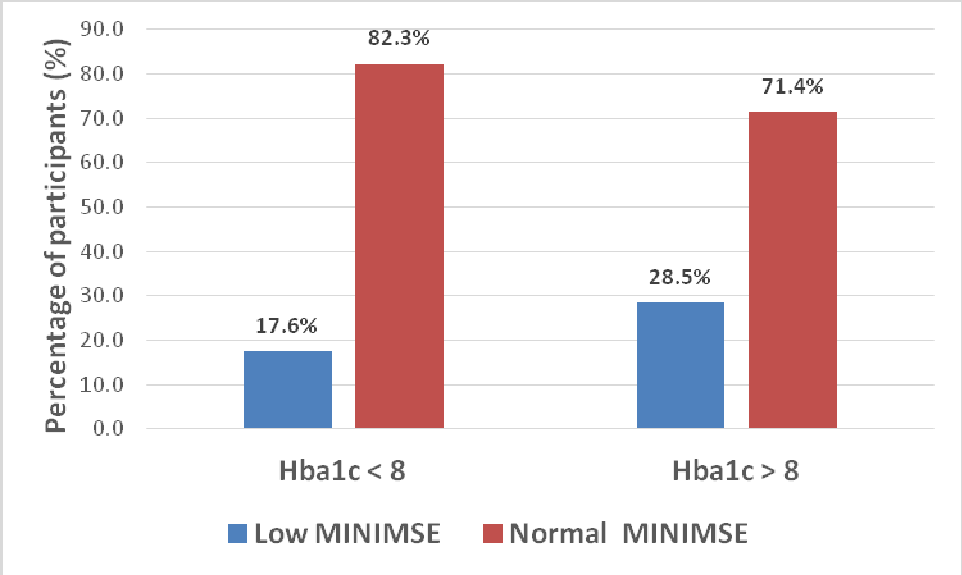
The mean scores for language ranged from 9.7 to 10.7 in the group of children with diabetes and from 10.1 to 10.6 in the non-diabetics compared to a maximum score of 15. There were no statistically significant differences in performance in the language sub-domain according to age group and diabetic or non-diabetic status.

The secondary objectives were to assess the relationship between scores on MMSE and glycaemic control and duration of diabetes in the diabetic sub-group of children. Findings are presented below.

Association between cognitive function and glycaemic control (HBA1C).

The diabetic subgroup was further studied to assess association between glycaemic control and MMSE scores. Glycaemic control was assessed using the HBA1C value over the previous 3 months. A total of 17 children out of the 66 (25%) has good glycaemic control, while 49 had HBA1C >8 (75%).

Figure 3: Relationship between MMSE scores and glycaemic control (HBA1C)

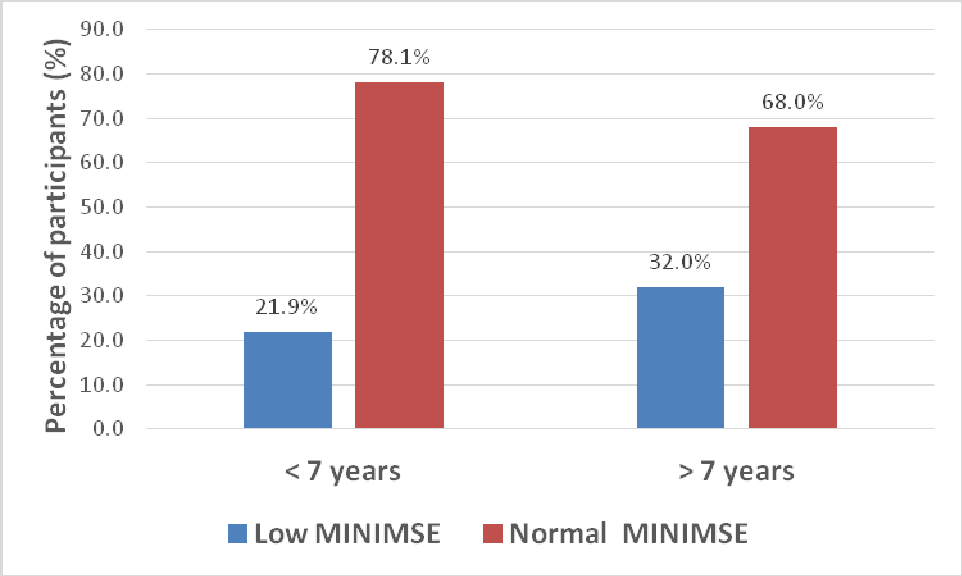


Of those with a good glycaemic control, 82.3% had normal MMSE (n=14), while 17.6% had low MMSE scores. (n=3). A total of 49 children had HBA1C level >8, and of these 28.5% had low MMSE scores. The difference between the 2 groups was however not statistically significant. (p=0.525)

Association between cognitive function and duration of diabetes.

In this study, the duration of diabetic diagnosis was not significantly associated with cognitive function. As shown in Figure 4 below, a total of 41 children had diabetes for < 7 years, while 25 % had diabetes for > 7 years.

Figure 4: Relationship between MMSE scores and duration of diabetes



Of those with diabetes for more than 7 years, 32% had low MMSE scores compared to 21% of those with diabetes for less than seven years. This difference was not statistically significant (p=0.365)

Adjusted Analysis

Adjusted analysis was carried out to control for differences in parental marital status, maternal and paternal level of education and level of income in the two groups. This is tabulated below:

Table 8: Adjusted Analysis

	Odds Ratio	P value	95% CI	
Non-diabetic	1.36	0.612	0.42	4.44
Unmarried parent	0.67	0.7	0.09	5.04
Patients age in years				
7 to 11 years	1.00			
12 to 16 years	0.70	0.002	0.57	0.87
Maternal education				
Primary education	1			
Secondary Education	0.31	0.233	0.04	2.14
Tertiary education	0.13	0.136	0.01	1.88
Paternal education				
Primary education	1			
Secondary education	0.21	0.129	0.03	1.57
Tertiary education	3.96	0.148	0.61	25.49
Paternal income (> Ksh 20 0000 per month)	1.61	0.433	0.49	5.28

After adjustment for the differences in demographic characteristics between the diabetic and non-diabetic groups, diabetic status did not predict MMSE scores (OR = 1.36; 95% CI 0.42-4.44).

Age was found to be significantly associated with mini MMSE scores with diabetic children aged 12-16 years having lower MMSE scores compared to their non diabetic peers (OR = 0.70, 95% CI 0.57-0.87; p=0.002).

9. DISCUSSION

In this hospital based cross sectional study of children with diabetes aged 7 to 16 years, the primary outcome of interest was the difference in cognition between children with Type 1 diabetes and those without diabetes as measured by the MMSE. A higher percentage of children with diabetes were found to have low scores on the MMSE compared to non-diabetic children. This difference was however, not statistically significant. (p=0.45).

In the sub-analysis of the different domains of cognitive function, there were no significant differences in scores in 4 sub-domains; however, diabetic children aged 12 to 14 years were found to have lower scores in recall (mean 2.5) compared to non diabetic children, (mean 2.8). Although this difference was not statistically significant p= 0.78, diabetes has been shown to affect recall in particular, especially in children who have early onset diabetes (6) which may account for this association.

The study also sought to assess the association between cognitive function and glycaemic control among children with T1DM. In this study, 75% of the children had poor glycaemic control (n=49). This is consistent with other local studies; in a study conducted by Ngwiri *et al* between May and October, 2003 in 3 out-patient clinics in Nairobi, prevalence of poor glycaemic control was high at 72%. Adolescents were found to be at particular risk for poor control.

In this study, 28.5% of children with HBA1C >8 were found to have low scores on the MMSE compared to 17% of children who had HBA1C <8%. Comparing this to studies carried out in other countries, Tolu-Kendir *et al* in Turkey (6) found that diabetic children with poor glycaemic control had poorer scores in visual perception and short term recall. While the difference in the two groups was not statistically significant in this study, (p=0.525); it informs the need to perform a baseline assessment on diabetic children at diagnosis, and periodically repeat the assessment to recognize decline in a particular child early.

No significant association was documented between poor scores on the MMSE and duration of diabetes. This is contrary to studies which have shown that children who have had diabetes for longer than 7 years are at greater risk of both structural and functional changes in brain function (6,14).

After adjustment for the differences in demographic characteristics between the diabetic and non-diabetic groups, diabetic status did not predict MMSE scores (OR = 1.36; 95% CI 0.42-4.44).

Age was found to be significantly associated with mini MMSE scores with older children, aged 12-16 years having lower MMSE scores (p=0.002) compared to their non diabetic peers. This is similar to findings in other studies and may reflect the cognitive decline associated with a longer duration of diabetes.
Ferguson *et al* demonstrated in Scotland that earlier age of onset of T1DM (less than seven years) was associated with lower scores on neuropsychological tests (14).

Cognitive development is affected by a wide range of factors including environmental factors such as socio-economic status, level of education of the parents and provision of a stimulating and emotionally safe home environment (28). In study done by Puri, *et al* in India in 2013, significantly lower cognitive scores were associated with a recent diagnosis, low socio-economic status, and higher levels of HBA1C (28). Al-Odayani *et al* carried out a study in Saudi Arabia in 2013 that looked at the relationship between glycaemic control and the mothers' level of education and knowledge. There was a significant association between mothers' level of education and glycaemic control (29), suggesting that higher levels of knowledge led to better glycaemic control. In a study carried out in Turkey, younger maternal age, higher paternal level of education and fewer siblings were found to be negatively associated with poor metabolic control (30).

In order to minimize differences in the 2 groups in socioeconomic status, the study was conducted at Kenyatta National Hospital and both the diabetic patients and non-diabetic comparative group were drawn from this environment.

It was however found that since patients seen at the Paediatric Endocrine clinic are drawn from all over the country, including private facilities, there were differences in maternal level of education, paternal level of education, parental marital status and family income. Parents of diabetic children were found to have a higher level of education with 30.3% of mothers of diabetic children having attained tertiary education compared to 11.6% of mothers of non-diabetics. Fathers of diabetic children also reported higher levels of education, with 36% having tertiary education compared to 26% of fathers of non-diabetic

children. The difference in the 2 groups was statistically significant, p=0.008. Fifty six percent of diabetic children came from households with monthly incomes above Ksh 20,000 compared to 31.3% of children without diabetes who came from families with incomes above Ksh 20,000. The two groups also differed in parental marital status, with eighty percent of the parents of diabetic children reporting that they were married compared to 91% of parents of non-diabetic children, p value = 0.001.

These differences in socio-economic status between the 2 groups of children have a potential for confounding the results, by introducing selection bias.

In addition, the parents of diabetic children who were compliant and attending clinics were overall found to be pro-active when they felt their children's performance was below par, and some had changed schools to private schools where they felt their children's unique need would be met. The children with Type 1 diabetes also had greater interaction with health care staff, and regular seminars organized were organized during school holidays where they would have sessions on self-motivation, changes in adolescence and time management. Skills acquired in such forums may have helped them to adapt to, and minimize effects of the cognitive changes caused by dysglycaemia.

When selecting the comparative group, children with chronic or severe illness were excluded. However, any child being reviewed in the clinic for acute illness may not be the ideal control.

The Mini Mental State Exam (MMSE) does not assess all cognitive domains (executive functioning, information processing) well and can only provide a gross estimate of cognitive capacity. More importantly, if the patient was pre-morbidly bright, the tasks involved in the MMSE may be too easy. Paediatric diabetes generally relates to mildly lower cognitive scores across most cognitive domains and cognition is greatly affected by the child's environment. While the test did not show any significant differences between the 2 groups, non-diabetic children were overall found to have slightly higher scores on the MMSE.

10. STUDY LIMITATIONS

- The 2 groups differed in socio-economic status, parental education and parental marital status, with parents of diabetic children having higher level of education and income compared to the comparative group. These factors have been found to affect cognition and this has the potential for confounding the results.
- The Mini Mental Status Examination has not been used specifically in diabetic patients in developing countries.
- The Tool requires the subject to understand and follow instructions. Thus, it can only be administered to children of school going age.
- The comparative group chosen for the study was drawn from the hospital and may thus not be representative of normal, well children.

11. CONCLUSIONS

- Though there were differences on the overall MMSE scores, more diabetic children achieving low MMSE scores, the difference in the two groups was not statistically significant.
- Within the population of children with Type 1 diabetes, there was no association between scores on the MMSE and duration of diabetes or glycaemic control.

12. RECOMMENDATIONS

- Serial assessments of diabetic children may be more useful than a single measurement.
- Children with low scores on the MMSE need close follow-up, and those who are exam candidates may benefit from having extra time allocated to them.

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13. APPENDICES

13.1. APPENDIX I - MODIFIED MINI MENTAL STATUS EXAMINATION

A. ORIENTATION – 1 point for each correct answer

	CORRECT ANSWER – 1	WRONG/NO ANSWER - 0
GENDER		
FIRST NAME		
LAST NAME		
RECOGNIZES RELATIVE		
TOTAL		
PLACE		
CITY		
COUNTY		
COUNTRY		
TOTAL		
DAY		
DATE		
MONTH		
YEAR		
TOTAL		

B. ATTENTION AND CONCENTRATION

Recite a minimum of 2 and a maximum of 5 digits forward

DIGITS RECITED	SCORE
NONE	0
1	0
2	
3	
4	
5	

Score 1 point for each number greater than 2, total score 4

Recite a minimum of 2 and a maximum of 4 digits backward

Score 1 point for each number greater than 2, total score 3

C. REGISTRATION AND SENSORY PERCEPTION

Identify 3 objects by name

Score 1 point each, total score 3

D. RECALL

Recall 3 objects previously presented

Score 1 point each, total score 3

E. LANGUAGE

Name 5 body parts

Score 1 point each, total score 5

Follow a 3 step command,

Score 1 point each, total score 5

Repeat sentence, 'No ifs, ands or buts'

Total score 1

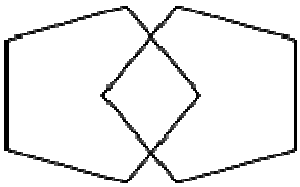
Reading his or her name

Total score 1

Writes his or her name

Total score 1

Copy a design



Total score 1

SCORES:

AGE GROUP	CUT-OFF
3-5 YEARS	24
6-8 YRS	28
9-11 YRS	30
12-14 YRS	35

13.2. APPENDIX II - WHO REPORTING QUESTIONNAIRE FOR CHILDREN

1. Is the child's speech in any way abnormal? (retarded, incomprehensible, Stammering)

   YES ☐ NO ☐

2. Does the child sleep badly?

   YES ☐ NO ☐

3. Does the child ever have a fit or fall to the ground for no reason?

   YES ☐ NO ☐

4. Does the child suffer from frequent headaches?

   YES ☐ NO ☐

5. Does the child run away from home frequently?

   YES ☐ NO ☐

6. Does the child steal things from home?

   YES ☐ NO ☐

7. Does the child get nervous or scared for no good reason?

   YES ☐ NO ☐

8. Does the child appear in any way backward or slow to learn compared with other children of about the same age?

   YES ☐ NO ☐

9. Does the child nearly never play with other children?

YES ☐ NO ☐

10. Does the child wet or soil himself?

YES ☐ NO ☐

Follow-up interview if one or more answer is yes.

13.3. APPENDIX III - SOCIODEMOGRAPHIC AND CLINICAL QUESTIONNAIRE

PARTICIPANT IDENTIFICATION NUMBER

AGE

GENDER

DATE

1. Parental Marital Status

Single	☐	Married	☐
Separated/Divorced	☐	Widowed	☐

2. Mother's age

15 – 20 yrs	☐	21 – 30 yrs	☐
31 – 40 yrs	☐	41 – 50 yrs	☐
>50 yrs	☐		

3. Mother's level of education

Primary school	☐	Completed Form 2	☐
Completed Form 4	☐	Technical training	☐
Bachelor's degree	☐	Master's degree	☐

4. Father's age

15 – 20 yrs	☐	21 – 30 yrs	☐
31 – 40 yrs	☐	41 – 50 yrs	☐
>50 yrs	☐		

5. Father's level of education

Primary school	☐	Completed Form 2	☐
Completed Form 4	☐	Technical training	☐
Bachelor's degree	☐	Master's degree	☐

6. Parental monthly income

< KSh. 3,000	☐	KSh. 3,000 – 5,000	☐
KSh. 6,000 – 10,000	☐	KSh. 11,000 – 20,000	☐
KSh. 21,000 – 30,000	☐	KSh. 31,000 – 40,000	☐
KSh. 41,000 – 50,000	☐	> KSh. 50,000	☐

Medical History

For Diabetic patients

7. Age at diagnosis

Less than 3 years	☐	3-4 years	☐
5-6 years	☐	7-8 years	☐
9-10 years	☐	> 10 years	☐

8. Duration since diagnosis of diabetes

Less than 1 year	☐	1 – 3 years	☐
4 – 6 years	☐	7 – 9 years	☐
10 – 12 years	☐	13 – 15 years	☐

9. Average HBa1C

< 6	☐	6-7	☐
8-9	☐	> 9	☐

10. Number of previous admissions

1	☐	2	☐
3	☐	4	☐
5	☐	> 5	☐

11 Type of medication (circle appropriately)

a)Insulin type…….. Dosage regimen……..

b) Who injects you? a) Self b) Parent c) Care giver

c) How many injections per day?

d) Oral hypoglycemic agent (specify)……

e) Have you been on alternative therapy? a) Yes b) No

Specify (herbal, nutritional supplements)……

12. Compliance to

Diet (do 24 hour recall)a)Yes b)No if no why? .....

Medication: a)Yes b) No if no why? .....

Follow-up: a)Yes b) No if no why? .....

13.Any other co-morbidity either related to diabetes or not

a)Central Nervous System(Retinopathy/Epilepsy/other)…….

b) Cardiovascular System (Hypertension/Stroke/ RHD/Other)……

c) Respiratory system (Asthma/Tuberculosis/other)……..

d) Gastrointestinal Tract (Duodenal Ulcers/Other……..

e)Genitourinary System (Renal disease/other)……

f) Musculoskeletal System (neuropathies/amputated/other)…….

g) Other medical /psychiatric conditions eg mental illness, HIV/AIDS……

……

14. Family history of chronic illness

15.Academic performance:

Before diagnosis:

Position in class …….. Total students in class…….

After diagnosis:

Position in class …….. Total students in class…….

For Surgical Patients:

16. Surgical diagnosis

17. Duration of illness

18. Intervention/Treatment received

13.4. APPENDIX IV - CONSENT AND ASSENT FORM

Study Number: ……. Hospital Number……..

Study Title: COGNITIVE FUNCTION IN CHILDREN AGED 7 TO 16 YEARS WITH TYPE 1 DIABETES AT KENYATTA NATIONAL HOSPITAL

Investigator: Dr. A. Macharia
Resident in Paediatrics and Child Health
Tel: 0722 726884

Supervisors: Dr. Lucy Mungai

Dr. Rachel Kang'ethe

Introduction: The purpose of this study is to assess the cognitive function in diabetic children and determine if there is a difference in cognition between children with Type 1 diabetes mellitus, and children who do not have Type 1 diabetes. The study also seeks to determine if cognition in diabetic children is affected by the level of control of diabetes.

The procedures to be undertaken in this study are:

- For diabetic children, a review of the patient's medical records to establish the diagnosis of diabetes, assess the HBA1C and exclude the presence of any other chronic medical illness
- For non-diabetic children, a review of the patient's medical records to exclude presence of diabetes or other chronic illness
- A structured questionnaire, the WHO Reporting Questionnaire for Children to exclude developmental disorders
- Random blood sugar results for the particular clinic visit will be reviewed for diabetic children to exclude hypo or hyperglycaemia
- For non-diabetic children, a random blood sugar will be done by obtaining a finger-prick sample
- A structured questionnaire will be administered to assess the past medical history and socioeconomic status

The mini-mental status exam is a structured tool that is used to assess cognitive function. This will be administered by the principal investigator and will take approximately 15 minutes

The information gathered will be used by the clinicians to improve the management of children with Type 1 diabetes. Any children identified to have cognitive disorders will also be assisted by sharing such information with their parents and teachers to determine the best way to ensure they fulfill their potential.

Risks: There will be no risks to you or your child during the study. There will be no harmful procedures carried out, and no discomfort except for pin-prick pain for collection of blood samples for random blood sugar and HBA1C where applicable. Refusal to participate will not jeopardize the treatment of your child in any way.

Voluntariness: The study will be fully voluntary. There will be no financial reward to you for participating in the study. You are free to participate or withdraw from the study at any point. Refusal to participate will not compromise the care of your child in any way.

Confidentiality: The information obtained about you, your child and your family will be kept in strict confidence. No information regarding you, your child or your family will be released to any person without your written permission. We will, however, discuss overall general findings regarding all children assessed, but nothing specific will be discussed regarding your child's condition. We will also not reveal the identity of you or your child during these discussions.

Problems or questions: If you have any questions about the study or about the use of the results, you can contact the principal investigator, Dr A. Macharia, on 0722 726884.

If you have any question on your rights as a research participant, you can contact the Kenyatta National Hospital Ethics and Research committee by calling 020 2726300 ext 44355.

I, _____being a guardian of _____name of child) have had the research information explained to me.

I AGREE/DISAGREE (cross out as appropriate) to participate/for my child to participate in the study.

I understand that our participation is fully voluntary and that I can withdraw my child from the study at any point and this will not affect my child's care in any

way. I have been given adequate opportunity to ask questions and seek clarification on the study and these have been addressed satisfactorily.

Participant/Guardian's signature: _____ Date: _____

I, _____ declare that I have adequately explained to the above participant/guardian the study procedure and risks and given him/her time to ask questions and seek clarification regarding the study. I have answered the questions raised to the best of my ability.

Investigator's signature: _____ Date: _____

ASSENT

Your parent/caregiver has agreed that you take part in our study where we are looking at the cognitive function of children aged 7 to 16 years with Type 1 diabetes.
By signing this form, you agree to voluntarily participate in this study.

Name of child……..Signature……..Date…..……

Name of investigator…… Signature……Date……

FOMU YA IDHINI

Nambari ya Utafiti…… Nambari ya Hospitali……

Swala kuu la utafiti: UTAMBUZI WA WATOTO WA MIAKA 7 HADI 16 WALIO NA UGONJWA WA SUKARI KATIKA HOSPITALI KUU YA KENYATTA

Mpelelezi Mkuu: Dkt. A. Macharia
Dept. of Paediatrics and Child Health
Tel: 0722 726884

Wasaidizi wakuu: Dr. Lucy Mungai
Dr. Rachel Kang'ethe

Lengo la utafiti huu ni kuweza kutambua utambuzi wa watoto walio na ugonjwa wa sukari; na kutambua kama kuna tofauti kwa utambuzi wa watoto hawa, na watoto ambao hawana ugonjwa wa sukari. Zaidi ni kuangalia kama matibabu watoto hawa wanapewa yanaweza kupungaza mabadiliko kwa utambuzi.

Mgonjwa au mlinzi atajibu maswali kuhusu utambuzi wa mtoto aliye na ugonjwa wa sukari anayeonakana kwa kliniki kwa Hospitali kuu ya Kenyatta. Utambuzi huu utatumia mbinu zifuatazo kuchunguza utambuzi wa watoto walio na ugonjwa wa sukari:

- Rekodi za matibabu zitachunguzwa kutambua watoto walio na ugonjwa wa sukari wa miaka saba hadi miaka kumi na sita
- Kipimo cha sukari cha hiyo siku kitachunguzwa kuhakikisha kwamba sukari ni zaidi ya 3.9 mmol/l na chini ya 11.1 mmol/l
- Mzazi/mlinzi atajibu maswali ya tuhakikisha kwamba mtoto hana shida ya kuchelewa na maendeleo
- Mtoto atajibu maswali yalio kwa 'Mini Mental Status Examination' MMSE, na usaidizi wa daktari
- Watoto wanaolinganishwa na wale walio na ugonjwa wa sukari watapata kipimo cha sukari kuhakikisha kwamba hawana ugonjwa wa sukari

Umuhimu

Umuhimu wa utafiti huu nikuboresha uchunguzi na matibabu ya watoto walio na ugonjwa wa sukari wanaotibiwa kwa Hospitali kuu ya Kenyatta.

Madhara na manufaa ya kushiriki

Hakuna madhara yoyote ambayo yatatokana na utafiti huu kwa afya ya mtoto. Hakuna gharama zaidi itakuja kwako juu ya kushiriki kwa utafiti huu. Baada ya utafiti hakuna malipo yoyote utakayopata bali shukrani kwa kukubali kushiriki katika utafiti huu.

Kushiriki kwa utafiti huu ni kwa hiari ya mgonjwa au mzazi/mlinzi. Mgonjwa atahudumiwa hata akikataa kuhusika na utafiti huu. Mgonjwa ama mzazi/mlinzi ana uhuru wa kutamatisha kuhusika kwake kwa wakati wowote bila madhara yoyote. Habari yoyote utakayotoa itawekwa kwa siri na jina la mgonjwa halitachepishwa popote.

Ikiwa ungetaka kupata maelezo zaidi, tafadhali wasiliana na mpelelezi mkuu kupitia nambari ya simu : 0722 726884 ; ama Hospitali kuu ya Kenyatta Department ya Utafiti kwa nambari ifuatayo : 020 2726300 ext 44355.

Mimi……. Nimeelewa maana na jinsi utafiti huu utakavyofanywa na nimepeana idhini yangu/ ya mtoto wangu nimemsimamia kushiriki.

Sahihi……..……. Tarehe……..

Mimi, Dr A. Macharia, nimepeana maelezo kuhusu utafiti huu vizuri niwezavyo, na nimepatie mzazi/mlinzi nafasi ya kuuliza maswali.

Mlinzi Mkuu…….. Tarehe…….....

Idhini ya mtoto anayehojiwa :

Mzazi wako amekubali ya kwamba uhojiwe kwenye utafiti tunaofanya; ambao unapima utambuzi wa watoto wa miaka 7 hadi 16 walio na ugonjwa wa sukari. Kwa kuweka alama kwenye hii fomu ni kutoa idhini ya kuhojiwa.

Jina la mtoto…….Alama……Tarehe……

Jina la mwenye kuhoji……Sahihi……Tarehe……

13.5. APPENDIX V - PROCEDURE FOR MEASURING Hemoglobin A1c

MACHINE: BAYER DCA 2000+

PRINCIPLE

A reagent containing an antibody specific for HbA1c coated on latex beads reacts with a synthetic agglutinator containing HbA1c antigen, resulting in the aggregation of the beads and increasing the turbidity of the reaction mixture. HbA1c in a blood sample is quantified by measuring the inhibition of the aggregation resulting from competition for the antibody by the HbA1c in the sample. The total hemoglobin is determined colorimetrically and the results are expressed as percent HbA1c.The glycosylated fraction of hemoglobin (HbA1c) reflects the glucose level in the blood and is a measure of long term glucose control.

SPECIMEN REQUIREMENTS

• No pre-visit preparation is necessary. Blood sampling and testing is done during the patient's visit. .

• The physician requests that a blood sample be obtained for testing while the patient is present.

• Only one patient sample is obtained at a time.

Specimen Type:

• Whole blood. A 1 µL blood sample from a finger stick is obtained at the time of the determination.

Handling Conditions:

• When the sample is obtained, the patient's name is written on a form and the form accompanies the sample to the testing instrument. The determination is performed immediately.

• After the capillary is filled with sample, the analysis must begin within 5 minutes.

TESTING PROCEDURE:

1. Check the temperature indicator on the box before taking a cartridge out.

2. Refrigerated cartridges should be warmed to room temperature for 10 minutes before use.

3. When a cartridge package is opened, the cartridge must be used within one hour.

• Check the cartridge and do not use it: if the cartridge is damaged, if the flexible cartridge pull-tab is loose or missing, or if the desiccant is missing or loose desiccant particles are found inside the foil pouch.

4. Room temperature must be between 59° and 90°F. Do not test if temperature exceeds this range. Room temperature must be recorded on the test log for each test that is done.

5. Allow the instrument enough time to warm up at the beginning of the day.

6. Pass cartridge through reader. A beep sound indicates a successful scan

7. Fill capillary holder with blood (1 micro liter from finger stick). Wipe away the first drop of blood before collecting the specimen).Wipe outside of holder. Testing must begin within 5 minutes of collecting the specimen.

8. If blood contacts the plastic outside of the holder, discard the holder and use another one.

9. Insert holder into the reagent cartridge with the rounded side of the holder to the outside.

10. Hold the cartridge with the foil to the left, and insert cartridge into instrument until it snaps into place.

11. Remove the tab and foil from the cartridge.

12. Close the door on the instrument. Reaction is complete in 6 minutes.

13. Read percent HbAlc before removing the cartridge. The range of the instrument is 2.5% to 14.0%. The result is displayed as percent HbA1c.

• Results proceeded by a < sign indicates a level below the range and a > indicates a level above the range, and should be recorded as such.

14. Record the result in the testing log, and in the patient's chart. All results are reported to the physician immediately.

15. Remove the cartridge by pushing down on the gray tab while sliding the cartridge to the right, toward the gray tab—then lift the cartridge out of the instrument and discard it in a biohazard container.

CALCULATIONS: None. The result is displayed as percent HbA1c.

REPORTING RESULTS: Reference Range: 4.2% to 6.5% HbA1c.

LIMITATIONS OF THE PROCEDURE:

• The results are accurate over a range of total hemoglobin of 7 to 24 g/dl.

• This test does not detect glycosylated hemoglobin F.

• Because of shortened red cell survival, results from patients with hemolytic anemia, polycythemia and homozygous HbS and HbC will not accurately reflect long term glycemic control.

Sample collection protocol and Infection control

The finger shall be wiped with alcohol swab before collection of blood.

Using a lancet the finger shall be pricked, the first drop of blood wiped off.

Fill capillary holder with blood (1 microliter from finger stick). Blood shall be handled as highly infectious and the lancet shall be disposed in the safety box for sharps. The other wastes: gloves, the capillary holder and the alcohol swabs shall be disposed in the highly infectious hospital waste yellow bin.