# SERUM MAGNESIUM LEVELS AMONG TYPE 2 DIABETES MELLITUS PATIENTS ATTENDING THE DIABETES OUT-PATIENT CLINIC IN KENYATTA NATIONAL HOSPITAL

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MBChB (UoN)

**H58/74588/2014**

*A Study Dissertation Submitted In Partial Fulfillment of the Requirements for the Award of the Masters of Medicine Degree in Internal Medicine*

**Department of Clinical Medicine and Therapeutics,**

**University of Nairobi**

**2017**

# DECLARATION

This research proposal is my original work and has been presented as a prerequisite for a Master's degree to the Department of Clinical Medicine and Therapeutics, University of Nairobi, Kenya. It has not been presented for any degree to any other university.

Signature…… Date…….

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| **Title of the work** | Serum magnesium levels in Type 2 Diabetes Mellitus |

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# ACKNOWLEDGMENT

I am indebted to the following for their contributions to this project:

*God, for His divine favour, strength and good health during the course of my study*

*My supervisors, Dr. A. J. O. Were, Prof. C.F. Otieno and Dr. S. M. Ngare for their unrelenting support, critical review and commitment throughout every stage of my study*

*My research assistant, Geoffrey Mandela, who worked tirelessly and helped me during data collection*

*Ken Mutai, Sylvia Onchaga and Nelson Lang'at who assisted me with data analysis*

*Nursing staff at the KNH Diabetes Clinic and members of staff at the KNH Biochemistry Laboratory for their assistance during the course of this work*

*The clients who graciously accepted to participate in my study*

*To all my friends and colleagues for their constant encouragement and motivation*

*Finally, I am grateful to my family for their love, patience and unwavering faith in me*

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# OPERATIONAL DEFINITIONS

**Hypomagnesaemia:** any decrease in serum magnesium levels below the lower limit of the reference value provided by the laboratory. The KNH Biochemistry Laboratory reference range is 0.66 - 1.07mmol/L using the magnesium colorimetric assay kit ***(Xylidyl Blue-I Method)*** run in the ***Biolys Superior 50i*** automated system.

**Renal function reserve** in this study refers to grading of the renal system's functional capacity based on the calculated / estimated Glomerular Filtration Rate (eGFR) using the Cockroft-Gault Formula.

**Quality of Glycaemic control:** graded as good (if the glycated hemoglobin level, $HbA_1c$ is ≤7%) or poor (if glycated hemoglobin level, $HbA_1c$ is >7%).

# LIST OF ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| ARIC | Atherosclerosis Risk In Communities |
| ATP | Adenosine Tri-Phosphate |
| CKD | Chronic Kidney Disease |
| DNA | Deoxyribonucleic Acid |
| DR | Diabetes Retinopathy |
| DOPC | Diabetes Out Patient Clinic |
| eGFR | Estimated Glomerular Filtration Rate |
| GLUT | Glucose Transporter |
| G | Gram |
| HbA1c | Glycosylated haemoglobin |
| IFG | Impaired Fasting Glucose |
| IGT | Impaired Glucose Tolerance |
| KNH | Kenyatta National Hospital |
| mEq/kg | milli-equivalent per kilogram |
| mg/Dl | milligrams per decilitre |
| Mg | Magnesium |
| mmol/L | millimoles per litre |
| $Na^{+}K^{+}$ATPase | Sodium Potassium Adenosine Tri-Phosphatase |
| OHAs | Oral Anti-glycaemic Agents |
| PI | Principal Investigator |
| SPSS | Statistical Package for Social Sciences |
| T1DM | Type 1 Diabetes Mellitus |
| T2DM | Type 2 Diabetes Mellitus |
| TRPM6 | Transient Receptor Potential Melastatin 6 |
| UoN | University of Nairobi |
| WHO | World Health Organisation |

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# ABSTRACT

**Background:** Magnesium plays a key role in many body cell processes. There is an established association between hypomagnesaemia, poor glycaemic control and diabetes complications. Hypomagnesemia in critically ill diabetic patients is associated with high mortality. Oral magnesium supplementation restores magnesium levels, improves insulin sensitivity and glycaemic control; hence slowing the progression to diabetes-related complications.

**Objective of the Study:** To determine the serum magnesium levels in patients with type 2 diabetes at the Kenyatta National Hospital. To correlate serum magnesium levels with: glycaemic control, renal function reserve and clinical characteristics.

**Methods:** This was a cross-sectional study done on type 2 diabetes patients attending the KNH Diabetes Out-Patient Clinic between August and September 2016. Consecutive sampling was used to recruit 190 study participants. Descriptive statistics were used to summarize the data. For continuous variables, histograms were plot; means (SD) or medians (IQR) reported. For categorical variables, bar / pie-charts were plot; frequencies and proportions were reported.

**Results:** The prevalence of hypomagnesemia was 12.1%. The average duration of diabetes was 11.5 years. Only 21.6% participants had good glycaemic control and 37.4% had Stage 3 CKD and beyond. Hypomagnesemia was significantly correlated with gender, $HbA_1c$, mean duration of diabetes and diuretic use **($p<0.05$)**.

**Conclusion:** Although the prevalence recorded appears to be low compared to studies done in other regions, there is still a significant burden of hypomagnesemia among our diabetic patients.

# CHAPTER ONE

## INTRODUCTION

Diabetes Mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. There are two major clinical entities; Type 1 and Type 2. Type 2 Diabetes Mellitus accounts for approximately 90% of all diabetes cases worldwide and is a global public health concern since it is on the rise due to change in lifestyle and dietary habits **(1).** International Diabetes Federation predicts a rise in the number of diabetes cases from 382 million (in 2013) to 592million (in 2035). This translates to a 170% rise in developing countries against a 42% rise in developed countries, causing a great economic burden in the developing countries. The International Diabetes Federation report also revealed that 80% of diabetic people live in the low- and middle income countries and most are between 40-59years of age **(2).** Motala *et al* in 2008 obtained a prevalence of 3.2% diabetes cases in South Africa while Christensen *et al* in 2009 recorded a prevalence of 4.2% diabetes cases in Kenya **(3, 4)**. Currently, 1.2 million Kenyans live with diabetes and this number is expected to rise to 1.5 million (4.5% of the population) by 2025 as predicted by the World Health Organisation in 2009.

Diabetes is the fourth commonest Non-Communicable Disease and is a major cause of morbidity and mortality world over. The hallmark of Type 2 Diabetes is chronic hyperglycemia which is associated with long-term damage, dysfunction and failure of different organs – kidneys, heart, blood vessels, nerves, eyes **(5).** Diabetes is associated with various electrolyte imbalances including magnesium. Magnesium is the most under diagnosed electrolyte deficiency and has been referred to as ‘the essential forgotten electrolyte’**(6)**. Type 2 Diabetes Mellitus is a

recognized independent risk factor for hypomagnesaemia with a reported incidence of 13.5-47.7% **(7).** It is associated with both extracellular and intracellular magnesium deficits; the principal causes of magnesium loss being gastrointestinal and renal losses **(8).** Notably, the kidney is the principal site for magnesium homeostasis. Hypomagnesaemia has been implicated in Type 2 Diabetes morbidity and its complications; there is an established association between hypomagnesaemia, poor glycaemic control and the diabetes-related complications including deterioration in renal function **(9).**

Oral magnesium supplementation has been shown to be beneficial since it restores magnesium levels, improves insulin sensitivity and glycaemic metabolic control eventually slowing down the rapid progression into the diabetes-related complications **(10).** It is therefore necessary to regularly monitor serum magnesium levels ideally in all Type 2 Diabetes patients but more so amongst those with poor metabolic control and those with diabetes-related complications. Hypomagnesemia in critically ill diabetic patients has been shown to be associated with high mortality **(11)**.

The prevalence of hypomagnesaemia in our diabetic population remains largely unknown yet most of our diabetic patients have poor metabolic control. A cross-sectional study carried out by Omari *et al* in 2013 to assess the level of knowledge, self care practice and glycaemic control among Type 2 diabetes patients attending the diabetes outpatient clinic in KNH, revealed that only 29.5% of the patients achieved a glycosylated hemoglobin equal to or less than 7%. With the documented prognostic implications of magnesium deficiency and the beneficial effects of magnesium supplementation, it is therefore prudent to know the magnitude of hypomagnesaemia in our Type 2 Diabetes patients.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Magnesium in health

### 2.1.1 Magnesium metabolism

Magnesium is principally an intracellular cation, the second most abundant after potassium **(12).** It is an essential ion and is involved in virtually all the metabolic and biochemical processes in the cell, including protein synthesis, DNA stability and energy homeostasis **(13).** A normal healthy adult has approximately 22-26g or 1,000mmol of magnesium with roughly 60% in bone out of which 30% is exchangeable and functions as a reservoir to stabilize the serum concentration. Another approximately 20% is found in muscle, 19% in other soft tissues and 1% in extracellular fluid. Total serum magnesium levels range between 0.7-1.0mmol/L. It exists in three forms - 20% protein bound to albumin and globulins; 15% complexed to anions including citrate, phosphate; and 65% as a free cation as depicted in **Figure 1** below **(14-16).**

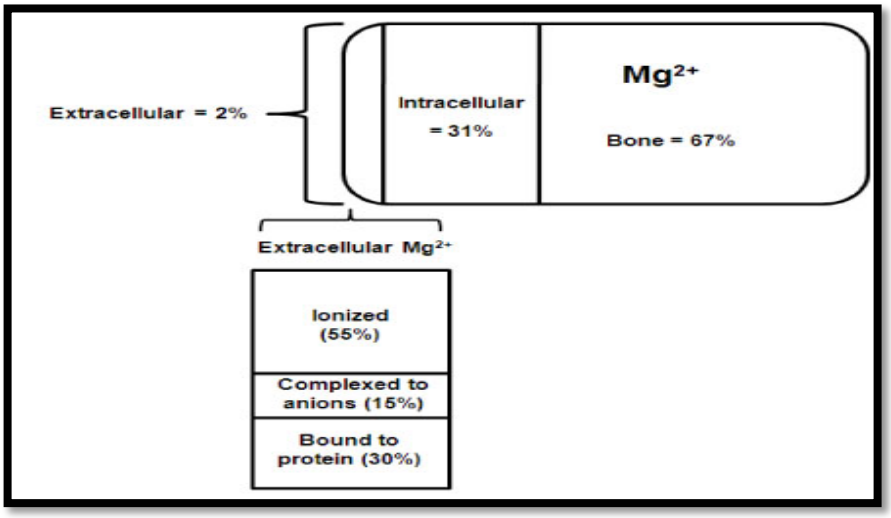



**Figure 1: Distribution of Magnesium in the body**

## 2.1.2 Magnesium homeostasis

Body magnesium homeostasis is dependent on interplay between intestinal magnesium absorption, bone magnesium storage and renal magnesium excretion **(13).** Intestinal absorption occurs principally along the jejunum and ileum via a passive paracellular mechanism accounting for approximately 90% and the rest is via an active transport process. Normally, 30-50% of dietary magnesium is absorbed. However, factors controlling magnesium absorption are still unknown. The kidney is the principal site of magnesium homeostasis.

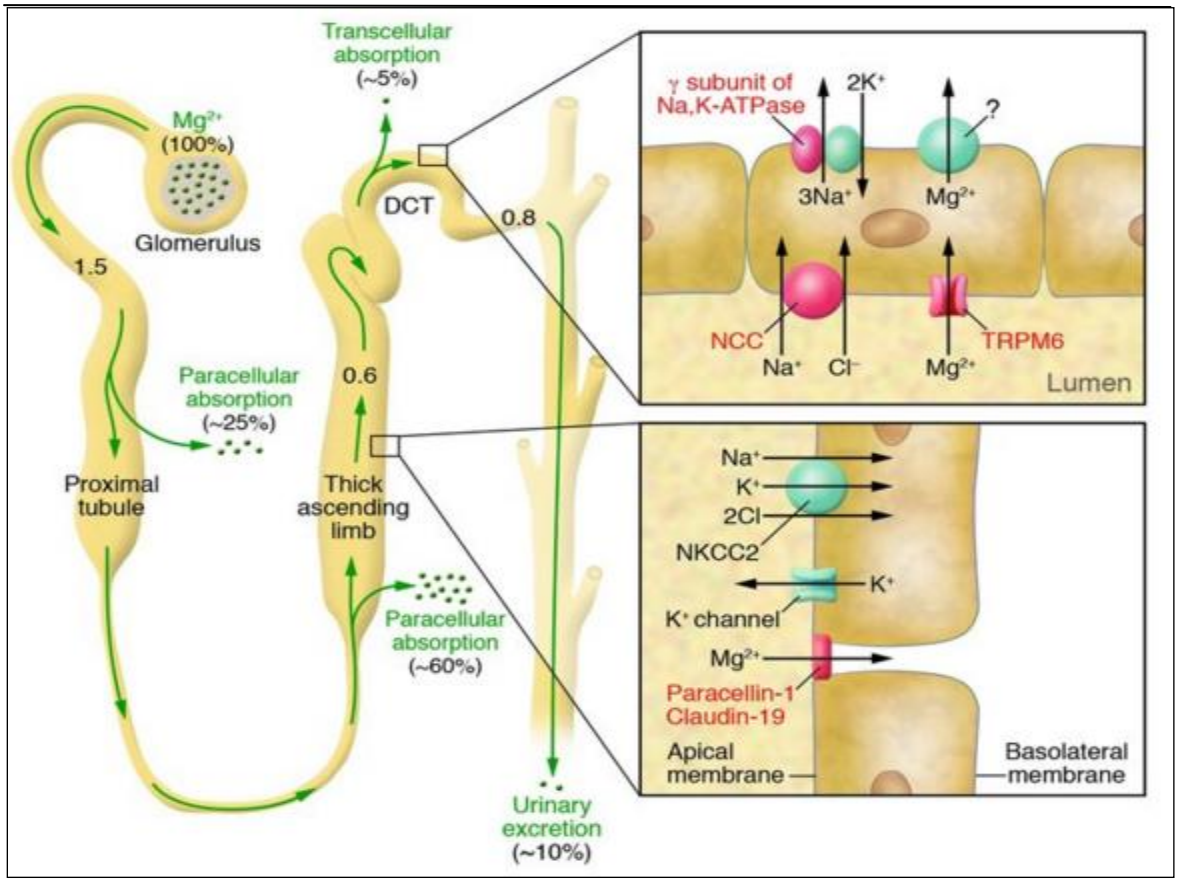



**Figure 2: Renal handling of Magnesium *(J Clin Invest. 2007 Aug 1; 117(8): 2086–2089)*(17)**

As shown in **Figure 2** above; approximately 10-25% of filtered magnesium is reabsorbed passively in the Proximal Convoluted Tubule and another 60-80% in the Thick Ascending Loop

of Henle modulated by the extracellular Calcium Sensing Receptor. In the Distal Convoluted Tubule there is transcellular reabsorption of the filtered magnesium (5-10%) from the pro-urine by the Transient Receptor Protein Melastatin 6 (TRPM6) channels and this determines the final urinary magnesium excretion since no reabsorption occurs beyond the Distal Convoluted Tubule. The Thick Ascending Loop of Henle is therefore the primary site of magnesium reabsorption.

In 2012, theTRPM6 channel was identified by Nair *et al* as the molecular target of insulin signaling and it was concluded that insulin regulates the renal $Mg^{2+}$ TRPM6 channel. When insulin binds to its receptor, it activates an intracellular signaling cascade resulting in increased insertion of TRPM6 in the plasma membrane **(18).** Single Nucleotide Polymorphisms (SNPs) in TRPM6 have been associated with increased risk of Type 2 Diabetes Mellitus **(19, 20).**

Dietary magnesium availability, ATP, pH, estrogen and Epidermal Growth Factor have been shown to regulate the TRPM6 channel. However, Vitamin D has not been shown to have a role in regulation of magnesium reabsorption in the Distal Convoluted Tubule **(21-23).** Hormones such as Insulin, Aldosterone and Prostaglandins; and drugs like Diuretics affect magnesium excretion at the level of the Thick Ascending Loop and the Distal Convoluted Tubule **(13).**

Insulin modulates the shift of magnesium from extracellular to intracellular space hence insulin is key in regulation of magnesium levels. Intracellular magnesium concentration has also been shown to have a role in modulating insulin action – mainly oxidative glucose metabolism.

A low intracellular magnesium concentration as seen in diabetic and hypertensive patients may result in a defective tyrosine kinase activity at the receptor level and a high intracellular calcium concentration. Consequently, there is impairment in insulin action, worsening of insulin resistance and an increase in vascular tone among the diabetic and hypertensive patients. Insulin activates intracellular signaling cascade in the Distal Convoluted Tubule leading to increased

$Na^+$ Chloride Co-transporter (NCC) phosphorylation with resultant increase in $Na^+$ reabsorption via the thiazide sensitive NCC. This is the physiology behind the co-existence of diabetes and hypertension as is seen in majority of the patients **(24).**

Out of the 2.4g of magnesium filtered daily in the kidney, only 120mg (~5%) is excreted implying that most of the magnesium is reabsorbed for use in the various cellular processes.

### 2.1.3 Physiological role of magnesium

Magnesium is an important cofactor in more than 600 enzymatic reactions, including all the enzymes of glycolysis, and an activator for more than 200 reactions. Magnesium plays a vital role in glucose metabolism and insulin homeostasis where it is involved in; regulation of insulin signaling, phosphorylation of the insulin tyrosine kinase receptor, post-receptor action of insulin and insulin mediated cellular glucose uptake **(1, 16, 25, 26).** Chronic magnesium deficit is therefore associated with post-receptor insulin resistance and reduced glucose utilization in the cells, worsening the already existing reduced insulin sensitivity present in diabetic patients **(27).** Magnesium, a known calcium antagonist, regulates insulin secretion by the pancreatic beta cell since release of the insulin vesicle during insulin secretion is dependent on calcium binding which then triggers the exocytosis **(28, 29)**, as is depicted in **Figure 3** below. In skeletal muscle, magnesium increases GLUT4 expression thereby increasing glucose uptake **(30, 31).** Adenylate Cyclase and the $Na^+K^+$ATPase are also highly dependent on magnesium.

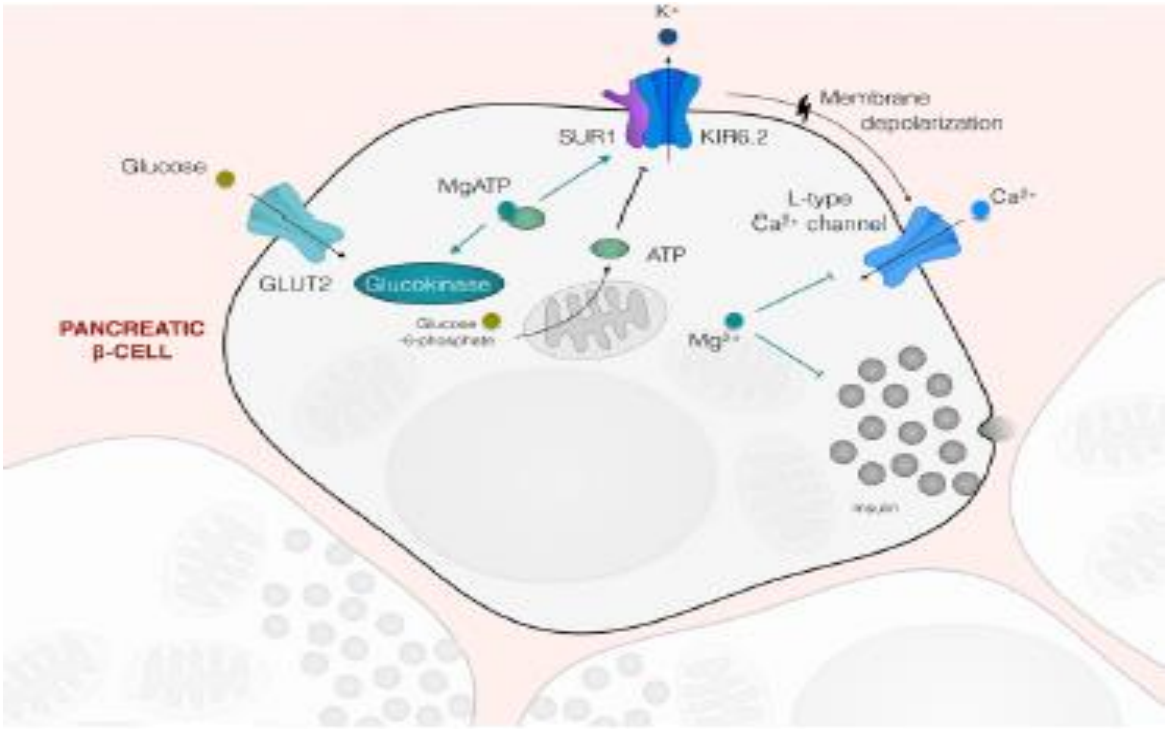



**Figure 3: Regulation of Insulin secretion by magnesium in the pancreatic beta cell *(Gommers et al, 2016)*(32)**

Magnesium being a calcium antagonist plays a role in smooth muscle tone regulation and has been implicated in hypertension, coronary vasospasm, seizures, bronchoconstriction and neuromuscular hyperexcitability. Magnesium is also an anti-inflammatory molecule, it plays an important role in both cellular and humoral immune reactions altering the levels of several cytokines including Interleukin-1, Tumor Necrosis Factor alpha and Interferon gamma. While oxidative stress and inflammation are associated with magnesium deficiency and reduced insulin sensitivity, free radicals have been shown to be increased in conditions associated with magnesium deficits namely diabetes, hypertension, metabolic syndrome and aging **(33-35).** Due to its diverse functions, serum magnesium levels are tightly regulated between 0.7-1.0mmol/L (1.7-2.4mg/dL), however numerous factors such as impaired intestinal magnesium

absorption, renal magnesium wasting, genetic and environmental factors as well as sex hormones can contribute to hypomagnesaemia **(13, 36).**

## 2.2 Magnesium and Diabetes

Magnesium deficiency is commonly associated with endocrine and metabolic disorders, especially Type 2 Diabetes Mellitus although the mechanism of hypomagnesaemia in diabetes is not well understood. There is a close association between metabolic control of diabetes and impaired magnesium balance. Chronic latent magnesium deficit or overt clinical hypomagnesaemia has been found to occur commonly in diabetic patients especially among those with poor glycaemic control **(37).**

**Table 1: Causes of Magnesium deficiency in Type 2 Diabetes Mellitus(38)**

| |
|---|
| 1. Gastrointestinal losses – malabsorption, diarrhea due to autonomic neuropathy |
| 2. Renal losses – osmotic dieresis, glomerular hyperfiltration, diuretic administration |
| 3. Poor dietary intake |
| 4. Recurrent metabolic acidosis |
| 5. Shift hypomagnesaemia – insulin administration |

Low serum magnesium level is a strong independent predictor of incident diabetes as was concluded in the ARIC Study, which was conducted among a cohort of 12,128 nondiabetic middle aged adults **(39).** More importantly, it has been shown that hypomagnesaemia is independently associated with the development of IGT, IFG + IGT and T2DM but not with development of IFG. This was demonstrated in a 10year follow up study done among 1,122 subjects by Guerrero-Romero *et al* in 2008 **(40).** The CARDIA Study, a 20 year follow-up study

of 4,497 Americans who had no diabetes at baseline revealed that magnesium intake was inversely associated with incidence of diabetes which could be attributed to the inverse correlations of magnesium intake with systemic inflammation and insulin resistance **(41).**

There are various causes of magnesium deficiency in diabetes as depicted in **Table 1** above, however low magnesium intake and increased magnesium urinary loss are the most important mechanisms favoring magnesium depletion among Type 2 Diabetes Mellitus patients **(8, 42).** In a meta-analysis of 13 prospective cohort studies, Dong *et al* in 2011 revealed that there is an inverse relationship between dietary magnesium intake and the risk of developing Type 2Diabetes Mellitus **(43).** In yet another meta-analysis of 7 prospective cohort studies, Larsson *et al* 2007 showed that intake of magnesium-rich foods like whole grains, nuts and green leafy vegetables was inversely associated with incidence of Type 2 diabetes **(44).** To note, these foods may not be readily available to the whole Kenyan population.

Hyperglycaemia and hyperinsulinaemia increase urinary magnesium excretion. Insulin has been shown to affect magnesium excretion at the level of the Thick Ascending Loop of Henle and the Distal Convoluted Tubule **(13).** Therefore it is possible that obese patients with hyperinsulinaemia in the early stages of Type 2 Diabetes could actually exhibit hypomagnesaemia. McNair *et al* in 1982 studied 215 insulin-treated diabetic patients and found that the net tubular reabsorption of magnesium was decreased in the presence of hyperglycemia resulting in hypermagnesiuria and hypomagnesaemia **(45).** Adequate metabolic control has been associated with a reduction in urinary magnesium wasting. However, a study done by Schnack *et al* in 1992 amongst diabetic patients at different time frames i.e. before, 1 and 3 months after initiation of insulin therapy or intensified treatment with Oral Hypoglycemic Agents(OHAs) showed that even marked improvement of glycaemic control does not correct hypomagnesaemia

in diabetic patients implying that these patients might benefit from chronic magnesium administration **(46).**

## 2.3 Implications of hypomagnesaemia in Diabetes

Hypomagnesaemia has been implicated in Type 2 Diabetes Mellitus morbidity and its complications. There is an established association between hypomagnesaemia, poor glycaemic control and diabetes-related complications including deterioration in renal function **(9, 37).** Hypomagnesaemia is associated with a more rapid and permanent decline in renal function among Type 2 Diabetic patients. A retrospective cohort study done by Sakaguchi *et al* in 2012 amongst 455 patients with Chronic Kidney Disease (CKD) revealed that among the diabetic CKD patients, those with hypomagnesaemia progressed to End Stage Renal Disease faster than those with higher magnesium levels. Their counterparts with non-diabetic CKD on the contrary had an even slower progression. Hypomagnesaemia is therefore considered an accurate predictor of diabetic nephropathy **(47).** In the year 2000, Corsonello *et al* in a comparative study involving three distinct groups of Type 2 Diabetic patients i.e. those without microalbuminuria, those with microalbuminuria and those with overt proteinuria; observed a significant decline in serum magnesium in both the overt proteinuria and microalbuminuria groups compared with the non-microalbuminuric group **(48).** Pham *et al* in 2005 carried out a retrospective study designed to determine whether there is any association between serum magnesium concentration and the rate of renal function deterioration; they reported an association between low serum magnesium levels and a significantly faster rate of renal function deterioration in Type 2 diabetics **(49).**

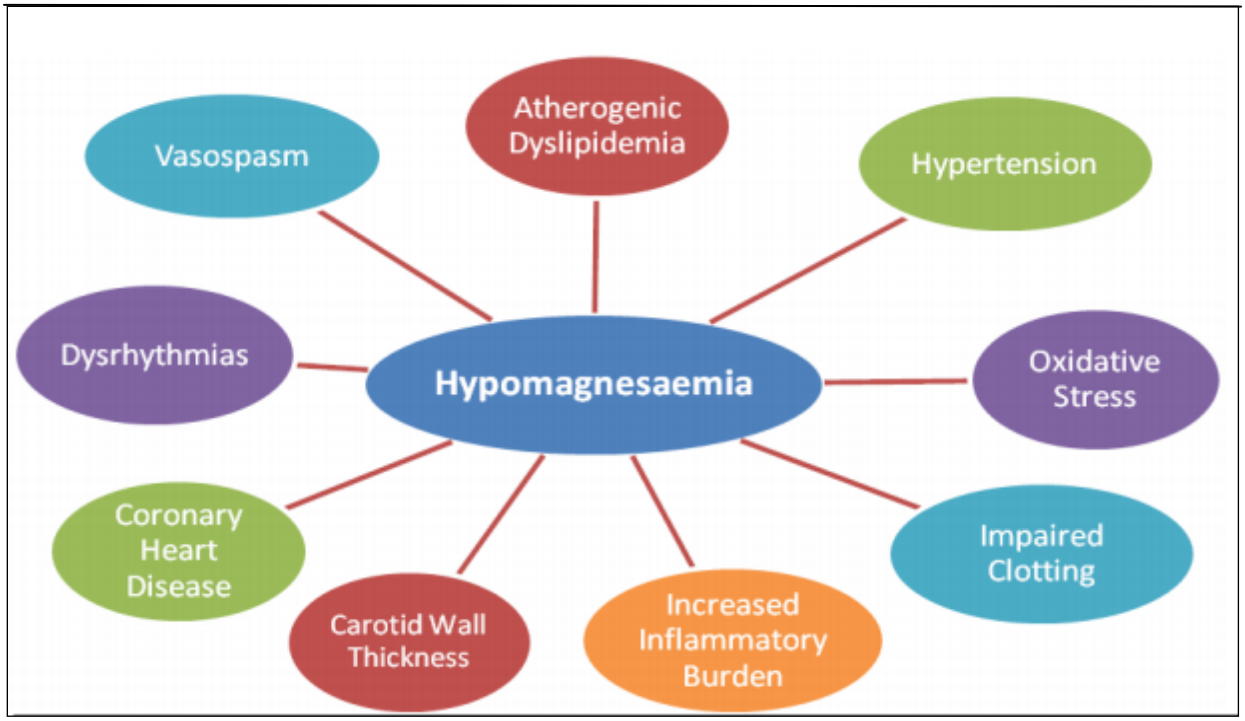



**Figure 4: Hypomagnesaemia induced cardiovascular complications (50)**

Cardio-metabolic disease states are a common occurrence amongst Type 2 diabetic patients and hypomagnesaemia has been found to play a significant role. He *et al* in 2006 found an inverse relationship between magnesium intake, metabolic syndrome and fasting insulin levels **(51).** Hypomagnesaemia may therefore be considered an additional cardiovascular risk among diabetic patients with Type 2 Diabetes. As depicted in **Figure 4** above, low circulating levels of magnesium are associated with atherogenic dyslipidaemia, ischemic cerebrovascular accidents, carotid wall thickness, coronary artery disease, arrhythmias, Premature Ventricular Complexes, hypertension, oxidative stress, increased inflammatory burden and impaired clotting **(50)**. Magnesium intake causes a decrease in triglycerides and an increase in High Density Lipoprotein cholesterol; and as a calcium antagonist it regulates smooth muscle tone therefore magnesium deficiency results in increased calcium levels, increased smooth muscle vascular tone resulting in

increased blood pressure and hypertension. Magnesium deficiency is also associated with defective tyrosine kinase activity at the insulin receptor level resulting in insulin resistance and eventually diabetes. These two pathways are illustrated in **Figure 5** below **(52).**

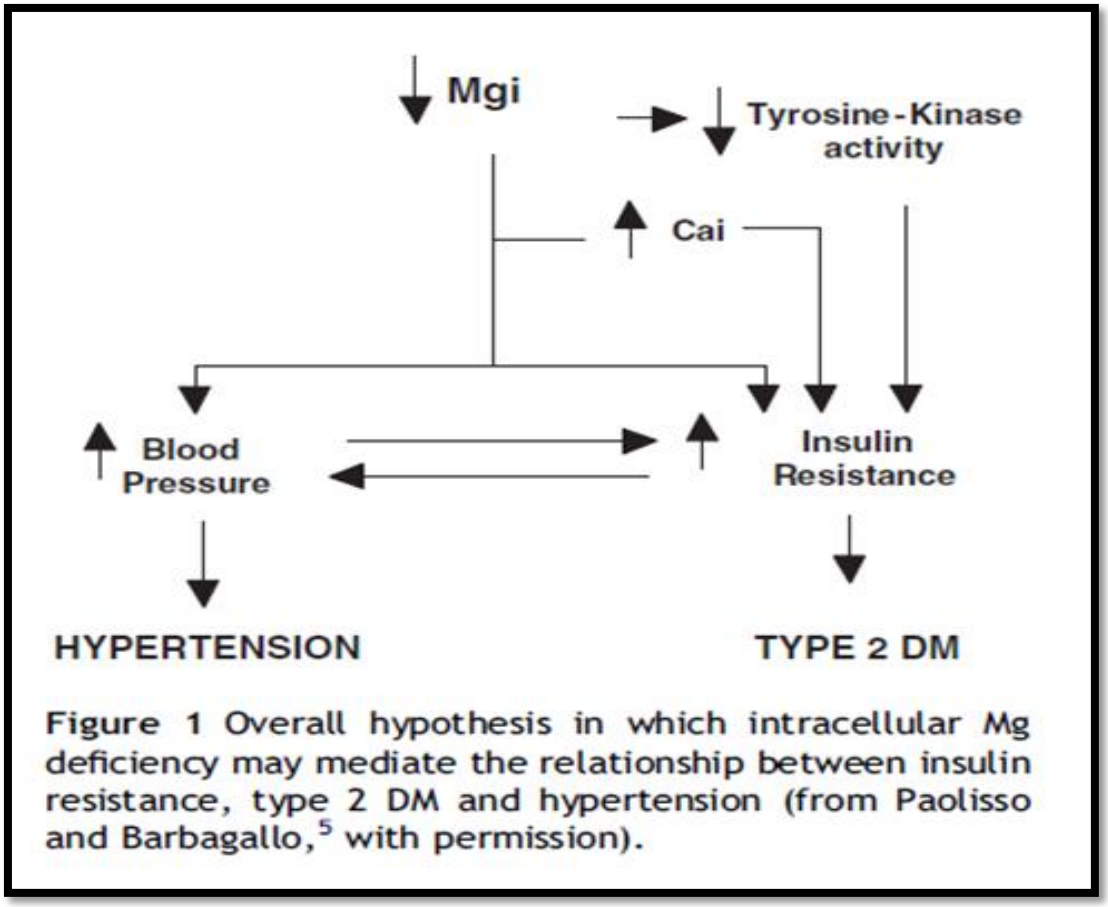



Figure 1 Overall hypothesis in which intracellular Mg deficiency may mediate the relationship between insulin resistance, type 2 DM and hypertension (from Paolisso and Barbagallo,[5] with permission).

**Figure 5: Relationship between Hypertension, Diabetes and Hypomagnesmia(27)**

In a cross-sectional health survey, Del Gobbo *et al* in 2012 found a higher prevalence (50% versus 21%) of Premature Ventricular Complexes amongst the hypomagnesaemic subjects, all of whom had diabetes as well **(53).**The use of magnesium in cardiac disease among diabetic

patients is still controversial, with no conclusive studies especially in diabetic patients with myocardial infarction.

Barbagallo *et al* in 1996 carried out a study on normotensive and hypertensive insulin-dependent diabetic patients. The aim was to establish the relation of calcium to hyperglycemia and cardiac mass. The study suggested that hypomagnesaemia resulted in an increase in glucose-related cytosolic free calcium in myocardial and vascular smooth muscle cells. This in itself was found to contribute to elevated blood pressure and increase cardiac mass with attendant left ventricular dysfunction and hypertrophy. This further supported the already established association between reduced cellular magnesium and cardiac hypertrophy among Type 2 diabetic patients **(54).**

There is an evident association between hypomagnesaemia and the various diabetes-related complications including neuropathy, retinopathy, foot ulcers and albuminuria. A cross-sectional study carried out by Dasgupta *et al* in India in 2012 among stable Type 2 diabetic patients revealed an 11.33% prevalence of hypomagnesaemia with a female: male ratio of 9:8. The mean glycosylated hemoglobin level was 11.9% in the hypomagnesaemic patients compared with 9.8% in the normomagnesaemic group. Among the hypomagnesaemic patients, the incidence of the microvascular complications was found to be higher compared to the normomagnesaemic group. Retinopathy, microalbuminuria, macroalbuminuria, foot ulceration and neuropathy was present in 64%, 47%, 17.64%, 58.8%, and 82.35% respectively, of the hypomagnesaemic patients compared with 45.8% ($P$ =0.118), 38.34% ($P$ =0.704),15.03% ($P$ =0.566), 22.55% ($P$ =0.011) and 82.7% ($P$ =0.976) in those without hypomagnesaemia **(9).** McNair *et al* in a cross-sectional study done in 1978 among 71 diabetic patients with varying degrees of retinopathy revealed that the levels of serum magnesium had an inverse correlation with the degree of retinopathy **(55).**

In a comparative study between diabetic patients with foot ulcers and those without, Rodriguez-Moran *et al* in 2001, observed a higher incidence of hypomagnesaemia among patients with foot ulcers compared with those without (93.9% versus 73.1%, p=0.02). Polyneuropathy and platelet dysfunction are among the risk factors that were thought to contribute to the development of diabetic foot ulcers **(56).**

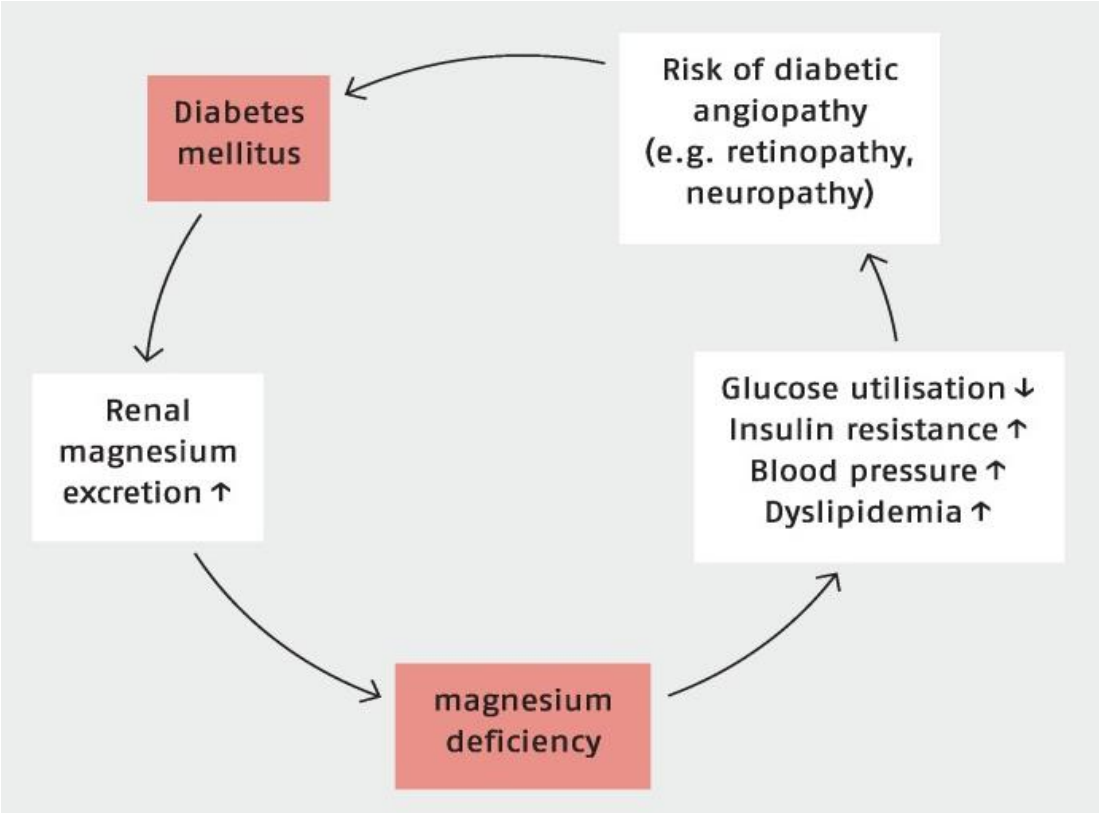



**Figure 6: Magnesium deficiency in Diabetes – A Vicious Circle (57)**

In addition, there is data to suggest an association between hypomagnesaemia and other diabetes-related complications, including dyslipidaemia and neurologic abnormalities **(58).** It is therefore paramount to minimise hypomagnesaemia in the routine management of Type 2 Diabetes patients since there is an established link between hypomagnesaemia and various micro- and

macrovascular complications. In conclusion, a vicious circle has been shown to exist between diabetes and magnesium deficiency as is shown in **Figure 6** above.

## 2.4 Assessment of magnesium status and hypomagnesaemia

Magnesium deficiency is defined as a total body deficiency of magnesium **(59).** Hypomagnesaemia reflects a low total body concentration but normomagnesemia does not necessarily indicate normal or high total body magnesium **(60).** Hypomagnesaemia is defined as serum magnesium concentration < 1.6mg/dL or >2 Standard Deviation (SD) below the mean of the general population **(49).** Clinical evaluation of magnesium status is associated with many challenges since there is no laboratory test as yet which measures total body magnesium levels. Secondly, serum (extracellular) magnesium levels may not necessarily reflect the intracellular magnesium levels. Thirdly, serum ionised magnesium which is the biologically significant portion of magnesium is not routinely measured.

There are different methods to assess magnesium levels as shown in **Table 2** below. Intracellular magnesium which is estimated using circulating red blood cells, mononuclear cells and skeletal muscle cells, is more accurate than the total serum magnesium concentration as an indicator of the magnesium status **(60).** Of note, only 1% of the whole body magnesium is found extracellularly. Lymphocytic and erythrocyte magnesium levels are the most accurate intracellular magnesium measurements and they also reflect the intramyocardial magnesium content. Lymphocytic is more accurate compared to erythrocytic which is less accurate and cell age dependent **(61).** Total serum magnesium concentration may not adequately reflect body magnesium stores since patients can actually have a normal serum magnesium level with total-body magnesium depletion.

Changes in serum protein concentrations can also affect the total serum magnesium concentration without necessarily affecting the ionised fraction or total body magnesium status. Intracellular magnesium cannot be used sufficiently as a discriminatory test to diagnose magnesium deficiency hence to make a timely diagnosis of hypomagnesaemia; patients require a thorough clinical assessment. A low serum magnesium concentration is enough to confirm the diagnosis in patients with suspected magnesium deficiency. More sensitive tests are required in patients with suspected magnesium deficiency but normal serum magnesium concentration. There is still no consensus on how best to determine hypomagnesaemia; nonetheless most studies have relied predominantly on total serum magnesium concentration **(7, 62).**

**Table 2: Laboratory assessment of Magnesium status (*Swaminathan et al, 2003)*(63)**

| | |
|---|---|
| **Serum Magnesium Concentration** | Total magnesium; Ultra-filterable Magnesium; Ionised Magnesium |
| **Intracellular Magnesium content** | Red Cells; Mononuclear blood cells; Skeletal muscle |
| **Physiological Tests** | Metabolic balance studies; 24h urinary excretion of magnesium; Magnesium loading test |
| **Intracellular free magnesium ion concentration** | Fluorescent dye; Nuclear magnetic resonance spectroscopy |
| **Others** | Magnesium balance; Isotope studies; Functional assays; Hair or tooth magnesium |

It is desirable to measure magnesium levels directly in complex matrices like whole blood, plasma and serum as opposed to estimation of magnesium levels in serum or plasma by analysis of ultra-filtrates i.e. complexed $Mg^{+}$ $Mg^{2+}$. This is because the latter does not distinguish the ionised or free form of magnesium from the $Mg^{2+}$ bound to organic or inorganic anions. The

levels of these ligands i.e. citrate, sulphate, lactate, bicarbonate, acetate, phosphate can vary in different disease states **(64, 65).** Magnesium retention after oral magnesium or intravenous load test is accurate but too cumbersome. It involves measuring magnesium retention in a 24hour urine collection, followed by the administration of 2.4 mEq/kg of parenteral magnesium, followed by a second 24-hour urine collection for magnesium. In cases of normal magnesium balance and renal function, most of the magnesium load will be excreted in 24 hours. Retention of more than 20% of the administered magnesium is suggestive of magnesium deficiency **(66).** Despite all these challenges, total serum magnesium concentration remains the simplest, most useful, standard and readily available test in the evaluation of magnesium status in patients **(15, 67).**

With development of reliable analysers, ionised magnesium measurement may become feasible. Clinical assessment of patients at risk of magnesium deficiency remains key for early diagnosis. Difficulties in diagnosing magnesium deficiency and in evaluating response to treatment are the main hindrances leading to delays in accepting low serum magnesium as an important contributor to morbidity and mortality.

## 2.5 Prevalence of hypomagnesaemia in Type 2 Diabetes Mellitus

The prevalence of hypomagnesaemia has been estimated at 13.5 – 47.7% among Type 2 Diabetic patients compared to 2.5-15% among non-diabetic subjects. In the general population the prevalence of hypomagnesaemia lies at 6.9% **(7).** Seyoum *et al* in a cross-sectional study done in Ethiopia in 2008 recorded a prevalence of 65%. This is an outlier and could be attributed to the harsh weather and famine experienced in Ethiopia resulting in low dietary intake of magnesium.

The study population included 44 Type 1 and 69 Type 2 diabetic patients plus 46 non-diabetic controls **(68).**

A case control study done among obese patients with and without diabetes in Spain by Lecube *et al* in 2012 revealed a prevalence of 48% **(69).** This could be attributed to the fact that adipocytes produce pro-inflammatory mediators including Interleukin-1, Tumor Necrosis Factor and also stimulate production of reactive oxygen species creating an inflammatory environment which contributes to insulin resistance. Consequently, there is reduced insulin mediated magnesium reabsorption in the Thick Ascending Loop of Henle resulting in hypermagnesuria and hypomagnesaemia.

In a recent comparative study done in India in 2015 by Ramachandra *et al,* a high prevalence of hypomagnesaemia i.e. 85% was recorded among diabetic patients with complications whereas 21% prevalence was demonstrated among those without complications. On average the glycaemic control among those without diabetes-related complications was 8.9% while that of those with complications was 9.3%. It is therefore evident that hypomagnesaemia is associated with both microvascular complications and poor glycaemic control.

Ajibola *et al* in Nigeria in 2013 demonstrated hypomagnesaemia at a prevalence of 18% in a case control study done among Type 2 diabetes patients looking at various trace elements. A cross-sectional study done by Dasgupta *et al* among 150 Type 2 diabetes patients in India in 2012 revealed a prevalence of 11%, with a female: male ratio of 9:8. The mean glycosylated hemoglobin amongst the hypomagnesaemic group was 11.9% while that of the normomagnesaemic group was 9.8%. In this study, hypomagnesaemia was found to be associated with poorer glycaemic control, retinopathy, foot ulcers and nephropathy **(9).**

Similarly, Baig *et al* in a study involving 60 Type 2 Diabetes patients detected significantly low

serum magnesium levels among the diabetic patients with or without complications when compared with the control group. However, they showed that the serum magnesium level in cases with diabetes-related complications was significantly lower than those without complications. Overall, these studies reveal that quite a number of diabetic patients are hypomagnesaemic yet serum magnesium levels are not routinely measured in Type 2 Diabetes patients **(7).**

**Table 3: Various Studies on Hypomagnesaemia in Diabetes**

| Author | Study design | N | Serum Mg (mmol/L) Mean ± SD | HbA1c (%) | Prevalence |
|---|---|---|---|---|---|
| **Seyoum *et al* (68)** 2008 Ethiopia | Cross sectional study | 44 T1DM<br>69 T2DM<br>46 controls | 0.82 ± 0.02<br>0.86 ± 0.02<br>1.02 ± 0.02 | | **65%** |
| **Dasgupta *et al* (9)** 2012 India | Cross sectional study | 150 T2DM | 0.42 ± 0.13 (11% hypomagnesaemia)<br>(89%normomagnesemia) | 11.9 ± 2.26<br>9.8 ± 2.1 | **11%** |
| **Lecube *et al* (69)** 2012 Spain | Case control study | Cases 50<br>Controls 150 | 0.75 ± 0.07<br>0.81 ± 0.06 | 7.6 ± 1.87<br>5.76 ± 0.56 | **48%** |
| **Kundu *et al* (70)** 2013 India | Case control study | 30 without<br>30 with DR<br>60 controls | 0.83 ± 0.12<br>0.57 ± 0.16<br>1.08 ± 0.15 | 7.56 ± 0.59<br>10.54 ± 1.02<br>4.68 ± 0.88 | |

## 2.6 Magnesium supplementation in Type 2 Diabetes Mellitus management

Magnesium status is often ignored by many physicians yet the detection and correction of altered magnesium status among Type 2 diabetes patients is fundamental. Magnesium supplementation is potentially beneficial amongst Type 2 diabetes patients and in patients with risk factors for diabetes. This is based on the studies that have shown an increased risk of developing Impaired Glucose Tolerance and frank Type 2 diabetes in the presence of dietary or serum magnesium deficiency **(40, 43, 44)**.Several studies have shown a beneficial effect of magnesium supplementation in Type 2 Diabetes patients **(10, 71, 72)**. Rodriguez-Moran *et al* conducted a clinical randomized double-blind placebo-controlled trial involving 63 hypomagnesaemic Type 2 diabetes patients on glinbenclamide and either 50mls Magnesium Chloride solution or placebo daily for 16 weeks. Oral magnesium supplementation was found to restore serum magnesium levels, improve insulin sensitivity and metabolic control amongst the hypomagnesaemic Type 2 diabetes patients **(10).**

Magnesium plays a key role in modulating insulin-mediated glucose uptake and vascular tone. Daily magnesium administration restores intracellular magnesium concentration improving insulin-mediated glucose uptake among diabetic patients; and decreases smooth muscle vascular tone thereby reducing arterial blood pressure. Oral magnesium supplementation has been shown to improve endothelial function in elderly diabetic subjects. In 2010, Barbagallo *et al* studied the effects of magnesium oral supplementation (368mg/day of magnesium ion) amongst 68 elderly patients, and reported a significant improvement in post-ischemic endothelial-dependent flow-mediated dilation. Magnesium ions influence vascular tone and responsiveness and are also cofactors for Acetylcholine-induced endothelium-dependent relaxation resulting in

vasodilatation **(71).** Oral magnesium supplements have been shown to improve insulin sensitivity in non-diabetic subjects with insulin resistance. A 3month randomized double-blind placebo controlled trial among hypomagnesaemic subjects with insulin resistance done by Guerrero-Romero *et al* in 2004, revealed an improvement in serum magnesium levels and insulin sensitivity with 2.5mg Magnesium Chloride **(73).** Oral magnesium supplementation also reduces C - reactive protein (CRP) levels amongst hypomagnesaemic patients with pre-diabetes. Magnesium deficiency is associated with the triggering of acute phase response which may contribute to Type 2 Diabetes and cardiovascular disease risk. Simental-Mendia *et al* in a clinical randomized double-blind placebo controlled study showed that oral magnesium supplementation decreases high-sensitivity C - reactive protein (hsCRP) levels in apparently healthy subjects with pre-diabetes and hypomagnesaemia **(74).**

In summary, oral magnesium supplementation has been found beneficial in Type 2 diabetes patients to restore magnesium deficiencies and consequently improve insulin resistance, oxidative stress, and systemic inflammation; and above all, slow progression to the diabetes-related micro- and macrovascular complications.

## Study Justification

Type 2 Diabetes Mellitus ($T_2DM$) is the fourth commonest non-communicable disease and is associated with numerous macrovascular and microvascular complications. It is a major cause of morbidity and mortality world over. Its prevalence is on the rise with a 170% predicted rise in developing countries and 42% rise in developed countries as reported by the International Diabetes Federation **(2).** Currently, 1.2 million Kenyans live with diabetes and this is expected to rise to 1.5 million by 2025, accounting for 4.5% of the population (WHO 2009).

Hypomagnesaemia has been implicated in type 2 diabetes with a reported incidence of 13.5 – 47.7% **(7).** Low magnesium intake and increased magnesium urinary loss are the most important mechanisms favoring magnesium depletion among type 2 diabetes patients **(8).** Despite these alarming statistics, hypomagnesaemia remains the most under diagnosed electrolyte deficiency yet magnesium plays a crucial role in most if not all cell processes. Hypomagnesaemia is associated with poor glycaemic control and diabetes-related complications including retinopathy, foot ulcers and deterioration in renal function **(9, 47).** Oral magnesium supplementation has been shown to restore magnesium levels, improve insulin sensitivity and glycaemic control and hence slows the progression to diabetes-related complications **(10).** It is therefore necessary to regularly monitor serum magnesium levels in all type 2 diabetes patients and offer supplementation where indicated. There is paucity of data on the prevalence of hypomagnesaemia amongst patients with type 2 diabetes in our set up yet beneficial effects of magnesium supplementation have been demonstrated. The findings in this study will therefore increase the surveillance of magnesium levels among type 2 diabetes patients and will improve their quality of care.

# Research Question

1. What are the serum magnesium levels in type 2 diabetes mellitus patients attending the Diabetes Out-Patient Clinic at the Kenyatta National Hospital?

# Objectives

## Broad Objective

1. To determine the prevalence of hypomagnesemia among type 2 diabetes mellitus patients attending Diabetes Out-Patient Clinic in Kenyatta National Hospital.

## Specific Objective

1. To determine the serum magnesium levels among type 2 diabetes mellitus patients attending the Diabetes Out-Patient Clinic in Kenyatta National Hospital.

2. To determine glycaemic control and renal function reserve among type 2 diabetes mellitus patients attending the Diabetes Out-Patient Clinic in Kenyatta National Hospital.

## Secondary Objective

1. To correlate serum magnesium levels with: glycaemic control, estimated glomerular filtration rate and clinical characteristics (duration of diabetes and treatment therapy) among the type 2 diabetes patients attending the Diabetes Out-Patient Clinic in Kenyatta National Hospital.

# CHAPTER THREE

# METHODS

## 3.1 Study design

This was a descriptive cross-sectional study undertaken to evaluate serum magnesium levels among Type 2 Diabetes Mellitus patients attending the Kenyatta National Hospital Diabetes Out-Patient Clinic.

## 3.2 Study site

Kenyatta National Hospital Diabetes Out-Patient Clinic

## 3.3 Study population

The study population consisted of patients with type 2 diabetes mellitus attending the diabetes out-patient clinic in Kenyatta National Hospital.

### 3.3.1 Case Definition

- Patient with a documented file diagnosis of Type 2 Diabetes Mellitus.

### 3.3.2 Inclusion Criteria

- Patients aged 30 years and above with a documented diagnosis of Type 2 Diabetes Mellitus; and had been on treatment and follow up at the Diabetes Out-Patient Clinic for ≥1 year
- Patients who gave written informed consent

### 3.3.3 Exclusion Criteria

- Patients with Diabetes Ketoacidosis, acute illness requiring inpatient care 3 months prior to assessment

## 3.4 Sample size calculation

Sample size was calculated using the Daniel's formula (1999) for finite population (75)

$$n \geq \frac{NZ^2{}_{\alpha/2}P(1-P)}{d^2(N-1)+Z^2{}_{\alpha/2}P(1-P)}$$

Where:

n = minimum sample size required

N = Total estimated accessible population (N=400)

$Z_{\alpha/2}$ = Critical value for standard normal distribution at α-level of significance (α = 0.05, $Z_{\alpha/2}$ = 1.96)

P = Estimated prevalence of hypomagnesemia in type 2 diabetes mellitus patients (p=0.65 based on a study in Ethiopia **(68)**

d = Margin of error (d = 0.05)

The minimum sample size required was; n = 187 patients.

## 3.5 Sampling method

Patients were recruited consecutively at the Diabetes Out-Patient Clinic in Kenyatta National Hospital

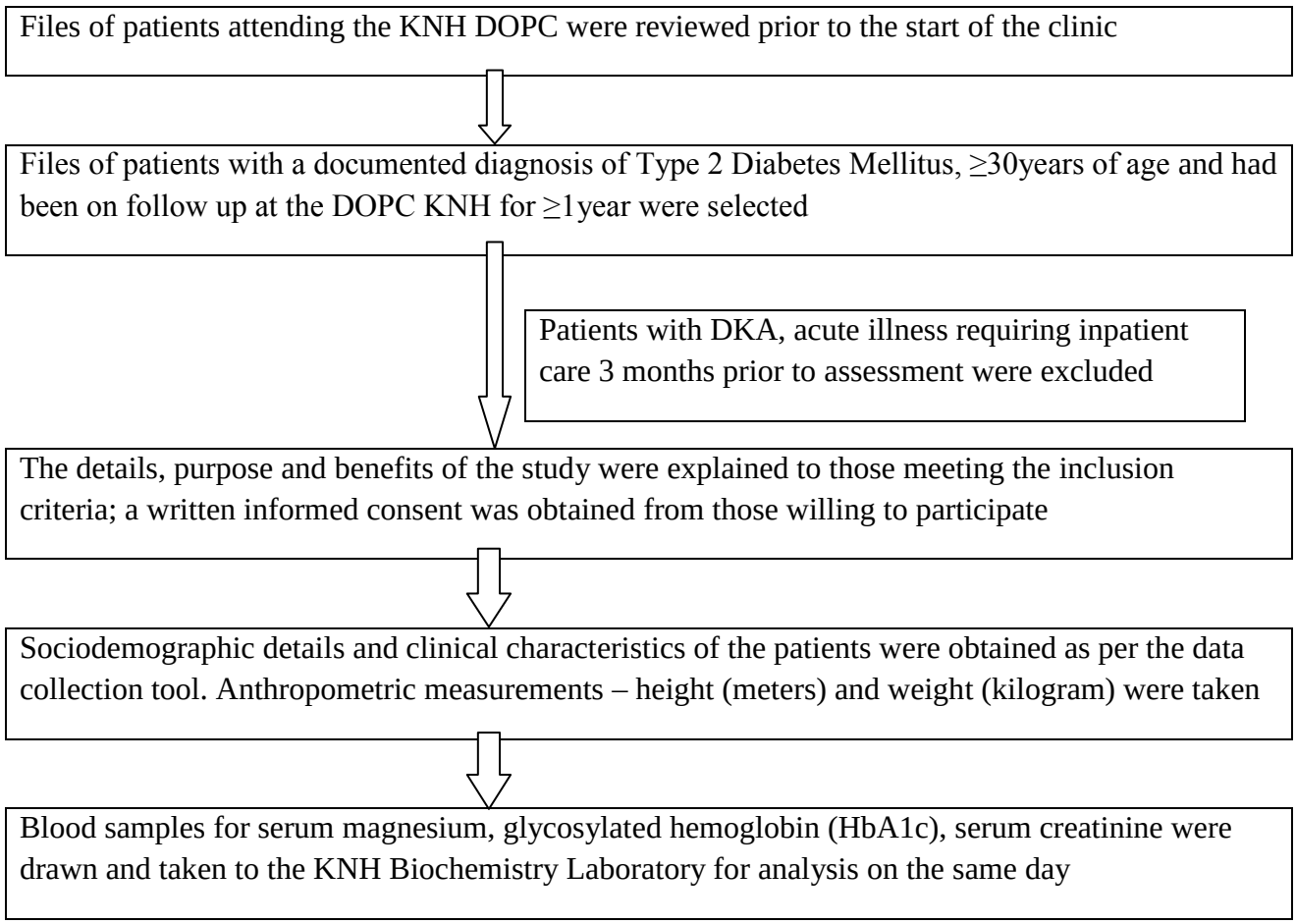
## 3.6 Participant screening and recruitment

The Principal Investigator (PI) with the help of one research assistant reviewed the files of the patients attending the clinic and selected the files of patients with a documented diagnosis of Type 2 Diabetes prior to the start of each clinic. The patients who met the inclusion criteria were

then called into the interviewing room after their routine visit and given all the relevant information about the study. Those who gave informed consent were then recruited.

### 3.6.1 Patient's Flow Chart

Below is a schema of the study processes involved in subject recruitment into the study.



## 3.7 Study variables

- **Dependent Variables**

✓ Serum Magnesium levels

  - Hypomagnesemia was defined as magnesium levels less than 0.66 mmol/L
  - Normomagnesemia defined as magnesium levels between 0.66 and 1.07 mmol/L
  - Hypermagnesemia was defined as magnesium levels greater than 1.07 mmol/L

✓ Glycaemic control - This was assessed by measuring glycated hemoglobin ($HbA_1c$). $HbA_1c$ less than or equal to 7% was considered as good control and $HbA_1c$ greater than 7% as poor control (ADA 2015 Recommendations)

✓ Renal function reserve - Serum creatinine was measured and an estimated Glomerular Filtration Rate (eGFR) calculated. Based on the calculated eGFR patients were categorized in stages of chronic kidney disease as follows:

  - Stage 1 - eGFR more than or equal to 90 ml/min/1.73m²
  - Stage 2 - eGFR 60-89 ml/min/1.73m²
  - Stage 3a - eGFR 45-59 ml/min/1.73m²
  - Stage 3b - eGFR 30-44 ml/min/1.73m²
  - Stage 4 - eGFR 15-29 ml/min/1.73m²
  - Stage 5 - eGFR less than 15ml/min/1.73m²

- **Independent Variables**

✓ Body Mass Index (BMI)

✓ Duration of Diabetes since diagnosis

✓ Treatment modality for Diabetes

- ✓ Diuretic therapy for hypertension

## 3.8 Data collection

- **Clinical Methods**

The sociodemographic data and relevant medical history was obtained from the patient. A weighing scale was used to measure weight (kilograms), a stadiometer to measure height (metres); while the standard mercury sphygmomanometer was used to measure blood pressure (mmHg). A body mass index (BMI) was computed. This information was subsequently entered into the study proforma (**Appendix 3**) for later analysis.

- **Laboratory Methods**

5-6mls of blood was collected from the ante-cubital fossa in each study participant. 2mls were put in the EDTA vacutainer (purple top) for estimation of glycated hemoglobin level and the remaining amount was put in the plain vacutainer (red top) for estimation of serum creatinine and serum magnesium levels. The samples were then delivered to the KNH Biochemistry laboratory at the end of the days' collection and were analysed on the same day hence storage at cool temperatures was not a requirement. Analysis of the different parameters namely, Serum Magnesium, Glycosylated hemoglobin and Serum Creatinine was carried out using the *Biolys Superior 50i* which is an automated biochemistry analyser. The reference ranges used were:-

- ✓ Serum Magnesium 0.66 - 1.07mmol/L;
- ✓ $HbA_1c$ 3.5 – 5.8%;
- ✓ Serum Creatinine 40-130µmol/L

## 3.9 Quality control

The Standard Operating Procedures for specimen collection, labeling, storage and transport were strictly adhered to, to minimize pre-analytical errors. The machine *(Biolys Superior 50i)* used for analysis was properly calibrated using standard calibration methods and materials, and the tests were assayed against controls. Quality control measures were provided by the company servicing the machine. The KNH Biochemistry laboratory carried out internal and external quality control.

## 3.10 Data management and analysis

Each study proforma was assigned a unique study serial number to avoid duplication of data collection. All the data that was collected was entered into Microsoft access in a password protected computer. Data cleaning and verification was done weekly to ensure validation and completion of the information. Data coding and analysis was done using Statistics and Data (STATA) version 13 software. The descriptive statistics were used to summarize the data. For continuous variables, histograms were plotted to show the distribution; means (SD) or medians (IQR) were reported depending on the distribution. For categorical variables, bar / pie-charts were plot to show the distribution; frequencies and proportions were reported in tables. Scatter plots were used to graphically show the relationship between hypomagnesemia and: glycaemic control, renal function reserve and duration of diabetes. Pearson / Spearman rank test was done to quantify the relationship.

## 3.11 Ethical considerations

The study was undertaken after the approval by the Department of Clinical Medicine and Therapeutics, University of Nairobi and the KNH/UoN Scientific and Ethical Review Committee; Research Approval number **P335/04/2016**. The objectives and purposes of the study were clearly explained to eligible participants in a language they could understand. Only patients who gave informed consent were recruited into the study after signing the consent form. Patients were recruited on a voluntary basis and were free to withdraw from the study at any point without any discrimination. Confidentiality was maintained by storing the study proformas in a secure location and excluding the patients' names from the computerized data sheets. Only blood samples intended for the study were drawn and thereafter discarded after analysis. The results were disseminated to the health care providers to aid in patient care.

# CHAPTER FOUR

## RESULTS

During the study period extending from August to September 2016, 190 patients were recruited and subsequently interviewed, anthropometric measurements taken and blood samples drawn for analysis of serum magnesium levels, creatinine and glycated haemoglobin levels.

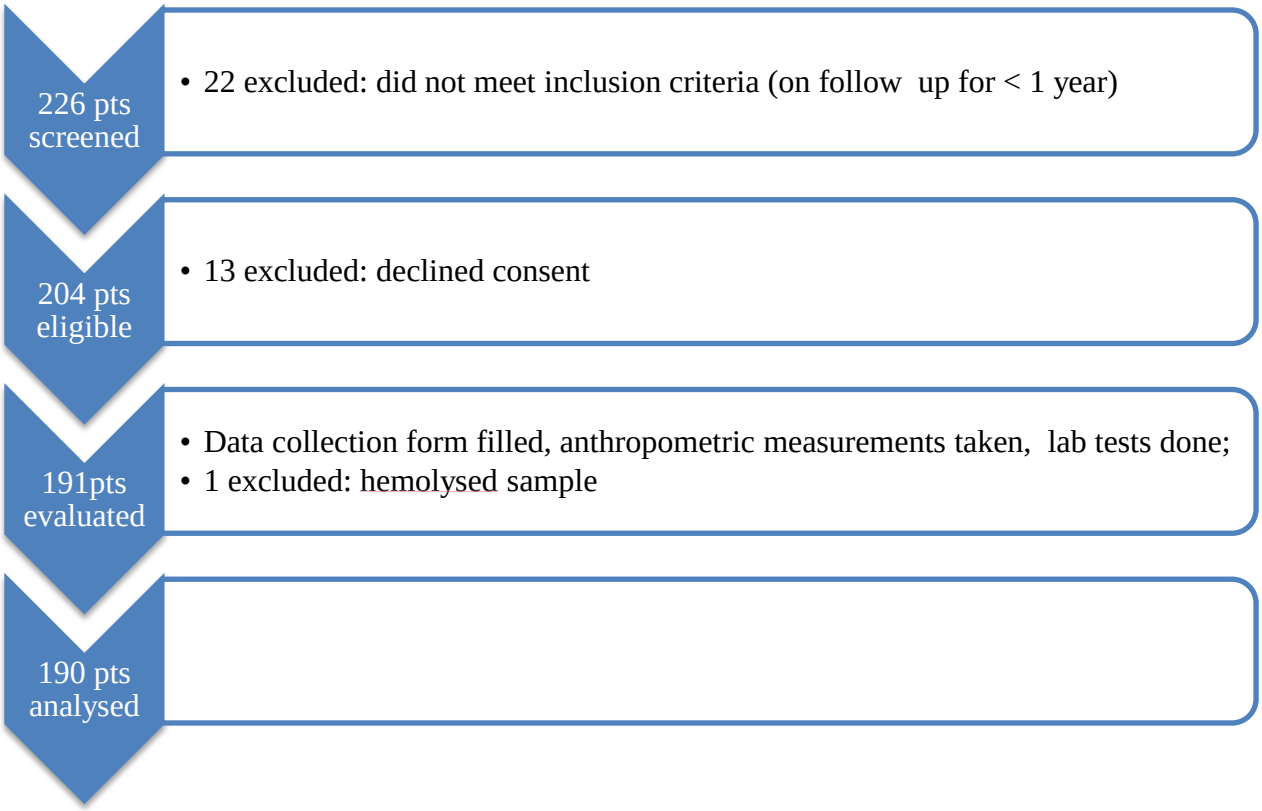



**Figure 7: The recruitment process**

## 4.1 Socio-demographic characteristics

Serum magnesium levels were evaluated in a total of 190 type 2 diabetes mellitus patients aged between 32 and 94 years. Age was normally distributed with mean (SD) age of 59.2 years (12.3 years).

More than half (57.4%) of the patients were female and about two thirds (66.8%) were employed (**Table 4 below**). A majority had either secondary (40%) or primary (38.9%) education. Over three-quarters (87.4%) of them were married while 2.6% were single.

**Table 4: Summary of patients' socio-demographic characteristics**

| Variable | Category | Frequency (n=190) | Proportion |
|---|---|---|---|
| **Age groups** | <45 | 29 | 15.3 |
| | 45-59 | 65 | 34.2 |
| | 60-74 | 75 | 39.5 |
| | ≥75 | 21 | 11.1 |
| **Gender** | Male | 81 | 42.6 |
| | Female | 109 | 57.4 |
| **Employment status** | Employed | 127 | 66.8 |
| | Unemployed | 63 | 33.2 |
| **Highest level of education** | None | 15 | 7.9 |
| | Primary | 74 | 38.9 |
| | Secondary | 76 | 40.0 |
| | Tertiary | 25 | 13.2 |
| **Marital status** | Single | 5 | 2.6 |
| | Married | 164 | 87.4 |
| | Divorced/ Widowed/ Separated | 21 | 10.0 |

## 4.2 Clinical profile of patients

Majority (80.5%) of the patients in this study had BMI above the normal; 41.1% were overweight and 39.4% were obese. Patients with normal BMI represented 17.9% of this population. 53.2% (101/190) of the patients had elevated blood pressure at the time of the study.

**Table 5: Summary of patients BMI and BP at recruitment**

| Variable | Category | Frequency | Proportion (%) |
|---|---|---|---|
| **BMI ($Kg/M^2$) (n=190)** | Underweight (<18.5) | 3 | 1.6 |
| | Normal weight (18.5-24.9) | 34 | 17.9 |
| | Overweight (25-29.9) | 78 | 41.1 |
| | Obese (≥30) | 75 | 39.4 |
| **Blood pressure (mmHg) (n=190)** | Normal (≤140/90) | 89 | 46.8 |
| | Elevated (>140/90) | 101 | 53.2 |

The duration of time since the diagnosis of diabetes ranged from 1 to 48 years with a median (IQR) duration of 10 years (4years- 16 years). Of the 190 patients, 72.6% (138/190) had been living with diabetes for more than 5 years.

Almost all the patients (186/190) were under medication for sugar control. Among the 186 patients under medication, 87 (46.8%) were taking oral hypoglycemic agents (OHAs) alone, 77 (41.4%) were on combination therapy (OHA & insulin) and the remaining 22 (11.8%) were using insulin alone.

**Table 6: Summary of diabetes history**

| Variable | Response | Frequency | Proportion (%) |
|---|---|---|---|
| **Duration of diabetes illness (n=190)** | <5 | 52 | 27.4 |
| | 5-10 | 49 | 25.8 |
| | >10 | 89 | 46.8 |
| **Any medication for sugar control? (n=190)** | Yes | 186 | 97.9 |
| | No | 4 | 2.1 |
| **Type of medication for sugar control (n=186)** | OHA | 87 | 46.8 |
| | Insulin | 22 | 11.8 |
| | OHA & Insulin | 77 | 41.4 |

More than three quarters (149/190; 78.4%) of the patients had a documented diagnosis of hypertension. The duration of illness ranged from 1 to 42 years, with a median (IQR) duration of 9 years (5years – 15 years). More than half (101/190; 67.8%) of the patients had been living with hypertension for more than 5 years.

Out of the 149 patients with hypertension, 58 (38.9%) reported taking diuretics. Majority (48/58; 82.8%) were on Thiazide and the remaining 10 (17.2%) on Loop diuretics.

**Table 7: Summary of hypertension history**

| Variable | Response | Frequency | Proportion (%) |
|---|---|---|---|
| **Documented history of hypertension?** | Yes | 149 | 78.4 |
| | No | 41 | 21.6 |
| **Any medication for BP control?** | Yes | 149 | 78.4 |
| | No | 41 | 21.6 |
| **Any diuretic therapy?** | Yes | 58 | 38.9 |
| | No | 91 | 61.1 |
| **Diuretic prescribed (n=58)** | Loop | 10 | 17.2 |
| | Thiazide | 48 | 82.8 |

### 4.2.1 Laboratory findings

Serum magnesium levels in this patient population ranged from 0.43mmol/L to 1.46mmol/L. The distribution of serum magnesium levels was right skewed with a peak at 0.68mmol/L. The median (IQR) serum magnesium concentration in this population was 0.8mmol/L (0.71mmol/L-0.92mmol/L).

Glycated hemoglobin level varied from 4.8% to 16.0%. Three quarters of the patients had glycated hemoglobin concentration above 7.2%. The median (IQR) glycated hemoglobin level was 8.9% (7.2%-11.4%). Estimated glomerular filtration rate was normally distributed with a mean (SD) of 68.1 ml/min/1.73m$^2$ (22.5 ml/min/1.73m$^2$).

The prevalence of hypomagnesemia in this patient population was 12.1% (95% CI 7.9 – 16.8%). Many patients (149/190; 78.4%) had poor glycaemic control. Less than half of the patients (91/190; 47.9%) had eGFR between 60-89, 23.2% (44/190) between 45-59, 14.7% (28/190)

above 90 and the remaining 27 below 45. There were no patients with eGFR less than 15 in this population.

**Table 8: Summary of laboratory findings**

| Variable | Category | Frequency | Proportion (%) |
|---|---|---|---|
| **Serum magnesium levels (mmol/L) (n=190)** | Hypomagnesemia (<0.66) | 23 | 12.1 |
| | Normomagnesemia (0.66-1.07) | 154 | 81.1 |
| | Hypermagnesemia (>1.07) | 13 | 6.8 |
| **Glycated hemoglobin (%) (n=190)** | Good (≤7%) | 41 | 21.6 |
| | Poor (>7%) | 149 | 78.4 |
| **Estimated glomerular filtration rate (ml/min/1.73m$^2$)** | Stage 1 (≥90) | 28 | 14.7 |
| | Stage 2 (60-89) | 91 | 47.9 |
| | Stage 3a (45-59) | 44 | 23.2 |
| | Stage 3b (30-44) | 19 | 10.0 |
| | Stage 4 (15-29) | 8 | 4.2 |

### 4.2.2 Relationship between serum magnesium and patients' profile

Pearson chi-square tests of association were done to evaluate the crude association between serum magnesium levels and patient profile (glycaemic control, eGFR, BMI, duration of diabetes illness, blood pressure, age, gender and type of diuretic therapy). Serum magnesium level was found to be significantly associated with: glycaemic control **(p = 0.029)**, gender **(p = 0.019),** diuretic therapy **(p < 0.001)** and mean duration of diabetes **(p = 0.014)**.

**Table 9: Association between serum magnesium and patients' profile**

| Variable | Category | Hypo-magnesemia | Normo/hyper Magnesemia | Pearson Chi-sq | P-value |
|---|---|---|---|---|---|
| **Glycaemic control** | Good | 9 | 32 | 4.764 | **0.029** |
| | Poor | 14 | 135 | | |
| **Diuretic therapy** | Loop | 5 | 5 | 16.382 | **<0.001** |
| | Thiazide | 2 | 46 | | |
| **Gender** | Male | 15 | 66 | 5.458 | **0.019** |
| | Female | 8 | 101 | | |
| **Mean duration of diabetes** | | 15.0 ± 11.0 | 11.0 ± 8.1 | | **0.014** |
| **eGFR** | Stage 1 | 2 | 26 | 2.555 | 0.645 |
| | Stage 2 | 12 | 79 | | |
| | Stage 3a | 4 | 40 | | |
| | Stage 3b | 4 | 15 | | |
| | Stage 4 | 1 | 7 | | |
| **BMI** | Underweight | - | 3 | * | * |
| | Normal | 4 | 30 | | |
| | Overweight | 7 | 71 | | |
| | Obese | 12 | 63 | | |
| **Blood pressure** | Normal | 12 | 77 | 0.299 | 0.585 |
| | Elevated | 11 | 90 | | |
| **Age group** | <45 | 1 | 28 | 4.210 | 0.240 |
| | 45-59 | 7 | 58 | | |
| | 60-74 | 13 | 62 | | |
| | ≥75 | 2 | 19 | | |
| **DM treatment** | OHA | 7 | 70 | 0.462 | 0.794 |
| | Insulin | 3 | 19 | | |
| | Both | 10 | 77 | | |

* *Test of association not done due to one empty cell*

A multiple logistic regression model was fit to evaluate the adjusted effect of selected patient baseline characteristics on the odds of having hypomagnesemia. Gender (p-value=0.039), glycemic control (p-value=0.005) and diuretic type used (p=0.014) were found to be significant after adjusting for the effect of other covariates. A female patient has 73% reduced odds of having hypomagnesemia relative to a male patient after adjusting for the effect of other covariates. In addition, a patient with poor glycemic control had 84% reduced odds of having hypomagnesemia relative to one with good glycemic control. Adjusting for the effect of other covariates, a patient who has been using thiazide diuretic has 95% reduced odds of having hypomagnesemia relative to one who has been using Loop diuretic.

**Table 20: Association between serum magnesium and patients' profile using multiple logistic regression**

| Covariate | Category | Odds Ratio | [95% CI] | P-value |
|---|---|---|---|---|
| Gender | **Male (ref)** | **1** | | |
| | Female | 0.27 | [0.08-0.93] | **0.039*** |
| Glycemic Control | **Good(ref)** | **1** | | |
| | Poor | 0.16 | [0.05-0.58] | **0.005**** |
| Diuretic used | **Loop (ref)** | **1** | | |
| | None | 0.19 | [0.03-1.34] | 0.095 |
| | Thiazide | 0.05 | [0.01-0.55] | **0.014*** |

# CHAPTER FIVE

## DISCUSSION

Our study population was predominantly mature adults; with a female preponderance at 57.4%, the mean (SD) age was 59.2 (12.3) years and the duration of diabetes was 11.5 years on average. 21.6% had achieved good glycaemic control ($HbA_1c$ less than 7%) and 37.4% had poor renal function reserve with Stage 3 CKD and beyond. 78.4% of the patients were hypertensive and 38.9% of them were on diuretic therapy.

This study evaluated 190 patients overall. The prevalence of hypomagnesemia defined as serum magnesium levels below 0.66mmol/L was found to be 12.1% with a mean (SD) serum magnesium concentration of 0.83mmol/L (0.15mmol/L). Serum magnesium concentration is closely regulated within the range of 0.7 - 1.0mmol/L (1.5 - 2mEq/L; 1.7 - 2.4mg/dL) but in our study, we used the magnesium colorimetric assay kit (*Xylidyl Blue-I Method)* which had a kit-dependent reference range of 0.66 - 1.07mmol/L. Studies have shown a significant fall in serum magnesium levels among diabetes patients compared with non-diabetic controls; the reasons for this are multi-factorial including inadequate magnesium intake, reduced magnesium intestinal absorption or hypermagnesuria (8). The recorded prevalence rates of hypomagnesemia range from 11-47.7% among patients with type 2 diabetes compared to 2.5 – 15% in the general population (7, 9).

Studies done in different African countries give varying prevalence. The Ethiopian study by Seyoum *et al*, recorded an overall prevalence of 65% with mean (SD) serum magnesium of 0.86mmol/L (0.02mmol/L). In this specific study they included both type 1 and 2 diabetes

patients; type 1 diabetes patients were found to have significantly lower magnesium levels compared to patients with type 2 diabetes (68). Insulin stimulates magnesium conservation in the loop of Henle and distal convoluted tubule therefore with insulin deficiency which is characteristic of type 1 diabetes there is increased urinary magnesium excretion. This could have contributed to the high prevalence recorded, although the mean serum magnesium levels were comparable to what we obtained in our study. Dietary differences could also explain this observation, at that time there was widespread famine and drought in Ethiopia rendering a large portion of the population to a magnesium-deficient diet.

Another study carried out in Nigeria by Ajibola *et al*, revealed a prevalence of 18.22% with a lower mean (SD) of 0.56mmol/L (0.20mmol/L); while Ramachandra *et al* in India found a prevalence of 21.7% with a mean (SD) of 0.79mmol/L (0.10mmol/L). The lack of concordance in results with the other studies may be as a result of the different population profiles with different dietary habits and choices, use of different laboratory methods as well as geographical / environmental and genetic differences. For instance, green leafy vegetables, grains and nuts which are a rich source of magnesium, are readily available and quite affordable in our setting. This could explain our lower prevalence and high mean serum magnesium levels although we did not set out to collect any dietary information, so this remains a speculation.

More than half of the study participants (19/23; 82.6%) of the study participants with hypomagnesemia had lived with diabetes for a period of at least 5 years. On further analysis, the mean (SD) duration of diabetes among patients with hypomagnesemia was 15 years (11.0 years) and 11.0 years ( 8.1 years) in patients with normal or high magnesium levels **(p = 0.014).** This implies that duration of diabetes could have an indirect relationship with serum magnesium

levels especially if the diabetes is poorly controlled. Patients who have had diabetes for long periods are likely to have an element of diabetes nephropathy hence are more prone to excess urinary magnesium loss. In 2015, Arpaci *et al* in Turkey showed a similar pattern with hypomagnesemic patients having a longer mean (SD) duration of illness; 8.58 years (7.92 years) compared to 6.5 years (7.08 years) among patients with normal magnesium levels (76); albeit the longer duration of diabetes in both patient groups in our study compared to the Turkey study.

Serum magnesium level was found to be significantly associated with diuretic therapy **(p-value <0.001)**; 30.4% of the patients with hypomagnesemia were on diuretic therapy. Rampant use of diuretics promotes urinary magnesium wasting. Loop diuretics inhibit the electrical gradient necessary for magnesium reabsorption in the thick ascending loop of Henle hence causing magnesium depletion especially with chronic use. Long term use of thiazide diuretics can cause substantial magnesium depletion due to secondary hyperaldosteronism, increased sodium load and interaction with calcium metabolism as well as causing reduced renal expression levels of the epithelial magnesium channel TRPM6 (77).

The glycaemic control in our study population was relatively poor with only 21.6% of the patients achieving $HbA_1c$ of 7% and below. This reflects the challenges that exist in management of diabetes where achieving glycaemic control requires a multi-disciplinary approach involving nutritional and social support among others.

60.9% of our patients with hypomagnesemia had $HbA_1c$ of 7% and above; with a mean of $HbA_1c$ 8.5%. This was comparable to a mean of 8.3% recorded by Xu J *et al* in a study done among type 2 diabetes patients in China (78). We also found that patients with poor glycaemic control were more likely to have hypomagnesemia compared to those with good glycaemic control **(p =**

**0.029)**. This could be explained by the fact that poor glycaemic control and glycosuria increase magnesium excretion via osmotic diuresis. In addition, glycosuria also impairs renal tubular magnesium reabsorption. There is an established inverse relationship between serum magnesium levels and glycaemic control (9). However, it is important to note that the poor glycaemic control can also be attributed to poor compliance to drugs as well.

The mean eGFR among the hypomagnesaemic patients was 72.7 (20.3) ml/min/1.73m² compared to 77.9 (26.8) ml/min/1.73m² among the patients who had normal magnesium levels. These findings differed with those of a study done in Turkey by Arpici *et al*. In this Turkish study, they found a mean eGFR of 115.3 ± 3.70 ml/min/1.73m² among the hypomagnesaemic patients compared to 118.0 ± 1.3 ml/min/1.73m² in normomagnesemic patients. This could mean that their study population was still in the 'early' hyperfiltration stage of diabetic nephropathy.

In a study done among the Chinese with type 2 diabetes, Xu J *et al* found a mean eGFR of 85.7 ml/min/1.73m² in those with hypomagnesemia compared to 94.0 ml/min/1.73m² among those with normal serum magnesium levels (78). This difference could be due to the poor glycaemic control with increased likelihood of developing diabetes kidney disease.

Hypomagnesemia is associated with accelerated loss of kidney function among diabetics (79). There have been controversial views regarding the relationship between microalbuminuria and magnesium deficiency. Some studies demonstrated a significant reduction in serum magnesium levels among diabetic cases with microalbuminuria (48); while others revealed that microalbuminuria and overt proteinuria did not affect serum magnesium levels (80). Although we were unable to assess for microalbunimuria; the eGFR trends we obtained indicate that hypomagnesemia was actually associated with a decline in eGFR.

# CONCLUSION

Although the prevalence recorded appears to be low compared to studies done in other regions, there is still a significant burden of hypomagnesemia among our diabetic patients. Patients with hypomagnesemia were noted to have poorer glycaemic control and a longer mean duration of diabetes.

# RECOMMENDATIONS

Larger and longitudinal studies to determine the direction of association between hypomagnesemia and: glycaemic control and renal function reserve.

# LIMITATIONS

This was a cross-sectional study hence no causal inference or temporal association could be drawn. It would have been ideal to compare the serum magnesium levels obtained in our study with those generated from the local population; however there is lack of locally generated data on serum magnesium. We were unable to investigate for causes of hypomagnesemia, poor glycaemic control and renal function reserve among our diabetic patients due to limited resources. This was a single centre study with a relatively small sample size so these results may not be generalisable to the entire population of patients with type 2 diabetes in Kenya.

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# APPENDIX 1: INFORMED CONSENT

## Informed consent for Serum Magnesium Levels in Type 2 Diabetes Mellitus

### INTRODUCTION

I am undertaking a study investigating magnesium levels in patients with Type 2 Diabetes Mellitus. This study is part of my university requirements. The results of this study will be used to offer recommendations which, if implemented, may result in improved management and quality of life of patients with Type 2 Diabetes.

This form is to give you the information you need before deciding on whether or not to participate in the study. As you read this study explanation form you may ask any questions or raise any concerns on what you do not understand.

**Purpose of the study**

I am carrying out a study amongst Type 2 diabetes patients to measure their magnesium levels and establish whether there is any association between magnesium levels, blood sugar control and kidney function.

**Procedures to be followed in the study**

Upon agreeing to participate in the study, you will sign the consent form. After which you will be asked questions regarding your medical condition as per the study questionnaire. Thereafter, approximately 6mls of blood will be withdrawn for purposes of laboratory analysis (2mls each for each test - magnesium levels, blood sugar control and kidney function analysis).

**Benefits to you as a participant**

Your participation in the study and the laboratory tests done are free of charge but the findings will be used for your individual benefit. Information obtained will improve knowledge amongst health care providers at the Kenyatta National hospital.

**Risks and discomforts**

You may feel slight pain/ discomfort when the blood sample is drawn. There may be slight swelling at the site of the needle prick, but this will disappear by itself after a few days. The amount of blood that will be drawn will not affect your health.

**Your rights as a participant**

Your participation in this research is a personal choice; if you refuse to participate in this study, your treatment will not be affected. If you choose to participate and not answer certain questions, you will not be penalized. You are free to terminate the interview and withdraw from the study at any time. You are free to ask questions before signing the consent form.

**Assurance of confidentiality**

All your responses as well as your results will remain confidential. Your individual responses will be stored in a locked place under my control and will only be seen by my statistician and myself.

**Contacts**

In case you need to contact me, my academic department or the Kenyatta National Hospital / University of Nairobi Ethics and Research Committee concerning this study, please feel free to do so using the contacts provided below.

I humbly request you to sign the consent form attached.

## Consent form

I have read the above information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant: ……..……. Date…….

Signature / Left thumbprint of subject: ……..……

**INVESTIGATOR'S STATEMENT:**

I, the Principal Investigator, have fully informed the research participant on the purpose and implication of this study.

Signed: ..... Date: ……

**CONTACTS**

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**Ethical Approval**

Kenyatta National Hospital /University of Nairobi Ethics and Research Committee,

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# APPENDIX 2: FOMU YA MAELEZO YA UTAFITI

**Fomu ya maelezo ya utafiti wa kiwango cha madini ya magnesi katika wagonjwa wenye ugonjwa wa kisukari**.

**Utangulizi**

Ninatarajia kufanya uchunguzi kuhusu kiwango cha ma dini ya magnesi katika wagonjwa wenye ugonjwa wa kisukari na ningependelea uhusike. Utafiti huu unahitajika kama sehemu ya masomo yangu lakini matokeo yatakayopatikana yatatumiwa kutoa maelezo, ambayo ikiwa itatumika italeta manufaa katika matibabu na hali ya maisha ya wagonjwa wa kisukari.

Fomu hii ni ya maelezo yote utakayohitaji ukiamua kama utajiunga na utafiti huu. Unapoisoma na baada ya kusoma fomu hii, uko huru kuuliza maswali yoyote kama kuna sehemu hujaelewa vyema.

**Je, utafiti huu unalenga kutambua nini?**

Ninafanya utafiti huu ili kudhibitisha kiwango cha madini ya magnesi katika wagonjwa wanaougua ugonjwa wa kisukari; na vile ambavyo kiwango cha madini haya yanalingana na kipimo cha sukari mwilini na utendakazi wa figo mwilini.

**Utaratibu wa utafiti:**

Mara utakapokubali kuhusika kwenye utafitu huu, utatia sahihi katika fomu ya ridhaa na matakwa ya utafiti. Itabidi ujibu maswali ya kibinafsi utakayoulizwa kisha utachunguzwa kimwili. Tutahitaji kuondoa mililita sita au kijiko moja ndogo ya damu (mililitambili kwa kila kipimo – kipimo cha kiwango cha magnesi, kipimo cha kiwango cha sukari mwilini na kipimo cha utendekazi wa figo mwilini).

**Manufaa ya utafiti huu**

Hakuna pesa utahitajika kulipa kwa kujihusisha kwa utafiti huu. Matokeo ya vipimo hivi vya madini ya magnesi, kipimo cha sukari na kipimo cha figo vitakufaidi kibinafsi. Matokeo ya utafiti yatasaidia wauguzi katika hospitali ya Kenyatta.

**Hatari na gharamainayohusika**

Unaweza hisi uchungu kidogo wakati damu inachukuliwa. Mahali unapodungwa panaweza fura kidogo, lakini itaisha yenyewe baada ya siku chache. Damu itakayoondolewa ni kidogo na haitakudhuru kiafya.

**Haki zako**

Kujiunga na utafiti huu ni kwa hiari yako. Hutabaguliwa kimatibabu ukikataa kujiunga na utafiti huu. Ukijiunga na utafiti huu na ushindwe kujibu mojawamo au maswali mengine tutakayouliza, ni sawa. Una uhuru wa kutoka kwenye mahojiano na kujitoa kwa utafiti huu wakati wowote. Una uhuru wa kuuliza maswali yoyote uliyo nayo kabla ya kutia sahihi fomu ya makubaliano. Maelezo yako yote yatawekwa pahali pa siri. Ni mtafiti mkuu na mwanatakwimu wake pekee ambao wataangalia maelezo yako.

## Cheti cha ridhaa

Nimesoma, au nimesomewa maelezo yaliyopewa. Nimepata fursa ya kuuliza maswali kuhusu utafiti na maswali yote niliyouliza yamejibiwa vyema. Ninakubali kuhusika katika utafiti huu.

Jina la mhusika: ……

Sahihi/Alama ya kidole gumba cha kushoto: ……Tarehe: ……

**KAULI YA MTAFITI:**

Miye, mtafiti mkuu, nimemweleza mhusika vilivyo kuhusu utafiti huu.

Sahihi: ..... Tarehe: ……..

**MAWASILIANO**

Ukiwa na maswali yoyote ya ziada, unaweza kuwasiliana na wafuatao:

**Taasisi:**Idhaa ya matibabu ya watu wazima, Chuo cha sayansi ya afya, Chuo kikuu cha Nairobi S.L.P. 30197-00400, Nairobi.

**Mtafitimkuu:**Dkt. Wanjiru Kibe, S.L.P. 30197-00400, Nairobi. Idhaa ya matibabu ya watu wazima, Simu – 072348665; shiro1018@gmail.com

**Msimamizi mkuu:**Dkt A.J.O. Were, Idhaa ya matibabu ya watu wazima, S.L.P. 30197-00400 Nairobi, Simu - 0722711444

**Ridhaa:** Kenyatta National Hospital /University of Nairobi Ethics and Research committee, S.L.P. 20723-00100, Nairobi. Tel 2726300/2716450 Ext 44102; uonknh_erc@uonbi.ac.ke

# APPENDIX 3: DATA COLLECTION TOOL

**SERUM MAGNESIUM LEVELS IN AMBULANT PATIENTS WITH TYPE 2 DIABETES MELLITUS ATTENDING THE DIABETES OUTPATIENT CLINIC AT THE KENYATTA NATIONAL HOSPITAL**

1. Study no ……. Date……..

2. Hospital no …….

3. Participant initials ……..

4. Participant's contacts ……

5. Age (in years) ……

6. Sex: Male ……. Female ……

7. Marital status: Single ……Married …... Separated...…. Divorced...…. Widowed ……..

8. Level of education: None …... Primary ……. Secondary ……. Tertiary …….

9. Occupation: Employed (Self / Non self)....…. Unemployed ……...

10. History of Diabetes:

When was the diagnosis made? ……Duration of Diabetes since diagnosis……

11. Any medications for sugar control? Yes …..... No…...….

12. If yes, what is the current treatment modality for Diabetes?

a) Oral Hypoglycemic Agents (OHAs) only ……..

b) Insulin only ……..

c) Both (OHAs and Insulin)…….……

13. Documented file diagnosis of hypertension: Yes ……No…….……

14. If yes, duration of hypertension since diagnosis …….years

15. Any medication for Blood Pressure control? Yes …… No……..

16. If yes, any Diuretic therapy? Yes…… No…….

17. If yes, Loop…… Thiazide ……

18. Weight in kilograms …… Height in meters……. BMI in kg/$m^2$.....

19. BP measurement in mmHg ……

20. Fasting Blood Sugar done on the clinic day ……..

**LABORATORY RESULTS**

1. Serum Magnesium measured in mmol/L……..

2. Serum HbA1c measured as a %.....

3. Serum Creatinine measured in µmol/L……

4. Estimated GFR (eGFR) in ml/min/1.73m²…….

# APPENDIX 4: LABORATORY REQUEST FORM

**SERUM MAGNESIUM LEVELS IN AMBULANT PATIENTS WITH TYPE 2 DIABETES MELLITUS ATTENDING THE DIABETES OUTPATIENT CLINIC AT THE KENYATTA NATIONAL HOSPITAL**

Study no …….  Date……..

Hospital no …….

Participant initials……..

Age (in years) ……

Sex: Male ……. …  Female ……

**LABORATORY RESULTS**

| | LABORATORY TEST | LABORATORY RESULT |
|---|---|---|
| 1. | Serum Magnesium (mmol/L) | |
| 2. | Serum Creatinine (µmol/L) | |
| 3. | Serum HbA1c (%) | |

Reported by …….

Signed…..…….  Date……..