

بسم الله الرحمن الرحيم

**Evaluation of Transthyretin Test as Indicator of Nutritional Status and
Glycemic Control of Type 2 Diabetic Patients
Jaber Abu Aliz Diabetic Center in Khartoum State, Sudan (2018)**

Hub Eldin Taha Ahmed

**(B.Sc: in Clinical Chemistry (1997), Faculty of Health Sciences, Omdurman
Ahlia University, M.Sc: in Clinical Chemistry (2013), Faculty of Medical
Laboratory Sciences, Omdurman Islamic University)**

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**Evaluation of transthyretin test as indicator of nutritional status and control
in type 2 diabetic patients'**

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Hub Eldin Taha Ahmed Abdallah

Supervision committee

Name	Position	Signature
Professor. Omer Fadl Idris	Main Supervisor
Dr. Khanssa Mohammed Elamin	Co- Supervisor
Dr. Shams Eldin Mohamed Ahmed	Co- Supervisor

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Examination committee

Name	Position	Signature
Professor. Omer Fadl Idris	Chair Person	
Professor. Essam Ahmed Mohammed	External Examiner	
Dr. Bakry Yousef M. Noor	External Examiner	

آيه قرانية



﴿اللَّهُ الَّذِي خَلَقَ سَبْعَ سَمَاوَاتٍ وَمِنَ الْأَرْضِ مِثْلَهُنَّ
يَتَنَزَّلُ الْأَمْرُ بَيْنَهُنَّ لِتَعْلَمُوا أَنَّ اللَّهَ عَلَىٰ كُلِّ شَيْءٍ قَدِيرٌ
وَأَنَّ اللَّهَ قَدْ أَحَاطَ بِكُلِّ شَيْءٍ عِلْمًا﴾
صدق الله العظيم

سورة الطلاق آيه (١٢)

Declaration

I hereby declare that this Ph.D. thesis entitled “Evaluation of transthyretin test as indicator of nutritional status and control in type 2 diabetic patients’ Jaber Abu Aliz Diabetic Center (JAZC in Khartoum State)” was carried out by me for the degree of Doctor of Philosophy in Clinical Pathology under the guidance and supervision of Professor. Omer Fadl Idris, and Dr. Shams Eldin Mohamed Ahmed

, Institute of Advanced Studies in English, University of Pune, India. The interpretations put forth are based on my reading and understanding of the original texts and they are not published anywhere in the form of books, monographs or articles. The other books, articles and websites, which I have made use of are acknowledged at the respective place in the text. For the present thesis, which I am submitting to the University, no degree or diploma or distinction has been conferred on me before, either in this or in any other University.

Place: Khartoum

(Mr. Hub Eldin Taha Ahmed)

Date: July 2018

Research Student

Dedication

This dissertation is dedicated to my family. For inspiration to pursue my doctoral degree,

Thank you to my academic adviser who guided me in this process and the committee who kept me on track.

I dedicate this dissertation to my sisters, who inspired my pursuit of economics.

For my parents, who helped me in all things great and small.

This dissertation is dedicated to my wife who encouraged me to pursue my dreams and finish my dissertation.

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Evaluation of Transthyretin Test as Indicator of Nutritional Status and Glycemic Control of Type 2 Diabetic Patients

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Hub Eldin Taha Ahmed Abdallah

Abstract

The aim of this study is to evaluate of transthyretin test as indicator of nutritional status and control in type 2 diabetic patients' TTR, FBS, and HbA_{1c}, and were measured in blood and serum, and microalbumin were measured in urine of 128 male and 257 females in Khartoum state. 5 ml of blood in SSTgel tube, and another 5 ml in EDTA₃ anticoagulant tube, fresh urine random specimen were collected from the volunteers. The volunteers were classified in sex groups (257 F, 128 M), aged (31 <40 Y/O and 354 >40 Y/O), with and without blood hypertension (211 DM and 174 DM/BP), microalbuminuria (183 negative and 202 positive), duration (163 < 5 years, 83 (5-10 Y/O), 58 (10-15 Y/O), 81 > 15 years), types of treatment (30 diet, 262 antihypoglycemic, 61 insulin, 32 insulin+anti-hypoglycemic). TTR in female compared to male were significantly different, ($p = 0.001$). TTR in microalbuminuria, were significantly different, ($p = 0.006$). FBS Duration ($p = 0.035$), FBS Duration < 5 years vs between 5 – 10 years ($p = 0.036$), FBS Duration < 5 years vs between 10 – 15 years ($p = 0.014$), FBS Duration < 5 years vs >15 years ($p = 0.027$), Correlation of FBS to HbA_{1c}, Pearson coefficient $r (.523)$, ($p = 0.000$). Correlation of FBS to sex, Kendall's tau_b coefficient $r (.150)$, ($p = 0.000$). Correlation of FBS to DMBP, ($p = 0.007$). HbA_{1c} Duration ($p = 0.006$), HbA_{1c} Treatment ($p = 0.026$), HbA_{1c} Duration < 5 years vs between 5 – 10 years ($p = 0.005$), HbA_{1c} Duration < 5 years vs between 10 – 15 years ($p = 0.014$), HbA_{1c} Treatment Diet vs oral hypoglycemia ($p = 0.039$), HbA_{1c} Treatment Diet vs Insulin+Oral hypoglycemia ($p = 0.006$). Correlation of HbA_{1c} to sex, ($p = 0.001$). Correlation of HbA_{1c} to DMBP, ($p = 0.013$). Treatment vs Duration ($p = 0.000$). Correlation of DMBP to sex, ($p = 0.033$). Correlation of DMBP to age, ($p = 0.000$). The results of this study revealed that TTR is act as management, control and nutritional biomarker test for type2 DM patients, TTR is verifying and accurate for managing and controlling type 2 diabetes mellitus..

تقييم إختبار الترانزثايريتين لحالة التغذية و التحكم لدي مريض السكري من النوع الثاني

حب الدين طه أحمد عبد الله

ملخص الدراسة

هدف هذه الدراسة هو تقييم مستوي الترانزثايريتين في الدم مع إدارة وضبط السكر لمرضي السكري النوع الثاني. تم قياس الترانزثايريتين ومستوي السكر في الدم و معدل السكر التراكمي في الدم. و مستوي الألبومين المصغر في البول ل ١٢٨ من الذكور و ٢٥٧ من الإناث في ولاية الخرطوم تم جمع ٥ ملليمتر من الدم في أنبوب خالي من مواد مضادة للتجلط من المتطوعين و ٥ ملليمتر أخرى في أنبوب مضاد للتجلط ($EDTA_3$)، كما جمعت كمية مناسبة من البول لإختبار الألبومين المصغ أثناء وجود المتطوع في المكان. المتطوعين في هذا البحث قسموا إلى ستة مجموعات حسب الجنس والعمر (٣١ أقل من الأربعين . و ٣٥٤ أكبر من الأربعين سنة) ووجود أو نفي الألبومين المصغر في البول . و مع أو بدون مصاحبة إرتفاع ضغط الدم المفرط. و قياس طول فترة الإصابة بمرض السكري النوع الثاني (أقل من ٥ سنوات ، بين ٥ إلى ١٠ سنوات ، من ١٠ إلى ١٥ سنة ، أكثر من ١٥ سنة) . ونع خطة العلاج (النظام الغذائي. مضادات إنخفاض السكر . الإنسولين. إستخدام مضادات إنخفاض السكر+الإنسولين) إرتفع مستوي الترانزثايريتين في النساء أكثر منه في الرجال، و إرتفع مستوي الترانزثايريتين في موجب المايكروالبيوم في البول مقارنة بسلبتي النتائج. إنخفاض مستوي السكر صائم في الدم في المرضى الذين أصيبوا بمرض السكري النوع الثاني في فترة أقل من خمسة سنوات مقارنة بالذين تعدوا ال ١٥ سنة بعد الإصابة. كما إنخفاض عند المعالجة بالإنسولين لوحده أكثر من المعالجة بالإنسولين المصاحب للأدوية خافضة السكر عن طريق الفم. إنخفاض معدل السكر التراكمي في الدم للمستخدمين للحمية أكثر من الذين يأخذون أدوية خافضات السكر عن طريق الفم. السكر صائم يوجد إختلاف بين تقديرات مدة المرض بالسنوات ، كما يوجد إختلاف للسكر صائم مع مرضي السكر مع أو بدون إرتفاع ضغط الدم.السكري التراكمي يوجد إختلاف بين تقديرات مدة المرض بالسنوات ، كما يوجد إختلاف بين نوعية العلاج وبين الحمية و إستخدام مضادات السكري مع أو بدون الإنسولين.يوجد إختلاف بين نوعية علاج مرض السكر و بين عدد سنوات المرض.يوجد إختلاف بين مرض السكري مع أو بدون إرتفاع ضغط الدم مع عنصر الجنس و العمر. أظهرت النتائج ملائمة لإستخدام الترانزثايريتين كمدير و متحكم لحالة التغذية لمرضى السكر النوع الثاني .

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List of Abbreviations

α : alpha.

A β : Amyloid- β .

Abs: Absorbance.

AD: Alzheimer's disease.

ADA: American Diabetes Association.

AP: acute pancreatitis.

β : beta.

BMI: Body Mass Index.

$^{\circ}\text{C}$: heat degree centigrade.

Ca^{2+} : calcium.

CA19-9: cancer antigen tumour marker.

CBG: Corticosteroid binding globulin.

CNS: Central Nervous System.

CP: chronic pancreatitis.

CRP: C-reactive protein

CSF: Cerebrospinal Fluid.

CVRF: Cardiovascular Risk Factors.

DCCT: Diabetes Control and Complications Trial.

DM/BP: Diabetic hypertensive status

E: Decimal system.

ELISAs: Enzyme-linked Immunosorbent Assays.

et al: et alia: and others.

FAP: Familial Amyloidotic Polyneuropathy.

FBS: Fasting Blood Sugar.

GDM: Gestational diabetes mellitus.

g/mg: gram per milligram.

Grps: glucose-regulated proteins

Hb: Hemoglobin.

HbA1c: Glycosylated hemoglobin A1c.

HDL: High Density Lipoprotein.

HIV/AIDS: Human immunodeficiency virus infection and Acquired Immune Deficiency Syndrome.

HNF: hepatic nuclear factor.

HRP: Horseradish Peroxidase.

hrs: Hours.

IAPP: Islet Amyloid Polypeptide.

ICU: Intensive Care Unit.

ID: Identification

IDDM: insulin-dependent diabetes mellitus.

IGF-IR: Insulin-like growth factor receptor I

IgG: Immunoglobulin G.

Insulin + Oral hypoglycemic: Oral antihypoglycemic combined with insulin therapy.

in vivo: Latin for "within the living"; often not italicized in English.

JAZC: Jaber Abu Alzi Diabetic Center.

kDa: Kilo Dalton.

KO mice: A knockout mouse is a genetically engineered mouse in which researchers have Inactivated.

LBM:lean body mass.

MA: Microalbumin.

MAU: Microalbuminuria.

mcg/mg: micrograms into milligrams.

mg/24 h: milligram per 24 hours.

mg/dl: milligram per deciliter.

mg/l: milligram per liter.

mg: milligram.

mg/g: milligram per gram.

ml: milliliter.

mm: millimeter.

MODY: Maturity onset diabetes of the young.

MRDM: Malnutrition-related diabetes mellitus.

MW molecular weight.

mW: milliwatt.

NGSP: National Glycohemoglobin Standardization Program.

NIDDM: non-insulin dependent diabetes mellitus.

NIH3T3: Standard fibroblast cell line, used in the cultivation of keratinocytes.

nm: nanometer.

NSAIDs: Nonsteroidal anti-inflammatory drugs.

NST: Nutrition Supporting Team.

PA: prealbumin.

PCM: Protein calorie malnutrition.

PDAC: Pancreatic ductal adenocarcinoma.

PEM: Protein Energy Malnutrition.

PINI: prognosis inflammatory and nutritional indexes.

r : Pearson coefficient.

r_s : correlation spearman rho coefficient.

R^2 : Adjusted R.

RBP: Retinol-Binding Protein.

RCF: Relative Centrifugal Force unit.

Sepharose CL-6B: Well proven cross-linked agarose chromatography base matrix

sig: significant.

SGA C: Subjective Global Assessment (Severe malnutrition)

SPSS: Statistical Package for the Social Sciences.

SST gel: Serum-Separating.

STAT: Latin word statum, meaning 'immediately.

Std: Standard.

TBG: Thyroxine-binding globulin.

T1D: type 1 diabetes

T2D: type 2 diabetes

T2DM: Type 2 Diabetes Mellitus.

T_3 : triiodothyronine, thyroid hormones.

TM: Trade Mark.

TTR: Transthyretin.

µg/mg: microgram per gram.

µg/min: microgram per minute.

μ l : microliter.

UKPDS: United Kingdom Prospective Diabetes Study.

VIPomas: vasoactive intestinal peptide, rare tumor endocrine originate in non-beta cell pancreas

vs: versus.

Wks: weeks.

yrs: years.

\geq : equal or more than.

$>$: more than.

$<$: less than.

1:1: One to One ratio.

* : Multiply.

\pm : plus and minus.

\sim : nearly.

Chapter One

Introduction

Diabetes Worldwide It was estimated that in 2017 there are 451 million (age 18-99 years) people with diabetes worldwide. These figures were expected to increase to 693 million by 2045. (Cho NH and *etal*; Apr 2018). Management of diabetes by following diabetes meal plan (Bantle J.P and *etal*; 2006). Diabetes mellitus control is evaluated mainly by: Fasting glucose for 8 - 12 hours, which give a picture of glucose level for 24 hours. Postprandial glucose test two hours after first meal of the day which give a picture of glucose level part of the day.

Glycosylated hemoglobin is the test that give picture of glucose level through 2 - 3 months. Diabetes control means keeping the blood glucose levels as close to normal as possible can be a lifesaver (Gough, S, and *etal*; 2010). Diabetic Diet: foods that may help control blood sugar (Asif, M.; 2014). Tight control can prevent or slow the progress of many complications (Gubitosi-Klug, R.A. and DCCT/EDIC research group; 2014).

The control of diabetes just look for adjust the blood glucose level around the normal, regardless the patient nutrition status evaluation. The evaluation of diabetes status is mainly done by: Too long as Hb A_{1C} give a picture over 2 – 3 months. Or too short as fasting, and postprandial glucose test.

Transthyretin (TTR) is nutritional status biomarker used in evaluation of post-surgery patient (Chernecky, C.C. and Berger, B.J; 2007), decline with condition of malnutrition.

Transthyretin (TTR) is a plasma protein, mainly synthesized by the liver and also at certain minor locations, including the islets of Langerhans. Transthyretin has lifespan 2 - 4 days (Johnson, A.M., and *etal*; 2007) suitable time to check the nutritional status of diabetic patients (Beck, F.K. and Rosenthal, T.C; 2002). TTR is play a role in pancreatic alpha and beta cells.

Although transthyretin (TTR) is expressed in pancreatic alpha (glucagon) cells in islets of Langerhans, function of TTR in pancreatic alpha cells remains unknown (Su, Y., and *etal*; 2012). TTR expressed in pancreatic alpha cells may play important roles in glucose homeostasis via regulating the expression of glucagon. Lack of TTR induced significantly lower levels of glucagon in the islets of Langerhans. Role of alpha cells in diabetes is secretion of glucagon (Burcelin, R., and *etal*; 2008.). It is the major factor stimulating hepatic glucose output (LeRoith, D., and *etal*; 2004).

Rationale of the research:

Several researches, studies, papers, experiments were run on serum transthyretin give many conclusions to assess the nutritional status. Many results are advice and recommended for evaluate the nutritional control by new processes measuring serum transthyretin.

The Physicians are concerning the HbA_{1c} to evaluate the controlee's of diabetic patient, which will take about three months to evaluate. In quick survey in some hospitals in Khartoum state (Khartoum teaching hospital, Khartoum north teaching hospital, Omdurman teaching hospital, Ibn Aof hospital for pediatrics, Asia hospital, and Al-Baraha hospital) we found that the HbA_{1c} tests is the only test used to evaluate the control of diabetes.

Objectives

General objective:

To verify the assessment of diabetic type 2 controls and nutrition status by measuring transthyretin test.

Specific objectives:

1. To measure and determine the serum transthyretin, FBS, and HbA_{1c} in type 2 diabetic patient.
2. To assess nutrition status of diabetes using TTR.
3. To compare TTR with HbA_{1c}. FBS
4. To determine the microalbumin in type 2 diabetic patient.
5. To correlate between above and the duration of type 2 diabetic disease.

Chapter Two

Literature review

2.1 Diabetes mellitus:

Diabetes mellitus represents a heterogeneous group of metabolic disorders characterized by decreased insulin secretion, insulin action, or both (Hall, J.E. and Nieman, L.K; 2003).

2.2 Diabetes mellitus types:

2.2.1 Type 1 diabetes:

Previously referred to as insulin-dependent diabetes mellitus (IDDM): characterized by low or absent endogenous insulin production; and exogenous insulin dependence to prevent ketoacidosis. Onset is usually in youth or young adulthood, but can occur at any age (Westermarck, G.T. and Westermarck, P; 2008).

2.2.2 Type 2 diabetes:

Previously referred to as non-insulin dependent diabetes mellitus (NIDDM): insulin-resistant condition with an impaired insulin secretory component. Onset usually older in age, and often associated with obesity age (Westermarck, G.T. and Westermarck, P; 2008).

2.2.3 Gestational diabetes mellitus (GDM):

Glucose intolerance with onset or first recognition during pregnancy age (Westermarck, G.T. and Westermarck, P; 2008).

2.2.4 Latent Autoimmune Diabetes in Adults

Latent autoimmune diabetes in adults (LADA) is a disorder in which, despite the presence of islet antibodies at diagnosis of diabetes, the progression of autoimmune β -cell failure is slow. LADA patients are therefore not insulin requiring, at least during the first 6 months after diagnosis of diabetes. Among patients with phenotypic type 2 diabetes, LADA occurs in 10% of individuals older than 35 years and in 25% below that age. Prospective studies of β -cell function show that LADA patients with multiple islet antibodies develop β -cell failure within 5 years, whereas those with only GAD antibodies (GADAs) or only islet cell antibodies (ICAs) mostly develop β -cell failure after 5 years. Even though it may take up to 12 years until β -cell failure occurs in some patients, impairments in the β -cell response to intravenous glucose and glucagon can be detected at diagnosis of diabetes. Consequently, LADA is not a latent disease; therefore, autoimmune diabetes in adults with slowly progressive β -cell failure might be a more adequate

concept. In agreement with proved impaired β -cell function at diagnosis of diabetes, insulin is the treatment of choice. (Stenström, G., and etal; 2005)

2.3 Definition, diagnosis and classification of diabetes mellitus and its complications WHO consultation.

The classification of diabetes mellitus and the tests used for its diagnosis were brought into order by the National Diabetes Data Group of the USA and the second World Health Organization Expert Committee on Diabetes Mellitus in 1979 and 1980. Apart from minor modifications by WHO in 1985, little has been changed since that time. There is however considerable new knowledge regarding the aetiology of different forms of diabetes as well as more information on the predictive value of different blood glucose values for the complications of diabetes. A WHO Consultation has therefore taken place in parallel with a report by an American Diabetes Association Expert Committee to re-examine diagnostic criteria and classification. The present document includes the conclusions of the former and is intended for wide distribution and discussion before final proposals are submitted to WHO for approval. The main changes proposed are as follows. The diagnostic fasting plasma (blood) glucose value has been lowered to ≥ 7.0 mmol/l (≥ 6.1 mmol/l). Impaired Glucose Tolerance (IGT) is changed to allow for the new fasting level. A new category of Impaired Fasting Glycaemia (IFG) is proposed to encompass values which are above normal but below the diagnostic cut-off for diabetes (plasma ≥ 6.1 to < 7.0 mmol/l; whole blood ≥ 5.6 to < 6.1 mmol/l). Gestational Diabetes Mellitus (GDM) now includes gestational impaired glucose tolerance as well as the previous GDM. The classification defines both process and stage of the disease. The processes include Type 1, autoimmune and non-autoimmune, with beta-cell destruction; Type 2 with varying degrees of insulin resistance and insulin hyposecretion; Gestational Diabetes Mellitus; and Other Types where the cause is known (e.g. MODY, endocrinopathies). It is anticipated that this group will expand as causes of Type 2 become known. Stages range from normoglycaemia to insulin required for survival. It is hoped that the new classification will allow better classification of individuals and lead to fewer therapeutic misjudgements. (Alberti, K.G.M.M. and Zimmet, P.F; 1998).

2.4 Role of alpha cells in diabetes:

Role of alpha cells in diabetes is secretion of glucagon. Glucagon is stimulating hepatic glucose output. It does this by stimulating glycogenolysis and gluconeogenesis, especially from

alanine. In addition, in the absence of insulin, glucagon stimulates ketogenesis in the liver by activating the carnitine shuttle at the hepatocyte mitochondrial membrane. However, lack of insulin is not the only factor, as glucagon levels remain high even after starting insulin treatment and it is likely that the alpha cells are also insulin-resistant in some way.

2.5 Duration in diabetes:

Duration of the disease is very common factor that may increase or decrease outcomes in many researches, *Diabetes mellitus* has been associated with an increased risk of Bladder cancer risk increased with *duration* of diabetes (Turati, F., and etal; 2015). In other research describe the degree of glycaemic control and CVRF in relation to diabetes duration. (Franch-Nadal, J., and etal; 2014). In more recent research of control and management diabetes results obtained indicated that the HbA_{1c} levels showed a significant increase with the duration of diabetes as well as the insulin level showed a significant correlation (Verma, M., and etal; 2006.).

2.6 Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus

Multiple laboratory tests are used to diagnose and manage patients with diabetes mellitus. The quality of the scientific evidence supporting the use of these tests varies substantially.

Approach An expert committee compiled evidence-based recommendations for the use of laboratory testing for patients with diabetes. A new system was developed to grade the overall quality of the evidence and the strength of the recommendations. Draft guidelines were posted on the Internet and presented at the 2007 Arnold O. Beckman Conference. The document was modified in response to oral and written comments, and a revised draft was posted in 2010 and again modified in response to written comments. The National Academy of Clinical Biochemistry and the Evidence-Based Laboratory Medicine Committee of the American Association for Clinical Chemistry jointly reviewed the guidelines, which were accepted after revisions by the Professional Practice Committee and subsequently approved by the Executive Committee of the American Diabetes Association.

Content In addition to long-standing criteria based on measurement of plasma glucose, diabetes can be diagnosed by demonstrating increased blood hemoglobin A_{1c} (HbA_{1c}) concentrations. Monitoring of glycemic control is performed by self-monitoring of plasma or blood glucose with meters and by laboratory analysis of HbA_{1c}. The potential roles of noninvasive glucose

monitoring, genetic testing, and measurement of autoantibodies, urine albumin, insulin, proinsulin, C-peptide, and other analytes are addressed.

Summary the guidelines provide specific recommendations that are based on published data or derived from expert consensus. Several analytes have minimal clinical value at present, and their measurement is not recommended.

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized and overproduced, causing hyperglycemia. The disease is classified into several categories. Type 1 diabetes mellitus, formerly known as insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes mellitus, is usually caused by autoimmune destruction of the pancreatic islet β -cells, rendering the pancreas unable to synthesize and secrete insulin. Type 2 diabetes mellitus, formerly known as non-IDDM or adult-onset diabetes, is caused by a combination of insulin resistance and inadequate insulin secretion. Gestational diabetes mellitus (GDM), which resembles type 2 diabetes more than type 1, develops during approximately 7% (range, 5%–15%) of pregnancies, usually remits after delivery, and constitutes a major risk factor for the development of type 2 diabetes later in life. Other types of diabetes are rare. Type 2 is the most common form, accounting for 85%–95% of diabetes in developed countries. Some patients cannot be clearly classified as type 1 or type 2 diabetes. (Sacks, D.B., and etal; 2002).

2.7 Diabetes mellitus type 2 management

Current guidelines for treating patients with type 2 diabetes mellitus are based on glycemic standards derived from epidemiologic data; however, the course of the disease, from prediabetes to end-stage complications, is not the same in all patients. Microvascular complications, including nephropathy, retinopathy, and neuropathy, are strongly related to hemoglobin A_{1c} (HbA_{1c}). However, vascular complications may progress in patients who have HbA_{1c} <7.0% and may appear even in undiagnosed patients owing to transient increases in plasma glucose concentrations. Concomitant atherosclerosis and occult macrovascular disease may follow an accelerated course in type 2 diabetes. Macrovascular complications may develop early, and, like microvascular complications, do not correlate linearly with HbA_{1c}. Managing hyperglycemia in the later stages of type 2 diabetes does not appear to be associated with improved cardiovascular outcomes. The glucotoxicity and lipotoxicity that may precede prolonged hyperglycemia and β -cell dysfunction are early, reversible pathophysiologic events. This suggests that prompt management may modify the course of hyperglycemia and prevent or

delay long-term complications. The challenge remains to identify patients with early type 2 diabetes who are at risk for rapid progression of β -cell decline and premature development of microvascular complications. Ongoing research into the mechanisms responsible for diabetic complications may provide new markers to help identify patients with type 2 diabetes who can benefit from earlier antidiabetes treatments.(Stolar, M; 2010).

2.7.1 Management of type 2 diabetes: evolving strategies for the treatment of patients with type 2 diabetes.

The prevalence of type 2 diabetes continues to increase at an alarming rate around the world, with even more people being affected by prediabetes. Although the pathogenesis and long-term complications of type 2 diabetes are fairly well known, its treatment has remained challenging, with only half of the patients achieving the recommended hemoglobin A (1c) target. This narrative review explores the pathogenetic rationale for the treatment of type 2 diabetes, with the view of fostering better understanding of the evolving treatment modalities. The diagnostic criteria including the role of hemoglobin A (1c) in the diagnosis of diabetes are discussed. Due attention is given to the different therapeutic maneuvers and their utility in the management of the diabetic patient. The evidence supporting the role of exercise, medical nutrition therapy, glucose monitoring, and antiobesity measures including pharmacotherapy and bariatric surgery is discussed. The controversial subject of optimum glycemic control in hospitalized and ambulatory patients is discussed in detail. An update of the available pharmacologic options for the management of type 2 diabetes is provided with particular emphasis on newer and emerging modalities. Special attention has been given to the initiation of insulin therapy in patients with type 2 diabetes, with explanation of the pathophysiologic basis for insulin therapy in the ambulatory diabetic patient. A review of the evidence supporting the efficacy of the different preventive measures is also provided.(Nyenwe, E.A., and etal; 2011).

2.7.2 Nutritional overview on the management of type 2 diabetes and the prevention of its complications

Diabetes mellitus is an increasing world health problem; particularly the prevalence of type 2 diabetes has assumed epidemic dimensions in Western industrialized societies. It is mainly the environmental, dietary and lifestyle behavioral factors that are the control keys in the progress of this disease. Several epidemiological studies have linked over nutrition and lack of physical activity with type 2 diabetes. Indeed, the excessive consumption of energy dense foods

as source of carbohydrates and fats along with ineffective medical management has negative impact on controlling blood glucose levels and on insulin response. This usually leads to a hyperglycemic state, which is associated with the development of the devastating secondary complications. Dietary guidelines have always been important for people with diabetes mellitus. Nutrition management aims to improve health quality maintaining blood glucose levels in normal range so as to reduce the risk for diabetes complications. A well-balanced diet that provides the essential macro- and micro-nutrients is always an impaired need for a patient with diabetes. In this article nutrition recommendations will be displayed for the management of diabetes type 2 and the prevention of its complications. Particular emphasis will be given to the important role of micronutrients such as trace elements and vitamins as well as to the potentiality of some dietary agents to inhibit aldose reductase enzyme, implicated in the etiology of diabetes complications. (Pegklidou, K., and etal; 2010).

2.7.3 Dietary management in diabetes.

Type 1 diabetes is primarily an autoimmune disease and type 2 diabetes is primarily a metabolic condition. However, medical nutrition therapy is an integral part of management for both types of diabetes to improve glycaemic control and reduce the risk of complications.

Objective: To outline the principles of dietary management in type 1 and type 2 diabetes and provide strategies to assist in overcoming common difficulties related to diet.

Discussion: All people with diabetes should be provided with quality professional education on medical nutrition therapy upon diagnosis, and at regular intervals thereafter. For children and adolescent patients with type 1 diabetes, the challenge is to maintain good glycaemic control while providing adequate energy for growth and development. Modification in dietary advice is required, depending on developmental stage. In type 2 diabetes, the initial challenge is to achieve weight loss of 5-10% body weight, normalize blood glucose and reduce cardiovascular risk factors. Specific strategies include a kilojoule controlled diet with reduced saturated fat, trans fat and sodium; moderate protein; and high in dietary fibre and low glycaemic index carbohydrates. Carbohydrates should be spread evenly throughout the day and matched to medication. (Barclay, A., and etal; 2010).

2.7.4 Dietary patterns, insulin resistance, and incidence of type 2 diabetes in the Whitehall II study

A dietary pattern associated with insulin resistance and investigate whether this pattern was prospectively associated with type 2 diabetes, investigated using Cox proportional hazard regression models.

Higher dietary pattern scores were associated with increased risk of type 2 diabetes. This relationship was attenuated after adjustment for ethnicity, employment grade, health behaviors (smoking, alcohol use, and physical activity) but remained significant after further adjustment for blood pressure and BMI.

A dietary pattern associated with insulin resistance predicts type 2 diabetes risk after adjustment for a range of confounders. This adds to the evidence that dietary patterns are an important risk factor for type 2 diabetes. (McNaughton, S.A., and etal; 2008).

2.7.5 Assessment of insulin sensitivity/resistance

Insulin resistance is one pretty troublesome entity which very commonly aggravates metabolic syndrome. Many methods and indices are available for the estimation of insulin resistance. It is essential to test and validate their reliability before they can be used as an investigation in patients. At present, hyperinsulinemic euglycemic clamp and intravenous glucose tolerance test are the most reliable methods available for estimating insulin resistance and are being used as a reference standard. Some simple methods, from which indices can be derived, have been validated e.g. homeostasis model assessment (HOMA), quantitative insulin sensitivity check index (QUICKI). For the clinical uses HOMA-insulin resistance, QUIKI, and Matsuda are suitable, while HES, McAuley, Belfiore, Cederholm, Avignon and Stumvoll index are suitable for epidemiological/research purposes. With increasing number of these available indices of IR, it may be difficult for clinicians to select the most appropriate index for their studies. This review provides guidelines that must be considered before performing such studies. Estimation of impaired insulin sensitivity should be given importance mainly in individuals with risk factors. The importance of the indices lies in their use in large epidemiological studies for assessment of relations between selected variables. For fasting values, insulin resistance is defined by WHO as the highest quartile of the IR_{HOMA} index in non-diabetic subjects. Insulin resistance is also defined as the lowest decile of insulin sensitivity in the lean subgroup of non-diabetic population. In clinical practice, however, their application is limited due to the lack of exact reference values. (Gutch, M., and etal; 2015).

2.8 Management of blood glucose in type 2 diabetes mellitus:

Insulin resistance, decreased insulin secretion, and increased hepatic glucose output are the hallmarks of type 2 diabetes. Medications target one or more of these defects. Average absolute reductions in HbA_{1c} for each class of medication range from 0.5 to 1.0 percent for exenatide, pramlintide, and alpha-glucosidase inhibitors to 1 to 2.5 percent for sulfonylureas and metformin. Reviews have reported that mono-therapy with any oral hypoglycemic agent is superior to dietary management or placebo in reducing HbA_{1c} values, but the studies are so heterogeneous that the expected HbA_{1c} reduction attributed to any class of medication should be interpreted with caution. This suggests that short-term studies may not accurately reflect long-term results. It is also critical to remember that the goal of treatment is not only to reduce HbA_{1c} levels, but also to prevent premature mortality and morbidity. Not all agents have been proven to achieve this goal. The first step in managing type 2 diabetes is to normalize fasting glucose levels, with weekly or monthly adjustments in the regimen. Any of antihypoglycemic agents can be combined with another. Once fasting blood glucose approaches near-normal levels, postprandial glucose is addressed by increasing the dose of the current medications or by adding additional agents. Once maximal benefit is achieved from first-line medications, other agents, can be considered.

One reason for this is the reluctance of patients and physicians to start insulin therapy, perceiving it as a treatment failure. However, progressive failure of the beta cells often occurs even with proper diet, exercise, and oral medications, so patients should be counseled that insulin is simply another management tool (Ripsin, C.M., and etal; 2009).

Evidence-based guidelines for the treatment of type 2 diabetes mellitus focus on three areas: intensive lifestyle intervention that includes at least 150 minutes per week of physical activity, weight loss with an initial goal of 7 percent of baseline weight, and a low-fat, reduced-calorie diet; aggressive management of cardiovascular risk factors (i.e., hypertension, dyslipidemia, and microalbuminuria) with the use of aspirin, statins, and angiotensin-converting enzyme inhibitors; and normalization of blood glucose levels (hemoglobin A1C level less than 7 percent). Insulin resistance, decreased insulin secretion, and increased hepatic glucose output are the hallmarks of type 2 diabetes, and each class of medication targets one or more of these defects. Metformin, which decreases hepatic glucose output and sensitizes peripheral tissues to insulin, has been shown to decrease mortality rates in patients with type 2 diabetes and is considered a first-line

agent. Other medications include sulfonylureas and nonsulfonylurea secretagogues, alpha glucosidase inhibitors, and thiazolidinediones. Insulin can be used acutely in patients newly diagnosed with type 2 diabetes to normalize blood glucose, or it can be added to a regimen of oral medication to improve glycemic control. Except in patients taking multiple insulin injections, home monitoring of blood glucose levels has questionable utility, especially in relatively well-controlled patients. Its use should be tailored to the needs of the individual patient. (Ripsin, C.M., and etal; 2009).

2.9 Limitations of Blood Glucose:

The results show that the majority of patients with diabetes cannot accurately estimate their blood glucose level and show a tendency for under-rather than overestimation. This pattern is also apparent when examining only those patients not using insulin. The results also showed that those who were accurate had lower blood glucose levels, were more likely to home test, were more likely to attend the clinic in a fasting state and reported feeling no symptoms when their blood glucose was high. In contrast, patients who overestimated were more likely to have vascular disease (ischemic heart disease or cerebrovascular disease) and be on β blockers and reported experiencing no symptoms when their blood glucose was low (Frankum, S. and Ogden, J; 2005).

2.10 Treatment of type 2 diabetes mellitus:

The overall goals of diabetes management in older adults are similar to those in younger adults and include management of both hyperglycemia and risk factors. Older adults with diabetes are a heterogeneous population that includes persons residing independently in communities, in assisted care facilities, or in nursing homes. They can be fit and healthy or frail with many co morbidities and functional disabilities. Thus, management of diabetes in older adults should be individualized, taking into account these variables.

In frail older patients with diabetes, avoidance of hypoglycemia, hypotension, and drug interactions due to polypharmacy are of even greater concern than in younger patients with diabetes. In addition, management of coexisting medical conditions is important, as it influences their ability to perform self-management (Ligthelm, R.J., and etal; 2012).

2.11 Glycohemoglobin:

The aim of diabetic management is to maintain the blood glucose concentration within or near the nondiabetic range with a minimal number of fluctuations. Serum or plasma glucose

concentrations can be measured by laboratories in addition to patient self-monitoring of whole blood glucose concentrations. Long-term blood glucose regulation can be followed by measurement of glycosylated hemoglobin.

Glycosylated hemoglobin is the term used to describe the formation of a hemoglobin compound produced when glucose (a reducing sugar) reacts with the amino group of hemoglobin (a protein). The glucose molecule attaches nonenzymatically to the hemoglobin molecule to form a ketoamine. The rate of formation is directly proportional to the plasma glucose concentrations. Because the average red blood cell lives approximately 120 days, the glycosylated hemoglobin level at any one time reflects the average blood glucose level over the previous 2 to 3 months. Therefore, measuring the glycosylated hemoglobin provides the clinician with a time-averaged picture of the patient's blood glucose concentration over the past 3 months.

Hemoglobin A_{1c} (HbA_{1c}), the most commonly detected glycosylated hemoglobin, is a glucose molecule attached to one or both N-terminal valines of the polypeptide chains of normal adult hemoglobin. HbA_{1c} is a more reliable method of monitoring long-term diabetes control than random plasma glucose. Normal values range from 4.5 to 8.0. However, this information needs to be used carefully, as a recent study has shown that the relationship between average plasma glucose and HbA_{1c} can differ substantially depending on the glycemic control of the population studied. It is also important to remember that two factors determine the glycosylated hemoglobin levels: the average glucose concentration and the red blood cell life span. If the red blood cell life span is decreased because of another disease state such as hemoglobinopathies, the hemoglobin will have less time to become glycosylated and the glycosylated hemoglobin level will be lower. Current ADA guidelines recommend that an HbA_{1c} test be performed at least two times a year with patients who are meeting treatment goals and who have stable glycemic control. For patients whose therapy has changed or who are not meeting glycemic goals, a quarterly HbA_{1c} test quarterly is recommend (Bishop, M.L., Fody, E.P. and Schoeff, L.E; 2013).

2.12 HbA_{1c} as controls management and nutritional indicator:

Diabetes is a global endemic with rapidly increasing prevalence in both developing and developed countries. The American Diabetes Association has recommended glycosylated hemoglobin (HbA_{1c}) as a possible substitute to fasting blood glucose for diagnosis of diabetes. HbA_{1c} is an important indicator of long-term glycemic control with the ability to reflect the cumulative glycemic history of the preceding two to three months. HbA_{1c} not only provides a

reliable measure of chronic hyperglycemia but also correlates well with the risk of long-term diabetes complications. Elevated HbA1c has also been regarded as an independent risk factor for coronary heart disease and stroke in subjects with or without diabetes. The valuable information provided by a single HbA1c test has rendered it as a reliable biomarker for the diagnosis and prognosis of diabetes. This review highlights the role of HbA1c in diagnosis and prognosis of diabetes patients. (Sherwani, S.I., and etal; 2016).

Disease-related malnutrition is common in diabetic patients. It is present in 21.2% of hospitalized older diabetics. Hospital related malnutrition is associated with treatment intolerance, poor prognosis, increased hospital-acquired infections, poor wound healing and longer hospitalizations.

Diabetes-specific enteral nutritional formulas are postulated as effective alternatives for nutritional treatment in diabetic subjects, being associated with maintenance of glycemic control, due to their content of slowly digested and absorbed carbohydrates and monounsaturated fats. Nevertheless, the long-term benefits on glycemic control or the economic impact of such formulas are unclear. For a product intervention to represent a good value, it should not only be efficacious but also be worth the scarce resources that were given up to purchase it.

The objective of this study is to assess the effect of enteral supplementation with the hypercaloric diabetes-specific formula (HDSF) Glucerna[®] 1.5 Cal, from Abbott Nutrition, on the use of health-care resources, health-care costs, glucose control (short- and long-term) and nutritional status in type-2 diabetes mellitus (T2DM) older malnourished patients in a real life setting (Sanz-Paris, A., and etal; 2016).

2.13 Limitations of Hemoglobin A_{1c}:

Hemoglobin A_{1c} is the measurement of glycated hemoglobin and can aid in both the diagnosis and continued management of diabetes mellitus. Accurate glycosylated hemoglobin A_{1c} (A_{1c}) measurements are an essential part of decision making in the diagnosis and treatment of type 2 diabetes mellitus. Although national standards exist to eliminate technical error with A_{1c} testing, multiple patient conditions can falsely decrease or elevate the A_{1c}. In this review, we discuss the methods to measure A_{1c} and the corresponding conditions that can affect the clinical utility of the test. Conditions that affect the A_{1c} can be either those that impair erythrocyte

production or alter the normal process of glycation. Some variation also has been associated with patient ethnicity and even with normal aging. We describe alternatives to A_{1C} testing for the above clinical scenarios in an effort to make the practicing clinician aware of alternatives for glucose evaluation.

The A_{1C}, however, may not always be accurate because of several limitations of the test itself. This creates a dilemma in that accurate A_{1C} measurements are not only an essential part of decision making but also serve as standards for quality of care and reimbursement structures under accountable care models and pay-for-performance measures. Although the National Glycohemoglobin Standardization Program (NGSP) was initiated in 1996 to eliminate many potential technical errors that can occur with A_{1C} testing, various clinical conditions/patient dispositions can lead to falsely low or high A_{1C}. The conditions that may affect A_{1C} values include changes in erythrocyte lifespan, hemoglobin variants, chemically modified hemoglobin, altered rate of glycation, ethnicity, and aging (Shepard, J.G., and etal; 2015).

2.14 Translating the A_{1C} assay into estimated Average Glucose values (eAG)

The A_{1C} assay, expressed as the percent of hemoglobin that is glycated, measures chronic glycemia and is widely used to judge the adequacy of diabetes treatment and adjust therapy. Day-to-day management is guided by self-monitoring of capillary glucose concentrations (milligrams per deciliter or millimoles per liter). We sought to define the mathematical relationship between A_{1C} and average glucose (AG) levels and determine whether A_{1C} could be expressed and reported as AG in the same units as used in self-monitoring.

Approximately correlations ($AG_{mg/dl} = 28.7 * A_{1C} - 46.7$), allowing calculation of an estimated average glucose (eAG) for A_{1C} values.

A_{1C} levels can be expressed as eAG for most patients with type 1 and type 2 diabetes. (Nathan, D.M., and etal; 2008).

2.15 The clinical utility of C-peptide measurement in the care of patients with diabetes

C-peptide is produced in equal amounts to insulin and is the best measure of endogenous insulin secretion in patients with diabetes. Measurement of insulin secretion using C-peptide can be helpful in clinical practice: differences in insulin secretion are fundamental to the different treatment requirements of Type 1 and Type 2 diabetes. This article reviews the use of C-peptide measurement in the clinical management of patients with diabetes, including the

interpretation and choice of C-peptide test and its use to assist diabetes classification and choice of treatment. We provide recommendations for where C-peptide should be used, choice of test and interpretation of results. With the rising incidence of Type 2 diabetes in younger patients, the discovery of monogenic diabetes and development of new therapies aimed at preserving insulin secretion, the direct measurement of insulin secretion may be increasingly important. Advances in assays have made C-peptide measurement both more reliable and inexpensive. In addition, recent work has demonstrated that C-peptide is more stable in blood than previously suggested or can be reliably measured on a spot urine sample (urine C-peptide: creatinine ratio), facilitating measurement in routine clinical practice. The key current clinical role of C-peptide is to assist classification and management of insulin-treated patients. Utility is greatest after 3–5 years from diagnosis when persistence of substantial insulin secretion suggests Type 2 or monogenic diabetes. Absent C-peptide at any time confirms absolute insulin requirement and the appropriateness of Type 1 diabetes management strategies regardless of apparent aetiology. (Jones, A.G. and Hattersley, A.T; 2013).

2.16 Microalbumin:

Microalbumin is ultraalbumin; permeability allows small (micro) amounts of albumin to pass into the urine. If detected in this early phase, rigid glucose control, along with treatment to prevent hypertension.

The term microalbuminuria describes small amounts of albumin in the urine. Urine microalbumin measurement is important in the management of patients with diabetes mellitus, who are at serious risk for developing nephropathy over their lifetime. In the early stages of nephropathy, there is renal hypertrophy, hyperfunction, and increased thickness of the glomerular and tubular basement membranes. In this early stage, there are no overt signs of renal dysfunction. In the next 7 to 10 years, there is progression to glomerulosclerosis, with increased glomerular capillary permeability. This permeability allows small (micro) amounts of albumin to pass into the urine. If detected in this early phase, rigid glucose control, along with treatment to prevent hypertension, can be instituted and progression to kidney failure prevented. Quantitative albumin-specific immunoassays, usually using nephelometry or immunoturbidimetry, are widely used. For a 24-hour urine collection, 30 to 300 mg of albumin is diagnostic of microalbuminuria. A 24-hour urine collection is preferred, but a random urine sample that uses a ratio of albumin to

creatinine can also be used. An albumin to creatinine in urine ratio of > 30 mg/g is diagnostic of microalbuminuria (Bishop, M.L., Fody, E.P. and Schoeff, L.E; 2013).

2.17 Microalbuminuria:

An early sign of nephropathy. It is useful to assist in diagnosis at an early stage and before the development of proteinuria. Microalbuminuria is defined as persistent albuminuria in the range of 30 to 299 mg/24 h or an albumin-creatinine ratio of 30 to 300 g/mg. Clinical proteinuria or microalbuminuria is established with an albumin-creatinine ratio of ≥ 300 mg/24 h or an albumin-creatinine ratio of ≥ 300 g/mg. The two other alternatives, a 24-hour collection or a timed 4-hour overnight collection, which are more burdensome to the patient and add little to prediction or accuracy, are seldom required (Chernecky, C.C. and Berger, B.J; 2007) (Schnell, Z.B., and etal; 2003).

Diabetes mellitus causes progressive changes to the kidneys and ultimately results in diabetic renal nephropathy.

This complication progresses over years and may be delayed by aggressive glycemic control. An early sign that nephropathy is occurring is an increase in urinary albumin. Microalbumin measurements are useful to assist in diagnosis at an early stage and before the development of proteinuria. An annual assessment of kidney function by the determination of urinary albumin excretion is recommended for diabetic patients. Microalbuminuria is defined as persistent albuminuria in two out of three urine collections of 30 to 300 mg/24 h, 20 to 200 μ g/min, or an albumin–creatinine ratio of 30 to 300 μ g/mg creatinine.

Clinical proteinuria or microalbuminuria is established with an albumin–creatinine ratio ≥ 300 mg/24 h, > 200 μ g/min, or ≥ 300 μ g/mg.

Although three methods for microalbuminuria screening are available, the use of a random spot collection for the measurement of the albumin–creatinine ratio is the preferred method. Using the spot method, without the simultaneous creatinine measurement, may result in false-positive and false-negative results because of variation in urine concentration. The two other alternatives, a 24-hour collection and a timed 4-hour overnight collection, which are more burdensome to the patient and add little to prediction or accuracy, are seldom required. A patient is determined to have microalbuminuria when two of three specimens collected within a 3 to 6 month period are abnormal. Factors that may elevate the urinary excretion of albumin include

exercise within 24 hours, infection, fever, congestive heart failure, marked hyperglycemia, and marked hypertension. (Pegklidou, K., and etal; 2010).

2.18 Prealbumin:

Transthyretin (TTR, Prealbumin, PA, Tryptophan-Rich Prealbumin). Serum Transthyretin is a transport protein synthesized in the liver that carries thyroid hormone and retinol in the body. Transthyretin half-life of 2 – 4 days makes it a much more sensitive marker for nutritional status and for liver dysfunction than albumin, which has a half-life of 22 days (Barclay, A., and etal; 2010) (McNaughton, S.A., and etal; 2008).

Table 2 - 1: Transthyretin normal range (Barclay, A., and etal; 2010).

	Conventional unit	SI Units
Adult	10 – 40 mg/dl	100 – 400 mg/l
Male	(mean) 21.5 mg/dl	(mean) 215 mg/l
Female	(mean) 18.2 mg/dl	(mean) 182 mg/l
Maternal	17 – 18.6 mg/dl	170 – 186 mg/l

2.18.1 Increased prealbumin:

Adrenal hyperfunction, Hodgkin's disease, shigellosis. Alcoholism, chronic renal failure, drugs include corticosteroids (high dose) and NSAIDs (high dose) (Barclay, A., and etal; 2010).

2.18.2 Decreased prealbumin:

Abdominal peritoneal dialysis, cirrhosis, chronic illness (with concomitant subnormal nutritional status), cystic fibrosis, diabetes mellitus, disseminated malignant disease, epithelial ovarian carcinoma, hereditary amyloidosis, protein and calorie malnutrition, tissue necrosis, diseases of the liver, acute-phase inflammatory response, drugs include amiodarone, estrogens, and oral contraceptives (containing estrogen) (Barclay, A., and etal; 2010) .

2.19 Malnutrition in diabetes mellitus:

2.19.1 The high prevalence of malnutrition in elderly diabetic patients: implications for anti-diabetic drug treatments.

Type 2 diabetes usually occurs in the context of obesity and associated insulin resistance. Current treatment recommendations are based on lifestyle modifications and incremental drug therapy. However, this approach could lead to inappropriate priorities upon ageing, when diabetes may be compounded by malnutrition and reduced insulin resistance.

Low Mini Nutritional Assessment scores and serum marker levels indicated a high prevalence of malnutrition and/or chronic disease, even in obese patients. Mini Nutritional Assessment scores were positively associated with HbA (1c) values.

Malnutrition is highly prevalent in elderly diabetic inpatients and, paradoxically, contributes to 'good' glycaemic control. Malnutrition should be screened for in these patients and, when present, should prompt a revision in diet and drug therapy. In particular, the possibility of reducing unnecessary drug therapy should be considered. (Vischer, U.M., and etal; 2010).

2.19.2 Nutrition in patients with diabetes

Diabetes is a chronic illness that requires a holistic approach in terms of care to prevent both acute and long-term complications. Nutritional management for diabetic patients has been evolving for 100 years as the pathophysiological basis of the complications incurred from diabetes becomes more explicit.

Medical nutrition therapy is extremely important for diabetic patients and prediabetic patients so that adequate glycemic control can be achieved. Nutrition counseling should be sensitive to the personal needs of the patient and how much effort the patient is willing to put in to making the change to eating appropriately.

The prevalence of type 2 diabetes, with most patients poorly controlled. Hence, this study aimed to determine nutritional and metabolic status as well as blood pressure with type 2 diabetes mellitus and identify associated risk factors for poor glycemic control.(Firouzi, S., and etal; 2015).

2.19.3 Prevalence of malnutrition among patients with diabetes mellitus type 2 admitted in a tertiary hospital

Malnutrition is a state of deficiency of the proper micro and macronutrients to meet daily nutritional requirement. Hospital malnutrition is associated with higher infection, impaired wound healing, and increased morbidity and mortality, especially in patients with type 2 diabetes mellitus (T2DM).

Malnutrition is highly prevalent in the acute hospital setting, 37% has moderate risk while 63% has high risk for malnutrition. While 55% has mild to moderate malnutrition and 45% of patients has severe malnutrition. Significant factors associated with malnutrition were SGA C, abnormal BMI, low albumin and low total lymphocyte count. Factors associated with severity of malnutrition were weight change, functional capacity, disease and nutritional requirements and presence of edema or ascites. (Carolyn, N.M., and etal; 2016).

2.19.4 Malnutrition-related diabetes mellitus among Ethiopian patients.

To search for evidence of MRDM among Ethiopian patients. The Under nutrition at presentation is probably caused by the untreated diabetic state and is reversible with treatment, even if the patient is poor and/or lives in a rural area. No convincing cases of MRDM fulfilling the published definition could be found.(Lester, F.T; 1993).

2.19.5 Nutritional and Health Status of Diabetic Patients

To assess the nutritional and health status of diabetics, nutritional and health status of 80 subjects (40-60 years) suffering with type 2 diabetes mellitus was determined using standard techniques. A questionnaire was designed to collect background information, anthropometric measurements, biochemical estimations and diet history. Data revealed that overweight/obesity, hypertension and eye problems were the health disorders associated with the subjects. Body mass index of subjects revealed that a higher number of female subjects were obese compared to their male counterparts. Mean fasting blood sugar and postprandial glucose level was noted to be 175.2 mg/dl and 258.4 mg/dl respectively. Diet survey of the subjects indicated high intake of fats, carbohydrates and energy and inadequate intake of proteins, fibre and iron as compared to their recommended values. Wide prevalence of associated health problems among the

hyperglycemic subjects clearly emphasized need of their diet and lifestyle modifications (Bhati, K. and Goyal, M; 2013).

2.19.6 Association of HbA1c level with nutritional status in community-based patients with type 2 diabetes.

Objective to explore the possible correlation between HbA1c level and nutritional status in community. Based patients with type 2 diabetes. Methods A total of 219 type 2 diabetes patients were assigned into 2 groups: one with HbA1c < 6.5 % (n: 108) and HbA1c \geq 6.5% (n = 111). Metabolic parameters, food components. And nutritional status were compared between 2 groups. Results (1) 49.32% of the participants attained HbA1c < 6.5%. (2) HbA1c level was positively correlated with fasting plasma glucose, postprandial plasma glucose, and homeostasis assessment for insulin resistance (HOMA-IR) (r were 0.56, 0.49, and 0.20, respectively, $P < 0.05$ or $P < 0.01$), but negatively correlated with high-density lipoprotein-cholesterol (HDL-C) ($r = 0.16$, $P < 0.05$). (3) Linear regression analysis showed that energy, carbohydrate, protein, and fat were the independent risk factors of HbA1c (all $P < 0.05$). (4) Patients with HbA1c < 6.5% consumed more fruits. The intake of pure energy-providing foods and protein-, fat-, or saturated fatty acid-rich foods were more frequent in patients with HbA1c \geq 6.5% ($P < 0.05$). (5) The linear regression revealed that HbA1c level were decreased 0.36% ($P < 0.10$) or 0.46% ($P < 0.01$) by intake of more fruits, roughage and beans, and HbA1c levels were also decreased 0.42% ($P < 0.05$) or 0.37% ($P < 0.10$) by intake of less meat or oils. Conclusions In community based patients with type 2 diabetes mellitus, the incidence of HbA1c < 6.5% remains low, There exists great difference in nutritional status between the groups with high and low HbA1c levels. The impact of diet on HbA1c level is great. It's necessary to emphasize the importance of diet therapy for better diabetes control (Danfeng, X.U., and et al; 2010).

2.20 Amyloid in type 2 diabetes mellitus:

2.20.1 Islet amyloid in type 2 (non-insulin-dependent) diabetes.

Amyloid deposits are found in pancreatic islets of 90% of type 2 (non-insulin-dependent) diabetic subjects at postmortem. Islet amyloid is formed from islet amyloid polypeptide (IAPP). IAPP is a 37 amino acid peptide which is a normal constituent of beta cells and is co-secreted with insulin in animals and in man. The causative factors for fibrillogenesis of IAPP are unclear, but could be related to the sequence of IAPP and abnormal production of the peptide. The lack of islet amyloid in rodent models of diabetes is due to proline substitutions in

the amyloidogenic region of IAPP. Amyloid fibrils are deposited between beta cells and islet capillaries: fibrils in invaginations of the plasma membrane may interfere with membrane signaling and insulin release. Amyloid fibrils are formed within 2 days in culture in islets isolated from transgenic mice expressing the gene for human IAPP, but not in vivo. Over expression and decreased clearance of human IAPP from islet spaces may be important factors. Progressive deposition of IAPP fibrils combined with the associated reduction in the insulin-secreting beta cells is likely to contribute to deterioration of islet function in the course of type 2 diabetes. (Clark, A., and etal; 1996).

2.20.2 Islet amyloid polypeptide and type 2 diabetes.

Type 2 diabetes is associated with progressive beta-cell failure manifest as a decline in insulin secretion and increasing hyperglycemia. A growing body of evidence suggests that beta-cell failure in type 2 diabetes correlates with the formation of pancreatic islet amyloid deposits, indicating that islet amyloid may have an important role in beta-cell loss in this disease. Islet amyloid polypeptide (IAPP; amylin), the major component of islet amyloid, is co-secreted with insulin from beta-cells. In type 2 diabetes, this peptide aggregates to form amyloid fibrils that are toxic to beta-cells. The mechanism(s) responsible for islet amyloid formation in type 2 diabetes is still unclear but it appears that an increase in the secretion of IAPP, per se, is not sufficient. Other factors, such as impairment in the processing of proIAPP, the IAPP precursor, have been proposed to contribute to the development of islet amyloid deposits. Inhibitors of islet amyloid fibril formation might prevent the progression to beta-cell failure in type 2 diabetes and should therefore be considered as a therapeutic approach to treat this disease. (Marzban, L., and etal; 2003).

2.20.3 Islet amyloid: a long-recognized but underappreciated pathological feature of type 2 diabetes.

Islet amyloid has been recognized as a pathological entity in type 2 diabetes since the turn of the century. It has as its unique component the islet beta-cell peptide islet amyloid polypeptide (IAPP), or amylin, which is cosecreted with insulin. In addition to this unique component, islet amyloid contains other proteins, such as apolipoprotein E and the heparan sulfate proteoglycan perlecan, which are typically observed in other forms of generalized and localized amyloid. Islet amyloid is observed at pathological examination in the vast majority of individuals with type 2 diabetes but is rarely observed in humans without disturbances of glucose

metabolism. In contrast to IAPP from rodents, human IAPP has been shown to form amyloid fibrils in vitro. Because all human subjects produce and secrete the amyloidogenic form of IAPP, yet not all develop islet amyloid, some other factor(s) must be involved in islet amyloid formation. One hypothesis is that an alteration in beta-cell function resulting in a change in the production, processing, and/or secretion of IAPP is critical to the initial formation of islet amyloid fibrils in human diabetes. This nidus of amyloid fibrils then allows the progressive accumulation of IAPP-containing fibrils and the eventual replacement of beta-cell mass by amyloid and contributes to the development of hyperglycemia. One factor that may be involved in producing the changes in the beta-cell that result in the initiation of amyloid formation is the consumption of increased dietary fat. Dietary fat is known to alter islet beta-cell peptide production, processing, and secretion, and studies in transgenic mice expressing human IAPP support the operation of this mechanism. Further investigation using this and other models should provide insight into the mechanism(s) involved in islet amyloidogenesis and allow the development of therapeutic agents that inhibit or reverse amyloid fibril formation, with the goal being to preserve beta-cell function and improve glucose control in type 2 diabetes. (Kahn, S.E., and etal; 1999).

2.21 Transthyretin:

Transthyretin (TTR) is a transport protein in the serum and cerebrospinal fluid that carries the thyroid hormone thyroxine (T_4) and retinol-binding protein bound to retinol. This is how transthyretin gained its *name: transports thyroxine and retinol*. The liver secretes transthyretin into the blood, and the choroid plexus secretes TTR into the cerebrospinal fluid.

TTR was originally called *prealbumin* (or thyroxine-binding prealbumin) because it ran faster than albumin on electrophoresis gels. (Thompson, E.J; 2005).

Transthyretin (TTR) is a plasma and cerebrospinal fluid protein mainly recognized as the transporter of thyroxine (T_4) and retinol. Mutated TTR leads to familial amyloid polyneuropathy, a neurodegenerative disorder characterized by TTR amyloid deposition particularly in peripheral nerves. Beside its transport activities, TTR is a cryptic protease and participates in the biology of the nervous system. Several studies have been directed at finding new ligands of TTR to further explore the biology of the protein. From the identified ligands, some were in fact TTR protease substrates. In this review, we will discuss the existent information concerning TTR ligands/substrates.

Transthyretin (TTR) is a homotetrameric protein originally discovered in the human cerebrospinal fluid (CSF). TTR is mainly synthesized in the liver and the choroid plexus of the brain, which contribute, respectively, to the plasma and CSF pool of the protein. The term TransThyRetin (TTR) reflects the main physiological roles of this protein, i.e., the transport of the thyroid hormone thyroxine (T_4) and of retinol, in this case through binding to retinol binding protein (RBP). Besides its transport activities, TTR is a protease. (Liz, M.A., and etal; 2010).

Transthyretin (TTR) is a transport protein in plasma which transport thyroxine, triiodothyronine, and retinol binding protein. Though found mainly in hepatocytes and the choroid plexus, alpha and beta cells located in the islet of Langerhans produce significant amounts of the protein, however, its function in endocrine pancreatic cells is unknown. Prohormones are precursors to hormones and can arise into multiple, different hormones at functional maturity. For instance, the same prohormone proglucagon becomes glucagon in the pancreas' alpha-cells, but becomes glicetin and GLP1 if further processed in the L-cells of the intestine. While the exact function of TTR in the islets of Langerhans is currently up for debate, its proximity to secretory granules indicates a possible role in prohormone processing. A role in hormone production would ultimately explain the presence of the potentially cytotoxic protein in the pancreas.

The goal of this project is to assess the extent to which transthyretin participates in the production of hormone from prohormone in the islets of Langerhans, specifically alpha cells, by blocking its synthesis using siRNA and comparing levels of prohormones and hormones to those of non-transfected cells. (Liz, M.A., and etal; 2010).

Transthyretin is sometimes called thyroxine-binding prealbumin or prealbumin because it migrates ahead of albumin in the customary electrophoresis of serum or plasma proteins. In normal situations, each transthyretin subunit contains one binding site for retinol-binding protein (RBP). Transthyretin and RBP are considered the major transport proteins for thyroxine and vitamin A, respectively.

Because of its short half-life and small body pool, transthyretin is a better indicator of visceral protein status and positive nitrogen balance than albumin and transferrin. Transthyretin is a superior indicator for monitoring short-term effects of nutritional therapy.

The concentration of transthyretin and RBP complex greatly decreased in protein-energy malnutrition, returns toward normal values after nutritional replenishment (Combs Jr, G.F. and McClung, J.P; 2016).

Transthyretin has a low pool concentration in the serum, a half-life of 2 days, and a rapid response to low energy intake, even when protein intake is inadequate for as few as 4 days. Serum transthyretin concentrations are decreased postoperatively by 50 – 90 mg/l in the first week, with the ability to double in 1 week or at least increase 40 – 50 mg/l in response to adequate nutritional support. If the transthyretin response increases less than 20 mg/l in 1 week as an outcome measure, this indicates either inadequate nutritional support or inadequate response.

When transthyretin decreases to levels of less than 80 mg/l, severe protein-calorie malnutrition develops; however, nutritional support can cause a daily increase in transthyretin of up to 10 mg/l. these concentrations do not appear to be significantly influenced by fluctuations in the hydration state. Although end-stage liver disease appears to affect all protein levels in the body, liver disease does not affect transthyretin as early or to the same extent as it affects other serum protein markers, particularly RBP.

Although transthyretin levels may be elevated in patients with renal disease, if a trend in the direction of change is noted, the changes are likely to reflect alteration in nutritional status and nitrogen balance. Steroids can cause a slight elevation in transthyretin, but the nutritional trend can still be followed because transthyretin responds to both overfeeding and underfeeding.

Transthyretin is also used as an indicator of the adequacy of a nutritional feeding plan because changes in plasma protein are correlated with nitrogen balance.

Transthyretin concentrations increase in patients with positive nitrogen balance and decrease in patients with negative nitrogen balance. When the transthyretin level is at ≥ 180 mg/l, this correlates with a positive nitrogen balance and indicates a return to adequate nutritional status.

It has been shown in both the pediatric and neonate population to be a highly accurate and relatively inexpensive marker for nutritional status and has been found to be the most sensitive and helpful indicator when looking at the nutritional status of very ill patients.

In summary, transthyretin effectively demonstrates an anabolic response to feeding and is a good marker for visceral protein synthesis in patients receiving metabolic or nutritional support (Pegklidou, K., and etal; 2010).

2.22 Transthyretin metabolism

2.22.1 Clinical interest of serum transthyretin (prealbumin) in dialysis patients.

Chronic renal failure is responsible for an increase in serum concentrations of transthyretin. Elevated serum transthyretin during renal insufficiency is secondary to the lack of retinol-binding protein degradation in renal tubules and to the subsequent increase in the fraction of transthyretin bound to retinol-binding protein. In both hemodialysis and peritoneal dialysis patients, serum transthyretin was demonstrated to be a reliable marker of nutritional status, exhibiting significant relationships with energy and protein intakes as well as with fat stores and lean body mass. Serum transthyretin levels less than 300 mg/l were shown to be associated with an increased risk of morbidity and mortality in dialysis patients. The predictive value of transthyretin was shown to be independent of serum albumin. Regular measurements of both serum albumin and transthyretin make it possible to detect patients whose prognosis is compromised by malnutrition and in whom an active nutritional therapy must be undertaken. Simultaneous measurements of inflammatory markers such as serum C-reactive protein are required to evaluate the role of inflammation in serum albumin and transthyretin variations. These low-cost protein parameters should be incorporated in the regular assessment of dialysis patients and measured every 1 to 3 months. (Cano, N.J; 2002).

2.22.2 Significance of transthyretin in protein metabolism.

Total body nitrogen (TBN) is mainly sequestered within the metabolically active lean body mass, in close relationship with total body potassium (TBK). TBN and TBK of growing children manifest superimposed accretion rates, display a sexual difference at the onset of adolescence and during adulthood, thereafter decreasing in elderly subjects. Plasma transthyretin (TTR) follows a comparable profile from birth to death in healthy individuals. Uncomplicated protein-energy malnutrition primarily affects the activity of nitrogen metabolic pool, reducing protein syntheses to levels compatible with survival. This adaptive response is well identified by declining TTR concentrations. In various stressful conditions, in vivo responses are characterized by upregulation in injured regions and with muscle proteolysis exceeding protein synthesis, resulting in a net body negative nitrogen balance. Again, this evolutionary pattern mirrors that of plasma TTR. Attenuation of stress and/or introduction of nutritional rehabilitation allows restoration to normal of both TBN and TTR values that follow parallel slopes. Despite distinct etiopathogenic mechanisms, TTR concentrations appear to reflect the loss or gain of TBN in body pools and they predict later outcome in malnutrition and in conditions of acute and/or chronic inflammation. (Ingenbleek, Y. and Young, V.R; 2002).

2.22.3 Studies on Transthyretin metabolism in the nervous system

Several points of evidence suggest that transthyretin (TTR) might play a significant role in the nervous system. First, TTR has been known to be present in CSF in disproportionately high concentrations, considering the molecular weight and hydrodynamic radius of TTR. The choroid plexuses of human and rat brain have been shown to be actively involved in the de novo synthesis of TTR. Initially this was first suggested by immunocheraical studies that showed TTR localized in choroid plexus epithelium. Very recently, the local synthesis of TTR in brain has been demonstrated through the use of specific cDNA probes for TTR mRNA; thus, TTR mRNA was detected in brain and specifically in the choroid plexus epithelium. (Saraiva, M.J.M., 1988).

2.23 Transthyretin induces insulin-like growth factor I nuclear translocation regulating its levels in the hippocampus.

Transthyretin (TTR) is the carrier protein of thyroxine (T_4) and binds to retinol-binding protein (RBP)-retinol complex. It is mainly synthesized by both liver and choroid plexuses of the brain. Besides these properties, it has a neuroprotective role in several contexts such as Alzheimer's disease (AD) and cerebral ischemia. Activation of insulin-like growth factor receptor I (IGF-IR) pathways and increased levels of TTR are associated with absence of neurodegeneration in an AD mouse model. In the present study, we verified that young/adult TTR null mice had decreased levels of IGF-IR in the hippocampus, but not in choroid plexus when compared with wild-type age-matched controls. Moreover, we could also demonstrate that conditional silencing of peripheral TTR did not have any influence in hippocampal IGF-IR levels, indicating that TTR effect on IGF-IR levels is due to TTR mainly synthesized in the choroid plexus. In vitro cellular studies, using NIH3T3 cell line and primary cultured hippocampal neurons, we showed that TTR up regulates IGF-IR at the transcription and translation levels and that is dependent on receptor internalization. Using a GFP-IGF-IR fusion protein, we also found that TTR triggers IGF-IR nuclear translocation in cultured neurons. We could also see an enrichment of IGF-IR in the nuclear fraction, after TTR stimulation in NIH3T3 cells, indicating that IGF-IR regulation, triggered by TTR is induced by nuclear translocation. In summary, the results provide evidence of a new role of TTR as a transcription inducer of IGF-IR in central nervous system (CNS), unveiling a new role in neuroprotection. (Vieira, M., and etal; 2015).

2.24 Transthyretin: a multifaceted protein.

Transthyretin is a highly conserved homotetrameric protein, mainly synthesized by the liver and the choroid plexus of brain. The carrier role of TTR is well-known; however, many other functions have emerged, namely in the nervous system. Behavior, cognition, neuropeptide amidation, neurogenesis, nerve regeneration, axonal growth and 14-3-3 ζ metabolism are some of the processes where TTR has an important role. TTR aggregates are responsible for many amyloidosis such as familial amyloidotic polyneuropathy and cardiomyopathy. Normal TTR can also aggregate and deposit in the heart of old people and in preeclampsia placental tissue. Differences in TTR levels have been found in several neuropathologies, but its neuroprotective role, until now, was described in ischemia and Alzheimer's disease. The aim of this review is to stress the relevance of TTR, besides its well-known role on transport of thyroxine and retinol-binding protein. (Vieira, M. and Saraiva, M.J; 2014).

2.25 Immunological and serological laboratory tests: transthyretin

Transthyretin (TTR) is a beta-sheet rich protein whose plasma half-life is 1.9 days. It behaves as a tetramer and binds to retinol binding protein (RBP) and thyroxine in plasma. Since TTR is a tryptophan-rich-protein, the protein is used as a useful marker protein for nutrition supporting team (NST). However, TTR is also an anti-acute phase protein, and the concentration is influenced by various conditions, such as inflammation and infection. Mutated forms of TTR are the precursor protein of familial amyloidotic polyneuropathy (FAP). Since plasma TTR is predominantly synthesized by the liver, liver transplantation has been performed as an effective therapy for FAP. Recent research revealed that TTR plays important roles in various central nervous system disorders, such as Alzheimer disease, depression, and lead intoxication. To elucidate the pathogenesis of those disorders, an accurate measurement of TTR concentrations in plasma and cerebrospinal fluids is of vital importance. (Ando, Y; 2005).

2.26 Transthyretin binds to glucose-regulated proteins and is subjected to endocytosis by the pancreatic β -cell

Transthyretin (TTR) is a functional protein in the pancreatic β -cell. It promotes insulin release and protects against β -cell death. We now demonstrate by ligand blotting, adsorption to specific magnetic beads, and surface plasmon resonance that TTR binds to glucose-regulated proteins (Grps) 78, 94, and 170, which are members of the endoplasmic reticulum chaperone family, but Grps78 and 94 have also been found at the plasma membrane. The effect of TTR on

changes in cytoplasmic free Ca^{2+} concentration ($[\text{Ca}^{2+}]$) was abolished if the cells were treated with either dynasore, a specific inhibitor of dynamin GTPase that blocks clathrin-mediated endocytosis, or an antibody against Grp78, that prevents TTR from binding to Grp78. The conclusion is that TTR binds to Grp78 at the plasma membrane, is internalized into the β -cell via a clathrin-dependent pathway, and that this internalization is necessary for the effects of TTR on β -cell function. (Dekki, N., and etal; 2012).

2.27 TTR assay use:

Transthyretin is a negative acute phase reactant and serum levels fall in inflammation, malignancy, cirrhosis of the liver and protein-wasting diseases of the gut or kidneys, owing to decreased synthesis. Elevated levels have been reported in Hodgkinson's disease.

Transthyretin is a specific clinical indicator of nutritional risk in the management of diseases such as HIV/AIDS, Renal disease, Diabetes, Pneumonia and Cancer. Nutritional assessment using Transthyretin has also been effective in surgical cases, pre-surgical screening, fractures, and wound healing.

2.28 Transthyretin function:

Transthyretin (TTR) is a glycosylated protein (MW 34.98 kDa) composed of four identical subunits, noncovalently bound to form a hollow core containing the T_3 and T_4 binding sites. It binds and transports approximately 10% of both hormones; with T_3 with higher affinity (Thyroxine binding globulin transport approximately 70% and albumin binds the "overflow" with low affinity). Because of negative cooperativity, TTR binding of the first hormone molecule decreases the binding affinity of the second, so only one site is normally occupied. TTR is synthesized in the liver and to a lesser extent in the choroid plexus of the CNS. Its synthesis is stimulated by glucocorticosteroid hormones, androgens, and many nonsteroidal antiinflammatory drugs (NSAID, including aspirin).

Retinol-binding protein is small (11 KDa), monomeric transport protein for all trans retinol, the physiologically active, alcoholic form of vitamin A. It's synthesized in the liver. Zinc is required for synthesis, and retinol is required for its transportation by the Golgi apparatus. When circulating in the plasma, RBP is in a 1:1 complex with transthyretin, preventing from RBP from being filtered by the renal glomeruli and stabilizing the binding of retinol, reducing its release to non-target cells. Uptake of retinol by target cells is followed by dissociation of the transthyretin-RBP complex and clearance of apoRBP (RBP without retinol) from the circulation

by the kidneys. It's reabsorbed by the proximal renal tubular cells and catabolized; the amino acids are reused (Sawyer, B.G; 2015).

2.28.1 Transthyretin constitutes a functional component in pancreatic β -cell stimulus-secretion coupling.

Transthyretin (TTR) is a protein that is synthesized in the liver, the choroid plexus of the brain, and the endocrine pancreas. It is a transport protein for thyroxine and, in association with retinol-binding protein, for retinol. It has been reported that 1 - 2% of plasma TTR circulates bound to high-density lipoprotein (HDL) and that the association to the HDL vesicle occurs through binding to apolipoprotein A₁.

TTR has a complex equilibrium between different quaternary structures in serum, but exists mainly as a tetrameric protein of 14 kDa subunits (160 - 380 mg/liter) with only a small amount of TTR monomer present *in vivo* in normal individuals.

Consequently, measurements of TTR in serum by conventional methods mainly reflect the tetrameric form. The TTR amyloidoses are human diseases in which misfolded TTR protein aggregates in different tissues. Several point mutations in TTR have been related to familial amyloidotic polyneuropathy (FAP). The fact that TTR is also produced within the pancreatic islet made us interested in evaluating a possible role of this protein in β -cell stimulus-secretion coupling.

Transthyretin (TTR) is a transport protein for thyroxine and, in association with retinol-binding protein, for retinol, mainly existing as a tetramer *in vivo*. We now demonstrate that TTR tetramer has a positive role in pancreatic β -cell stimulus-secretion coupling. TTR promoted glucose-induced increases in cytoplasmic free Ca^{2+} concentration ($[\text{Ca}^{2+}]$) and insulin release. This resulted from a direct effect on glucose-induced electrical activity and voltage-gated Ca^{2+} channels. TTR also protected against β -cell apoptosis. The concentration of TTR tetramer was decreased, whereas that of a monomeric form was increased in sera from patients with type 1 diabetes. The monomer was without effect on glucose-induced insulin release and apoptosis. Thus, TTR tetramer constitutes a component in normal β -cell function. Conversion of TTR tetramer to monomer may be involved in the development of β -cell failure/destruction in type 1 diabetes (Gutch, M., and etal; 2015).

2.28.2 Novel function of transthyretin in pancreatic alpha cells:

Although transthyretin (TTR) is expressed in pancreatic alpha (glucagon) cells in the islets of Langerhans, the function of TTR in pancreatic alpha cells remains unknown. In this study, by using TTR knockout (TTR KO) mice, we determined the novel role of TTR in glucose homeostasis.

We demonstrated that TTR KO mice evidenced impaired recovery of blood glucose and glucagon levels. Lack of TTR induced significantly lower levels of glucagon in the islets of Langerhans. These results suggest that TTR expressed in pancreatic alpha cells may play important roles in glucose homeostasis via regulating the expression of glucagon (Su, Y., and etal; 2012).

2.28.3 Transthyretin: it's miracle function and pathogenesis

Transthyretin (TTR) was previously called prealbumin because the band it formed on agarose gel electrophoresis at pH 8.6 was at the prealbumin position. However, it has been well documented that TTR of rodents does not show a prealbumin position on electrophoresis. Now, its name describes its function, binding to retinol binding protein (RBP) and T4. The serum concentration of the protein is 20-40 mg/dl, and TTR forms a tetramer. The plasma half-life of the protein is 1.9 days. TTR is synthesized by the liver, retina, pancreas, and choroid plexus. In cerebro-spinal fluid (CSF), it is the second most abundant protein, and is considered as an important protein in the pathogenesis of Alzheimer's disease, depression, and lead intoxication. In addition, TTR is a tryptophan-rich protein, it is used as one of the nutrition assessment proteins, it acts as an anti-acute phase protein, and its plasma concentration decreases during inflammation and bacterial infection. Since TTR is a highly amyloidogenic protein because it contains a beta-sheet structure, it becomes a precursor protein in familial amyloidotic polyneuropathy (FAP). Moreover, TTR plays important roles in various CNS disorders, diabetes mellitus, and lipid metabolism. (Ando, Y; 2009).

2.28.4 Transthyretin-its function and pathogenesis

Transthyretin (TTR) is a transport protein for retinol-binding protein and thyroxine, and works as a rapid turnover protein. Recently, it has been used as a nutrition assessment protein in the assessment of the acute phase nutritional status in various diseases because it contains four tryptophans in the tetramer of the protein and its plasma half-life is 1.9 days. However, the wild-type protein and its mutated form become a precursor protein of amyloid fibrils in senile systemic amyloidosis (SSA) and familial amyloidotic polyneuropathy (FAP), respectively.

Recent biochemical and pathological studies revealed that instability of the tetrameric form of TTR by mutation and post-translational modifications leads to amyloid formation in the tissues of SSA and FAP. In the process of TTR amyloid formation, misfolding of TTR, the trigger of amyloid formation, is also induced. For these amyloid formation mechanisms, Cr3+ administration, BSB (FSB) therapy, gene therapy, and antibody therapy are now on-going therapeutic projects for FAP and SSA. (Ando, Y., 2006).

2.29 TTR nutritional indicator:

Serum prealbumin is a marker of nutritional status or of risk of malnutrition, prealbumin, also known as transthyretin, has a half-life in plasma of ~2 days, much shorter than that of albumin. Prealbumin is therefore more sensitive to changes in protein-energy status than albumin, and its concentration closely reflects recent dietary intake rather than overall nutritional status. Because of this short half-life, however, the concentration of prealbumin falls rapidly as a result of the fall in its synthetic rate when there is a reprioritization of synthesis toward acute-phase proteins such as C-reactive protein (CRP), fibrinogen, or α_1 -acid glycoprotein. Moreover, prealbumin concentration in plasma, like that of albumin, is affected by changes in transcapillary escape. Hence, interpretation of plasma prealbumin is difficult in patients with infections, inflammation, or recent trauma. Despite this difficulty, interest in prealbumin as a potential marker of nutritional status in certain groups of patients led to the First International Congress on Transthyretin in Health and Disease in 2002 (Shenkin, A; 2006) (Ingenbleek, Y. and Young, V; 1994). To evaluate the use of serum transthyretin (TTR) as a valid indicator of nutritional status in the hemodialysis patient and to validate the correlation of low-serum (TTR) levels with established nutrition assessment parameters. Measuring serial serum TTR levels in hemodialysis patients is a reliable method for identifying patients in need of nutrition intervention (Duggan, A. and Huffman, F.G; 1998).

2.29.1 Is transthyretin a good marker of nutritional status?

The assay of plasma transthyretin (TTR), also known as prealbumin, is a key step in the assessment of nutritional status. However, it remains unclear whether it really is a useful nutrition marker, and when and how to use it and interpret TTR levels and variations. Risk of malnutrition, malnutrition severity, prognosis associated with malnutrition and effectiveness of refeeding are four parameters in nutritional assessment, and need clear separation to understand the associated utility of TTR. TTR does not have the same impact and potential on each of these

parameters: it can be helpful but not essential for evaluating the risk of malnutrition, and it can diagnose malnutrition and its severity in patients with no inflammation syndrome. TTR is a good marker for prognosis associated with malnutrition, and is even better for monitoring refeeding efficacy despite inflammation. Thresholds depend on the purpose for which it is used. We propose a simple algorithm to guide the interpretation of TTR levels as a helpful tool for day-to-day practice. (Dellière, S. and Cynober, L; 2017).

2.29.2 Is serum transthyretin a reliable marker of nutritional status in patients with end-stage renal disease?

To test the value of serum transthyretin (TTR) concentration as a nutritional marker in renal patients. Serum TTR concentrations were at normal range in renal patients despite evidence of malnutrition and inflammation. However, they were related to BMI and were significantly lowered in malnourished patients. Thus, serum TTR would reflect nutritional status in renal patients. However, the cutoff of malnutrition should be raised to 300 mg/L.S. (Fellah, H., and etal; 2008).

2.29.3 Validation of serum transthyretin (prealbumin) as a nutritional parameter in hemodialysis patients.

To evaluate the use of serum transthyretin (TTR) as a valid indicator of nutritional status in the hemodialysis patient and to validate the correlation of low-serum (TTR) levels with established nutrition assessment parameters. Measuring serial serum TTR levels in hemodialysis patients is a reliable method for identifying patients in need of nutrition intervention. (Duggan, A. and Huffman, F.G; 1998).

2.29.4 Transthyretin (prealbumin) in health and disease: nutritional implications.

The name "transthyretin" reflects the dual physiological roles of this tetrameric unglycosylated plasma protein. TTR is one of three specific carrier proteins involved in the transport of both thyroid hormones and of retinol through the mediation of RBP. TTR is a product of the visceral compartment, and its hepatic synthesis is exquisitely sensitive to both the adequacy and levels of protein and energy intakes--hence the proposal of TTR as a nutritional marker. To date, 38 TTR variants have been described, most of which are associated with variable degrees of cardiac and/or neural tissue amyloid deposits. All known variants arise from a single AA substitution due to single point mutation in the coding region of the TTR gene. Under acute stress conditions, the synthesis of TTR, RBP, and CBG is abruptly depressed by a cytokine-directed orchestration of new metabolic priorities, with a redistribution of organ and

tissue protein pools. It is proposed that TTR, RBP, and CBG behave as acute-booster reactants (ABRs), actively participating in the cascade of metabolic events characterizing the stress reaction along pathways best explained by the free hormone/vitamin hypothesis. The latter is governed by the law of mass action--the spontaneous dissociation and instant uptake by hepatocytes of the ligands freed from their specific carrier proteins, which creates a transient hyperthyroid, hyperretinoid, and hypercortisolic climate. This response generally does not exceed four or five days because the initial impact of injury normally subsides, but it may last longer if complications occur. The magnitude and adequacy of the stress responses depend on the preceding nutritional status as assessed by TTR plasma levels and are proportionate to the severity of insult. Clinical, animal, and molecular studies concur to demonstrate the dualistic stimulatory or inhibitory effects triggered by the ligands, whose unmetabolized fractions are excreted in the urinary output. Thyroid hormones and retinoids appear to control the early maturation processes and the synthesis of primary transcripts, whereas cortisol preferentially modulates the secondary responses and confers a protective effect on healthy tissues. During acute stress, the evolutionary patterns of visceral proteins and inflammatory markers exhibit compulsory mirror images. However, they change in independent ways under more chronic circumstances. A relatively simple biochemical micro method based on the simultaneous measurement of plasma TTR, albumin, CRP, and orosomucoid aggregated into a PINI is proposed for the early recognition and follow-up of both nutritional and inflammatory facets of the disease spectrum. (Ingenbleek, Y. and Young, V; 1994).

2.29.5 Assessment of nutritional status in organ transplant: is transthyretin a reliable indicator.

Transthyretin has been proposed as a nutritional index to screen for malnutrition and monitor the metabolic response to dietary intervention. In the presence of inflammation, circulating transthyretin levels drop regardless of optimal caloric intake. In this case, due to its rapid turnover, the pattern of transthyretin, monitored by means of repeated measures, could indicate the metabolic status (catabolism vs. anabolism). The aim of this review is to investigate the possible role of transthyretin as a nutritional parameter in organ transplantation. The literature on nutritional assessment in transplantation was reviewed and all the data regarding circulating transthyretin levels were analyzed. It appears that, on the one hand, the transthyretin level reflects closely dietary manipulations; on the other hand, it is affected by the inflammatory status. Consequently, interpretation could be difficult during the acute phase immediately after

the transplant. Moreover, the role of transthyretin in monitoring the hepatic synthetic function in liver transplant is discussed. In conclusion, transthyretin is a reliable indicator of nutritional status in transplant candidates and potentially useful in the post-transplant phase if the inflammatory status is taken into account. (Raguso, C.A., and etal; 2002).

2.29.6 Transthyretin: its response to malnutrition and stress injury. Clinical usefulness and economic implications.

Serum transthyretin is an ideal marker for monitoring patients who are malnourished or have metabolic consequences of acute stress injury because it has a short half-life, it measures the level of metabolic deficit, the response to nutritional metabolic support, and because it is a prognostic indicator. Mounting clinical evidence indicates that the use of transthyretin to assess and monitor a patient's nutritional status results in improved treatment outcomes and lower overall healthcare costs. (Bernstein, L.H. and Ingenbleek, Y; 2002).

2.29.7 Outcomes of continuous process improvement of a nutritional care program incorporating TTR measurement.

Early assessment of protein calorie malnutrition (PCM) can improve the outcome for hospitalized patients by allowing the initiation of nutrition support if required. In addition, monitoring nutritional status during the hospital stay can identify a decline in or improvement of PCM so that alterations to treatment regimens can be made if needed. The visceral protein albumin is the traditional laboratory indicator of PCM. In the past decade another protein has been lauded as a superior marker that can be used in conjunction. We undertook several studies to test the effectiveness of TTR as an aid in nutritional assessment. We found TTR to be a sensitive measure of nutritional status, allowing for earlier assessment and intervention, thus reducing length of stay and other hospital associated costs. Based on these findings, our hospital generated and implemented a multidisciplinary nutrition care program. Transthyretin is an integral portion of this program; levels are determined on admission and repeated twice weekly until discharge. (Mears, E; 2002).

2.29.8 Purification of transthyretin as nutritional biomarker of selenium status

Transthyretin has been proposed as nutritional biomarker of selenium intake. Previous transthyretin purification methods used different procedures to isolate transthyretin either from plasma or from pathological urine of humans. In general, the procedure for purification of transthyretin is laborious and expensive, and extensive sample recycling is necessary for purification in appreciable amounts. This work proposes a new, promissory, and

cheap two-step process to purify transthyretin from blood plasma, composed by a first aqueous two-phase system fractionation followed by affinity chromatography, using thyroxine immobilized on epoxy-activated Sepharose CL-6B. The aqueous two-phase system fractionation was demonstrated to perform better than commercial immunoaffinity-based kits for albumin depletion in blood plasma samples and is an effective first step for transthyretin purification. Thyroxine affinity chromatography was designed to bind transthyretin with high affinity, and was demonstrated to be useful to purify transthyretin, but was unable to completely resolve transthyretin from thyroxine-binding globulin and serum albumin, although the relative amount of albumin was lowered in the eluates. This purification process could be used in nutritional diagnosis tools or as a first step in structural and functional studies. (Mahn, A., and etal; 2012).

2.30 TTR malnutrition indicator:

2.30.1 Protein status in pancreatitis--transthyretin is a sensitive biomarker of malnutrition in acute and chronic pancreatitis.

Malnutrition may develop in acute pancreatitis (AP), accompanied by hypermetabolism and high nutritional requirements, and in chronic pancreatitis (CP). Transthyretin correlated positively with albumin and transferrin and negatively with CRP. Transthyretin seems to be a sensitive biomarker of protein status and metabolic stress. Monitoring nutritional status through measurement of serum proteins is important for optimal treatment of AP and CP. (Lasztity, N., and etal; 2002).

2.30.2 Prevalence of malnutrition among patients with diabetes mellitus Type 2 admitted in a tertiary hospital

Malnutrition is a state of deficiency of the proper micro and macronutrients to meet daily nutritional requirement. Hospital malnutrition is associated with higher infection, impaired wound healing, and increased morbidity and mortality, especially in patients with type 2 diabetes mellitus (T2DM).

Malnutrition is highly prevalent in the acute hospital setting, 37% has moderate risk while 63% has high risk for malnutrition. While 55% has mild to moderate malnutrition and 45% of patients has severe malnutrition. Significant factors associated with malnutrition were SGA C, abnormal BMI, low albumin and low total lymphocyte count. Factors associated with severity of malnutrition were weight change, functional capacity, disease and nutritional requirements and presence of edema or ascites. (Cabangon, M.R., and etal; 2016).

2.31 Transthyretin Prediction of disease

2.31.1 Transthyretin as a marker to predict outcome in critically ill patients

A determination of serum Transthyretin (TTR, Prealbumin) level is an objective method of assessing protein catabolic loss of severely ill patients and numerous studies have shown that TTR levels correlate with patient outcomes of non-critically ill patients. We evaluated whether TTR level correlates with the prevalence of PEM in the ICU and evaluated serum TTR level as an indicator of the effectiveness of nutrition support and the prognosis in critically ill patients.

TTR identified patients at highest risk for metabolic losses associated with stress hypermetabolism as serum TTR levels did not respond early to nutrition support because of the delayed return to anabolic status. It is particularly helpful in removing interpretation bias, and it is an excellent measure of the systemic inflammatory response concurrent with a preexisting state of chronic inanition.(Devakonda, A., and etal; 2008).

2.31.2 Serum transthyretin is a predictor of clinical outcomes in critically ill trauma patients.

In surgery patients, low preoperative serum transthyretin (TTR) level is associated with greater rates of infection and mortality. However, the predictive value of TTR on surgical outcomes after major trauma has not yet been studied. In critically ill trauma patients, low serum TTR level is associated with poorer clinical outcomes, and its prognostic utility warrants further study. (Cheng, V., and etal; 2015).

2.31.3 Familial ATTR amyloidosis: microalbuminuria as a predictor of symptomatic disease and clinical nephropathy.

Portuguese type familial amyloid polyneuropathy (FAP) is a neuropathic amyloidosis caused by a mutant transthyretin (TTR). Varying degrees of renal involvement have been reported. Our aim was to assess the value of microalbuminuria (MA) for predicting clinical neurological disease and overt nephropathy in TTR-related amyloidosis.

Microalbuminuria represents the first stage of clinical TTR amyloid nephropathy and is premonitory of neuropathy. Its presence identifies a subgroup of patients who are more prone

to develop overt nephropathy. Screening of MA may be important to assess disease onset and to recommend liver transplantation in individuals at risk. (Lobato, L., and etal; 2003).

2.31.4 Serum prealbumin (Transthyretin) predict good outcome in young patients with cerebral infarction

Low serum protein and albumin are considered to significantly associate with malnutrition, impaired functional status, poor outcome, and mortality. Prealbumin is an independent predictor of the good clinical outcome of young cerebral infarction patients. The serum prealbumin may be a useful prognostic indicator for judging the prognosis of cerebral infarction. (Gao, C., and etal; 2011).

2.31.5 Transthyretin predicts cardiovascular outcome in hemodialysis patients with type 2 diabetes.

BMI and albumin are commonly accepted parameters to recognize wasting in dialysis patients and are powerful predictors of morbidity and mortality. The visceral protein transthyretin (TTR) may be helpful in overcoming the diagnostic and prognostic gap.

The current study demonstrated that TTR is a useful predictor for cardiovascular outcome and mortality in diabetic hemodialysis patients. TTR was particularly useful in patients who were not identified to be at risk by BMI or albumin status. (Henze, A., and etal; 2012).

2.32 TTR Marker

2.32.1 Plasma transthyretin as a candidate marker for Alzheimer's disease.

Diagnosis of the progressive neurodegenerative disorder Alzheimer's disease (AD) can only definitively be made postmortem. The most promising AD biomarkers identified to date are found in cerebrospinal fluid (CSF). Among these, one of the most interesting candidates is transthyretin (TTR), the carrier of thyroxine and retinol, which also binds with amyloid- β ($A\beta$), and it has been suggested that it protects against $A\beta$ deposition. A biomarker detectable in plasma would have great diagnostic value and could be of use for determining disease progression and the monitoring of therapeutic efficacy due to its greater accessibility over CSF-based markers. We aimed to validate TTR as a prognostic marker in AD and to determine its relation with cognitive measures. AD biomarker that should be included in the development of blood based biomarker panels for disease diagnosis and also suggests that plasma TTR is a marker of disease severity and progression. (Velayudhan, L., and etal; 2012).

2.32.2 Identification and verification of transthyretin as a potential biomarker for pancreatic ductal adenocarcinoma

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers worldwide and is difficult to detect at its early stages when treatment is most effective. Therefore, we performed a comparative proteomic study to identify new biomarkers for the detection of PDAC.

These results suggest that the level of transthyretin is elevated in patients with PDAC. In combination with CA19-9, transthyretin may provide additional information for the detection of PDAC and should be further investigated. (Chen, J., and etal; 2013).

2.32.3 Plasma Transthyretin as a biomarker of lean body mass and catabolic states

Plasma transthyretin (TTR) is a plasma protein secreted by the liver that circulates bound to retinol-binding protein 4 (RBP4) and its retinol ligand. TTR is the sole plasma protein that reveals from birth to old age evolutionary patterns that are closely superimposable to those of lean body mass (LBM) and thus works as the best surrogate analyte of LBM. Any alteration in energy-to-protein balance impairs the accretion of LBM reserves and causes early depression of TTR production. In acute inflammatory states, cytokines induce urinary leakage of nitrogenous catabolites, deplete LBM stores, and cause an abrupt decrease in TTR and RBP4 concentrations. As a result, thyroxine and retinol ligands are released in free form, creating a second frontline that strengthens that primarily initiated by cytokines. Malnutrition and inflammation thus keep in check TTR and RBP4 secretion by using distinct and unrelated physiologic pathways, but they operate in concert to down regulate LBM stores. The biomarker complex integrates these opposite mechanisms at any time and thereby constitutes an ideally suited tool to determine residual LBM resources still available for metabolic responses, hence predicting outcomes of the most interwoven disease conditions. (Ingenbleek, Y. and Bernstein, L.H; 2015).

2.32.4 Transthyretin in endocrine pancreatic tumors.

The occurrence of transthyretin (TTR) in 25 endocrine pancreatic tumors was investigated by immunohistochemical methods using both polyclonal and monoclonal antibodies. All malignant insulinomas were strongly TTR immunoreactive, more so than their benign counterparts, which in some cases were TTR negative. All glucagonomas and nonfunctioning tumors were TTR immunoreactive, whereas gastrinomas and VIPomas were TTR negative. TTR, chromogranin A, and the argyrophil reaction (Grimelius' silver technique)

had similar distributions among the cells in many, but not all, tumors. Coexistence of TTR with glucagon, insulin, or pancreatic polypeptide in tumor cells was demonstrated. TTR was also quantitated in preoperative serum samples by electroimmuno assay in some cases. Although one patient with a glucagonoma had a markedly increased serum TTR level, five other patients with endocrine tumors, including two patients with glucagonoma, had TTR levels in serum that were within or below the reference range. (Jacobsson, B., and etal; 1989).

2.32.5 Plasma retinol-binding protein: structure and interactions with retinol, retinoids, and transthyretin.

Retinol-binding protein (RBP) is the retinol-specific transport protein present in plasma. The available crystal structures of different forms of RBP have provided details of the interactions of this binding protein with retinol, retinoids, and transthyretin (TTR, one of the plasma carriers of thyroid hormones). The core of RBP is a beta-barrel, the cavity of which accommodates retinol, establishing with its buried portions apolar contacts. Instead, the retinol hydroxyl is near the protein surface, in the region of the entrance loops surrounding the opening of the binding cavity, and participates in polar interactions. The stability of the retinol-RBP complex appears to be further enhanced when holo-RBP is bound to TTR. Accordingly, the region of the entrance loops represents the contact area of RBP interacting with the TTR counterpart, such that the hydroxyl of the RBP-bound vitamin becomes fully buried in the holo-RBP-TTR complex. Limited protein conformational changes affecting the entrance loops, which lead to a decrease or loss of the binding affinity of RBP for TTR, have been demonstrated for apo-RBP and RBP in complex with retinoids modified in the area of the retinol hydroxyl. A relatively small number of amino acid residues of RBP, essentially confined to the region of the entrance loops, and of TTR appear to play a critical role in the formation of the RBP-TTR complex, as established by crystallographic studies, mutational analysis, and amino acid sequence analysis of phylogenetically distant RBPs and TTRs. Overall, the available evidence indicates the existence of a high degree of complementarity between RBP and TTR, the contact areas of which are highly sensitive to conformational changes and amino acid replacements. (Zanotti, G. and Berni, R; 2004).

2.33 TTR with Diabetes Mellitus:

Transthyretin predicts cardiovascular outcome in hemodialysis patients with type 2 diabetes. The visceral protein transthyretin (TTR) may be helpful in overcoming the diagnostic

and prognostic gap of wasting in dialysis patients. The aim of this study was to assess the association of TTR with morbidity and mortality in hemodialysis patients. The TTR concentration was determined in baseline plasma samples of 1177 hemodialysis patients with diabetes mellitus type 2. The present study demonstrated that TTR is a useful predictor for cardiovascular outcome and mortality in hemodialysis patients. TTR was particularly useful in patients who were not identified to be at risk by BMI or albumin status (Henze, A., and etal 2012) (Henze, A., and etal 2012).

Transthyretin (TTR) is a transport protein for thyroxine and, in association with retinol-binding protein, for retinol, mainly existing as a tetramer *in vivo*. We now demonstrate that TTR tetramer has a positive role in pancreatic β -cell stimulus-secretion coupling. TTR promoted glucose-induced increases in cytoplasmic free Ca^{2+} concentration ($[\text{Ca}^{2+}]$) and insulin release. This resulted from a direct effect on glucose-induced electrical activity and voltage-gated Ca^{2+} channels. TTR also protected against β -cell apoptosis. The concentration of TTR tetramer was decreased, whereas that of a monomeric form was increased in sera from patients with type 1 diabetes. The monomer was without effect on glucose-induced insulin release and apoptosis. Thus, TTR tetramer constitutes a component in normal β -cell function. Conversion of TTR tetramer to monomer may be involved in the development of β -cell failure/destruction in type 1 diabetes (Refai, E., and etal; 2005).

2.33.1 Human serum transthyretin levels correlate inversely with Alzheimer's disease:

Alzheimer's disease (AD) is the fastest growing neurodegenerative disease in the elderly population, and the search for therapeutic targets and diagnostic AD biomarkers is an exigent issue. Because amyloid- β ($\text{A}\beta$) aggregation constitutes the epicenter of AD pathology, $\text{A}\beta$ -binding proteins that regulate $\text{A}\beta$ aggregation, such as transthyretin (TTR), have attracted much attention. TTR binds to $\text{A}\beta$, prevents its aggregation, and consequently inhibits $\text{A}\beta$ -induced cellular toxicity.

Decreased TTR levels in cerebrospinal fluid (CSF) from AD patients suggest that TTR is a biomarker of AD. But, studies on TTR as a biomarker have focused on CSF; no study has evaluated peripheral levels of TTR in AD. Here, we examined the relationship between serum TTR levels and AD. We measured TTR levels in serum samples from 90 nondemented controls and 111 AD patients and observed significantly lower serum TTR levels in AD ($p < 0.001$). Notably, females in the control group had lower serum TTR levels compared with male

in the control ($p = 0.006$), while no difference in gender was noted in the AD group. There were no age-related changes in serum TTR levels. Thus, this study demonstrates a clear negative correlation between serum TTR levels and AD, suggesting that TTR is not only involved in AD pathological process but also suggested as possible peripheral biomarker for AD diagnosis in serum level (Han, S.H., and etal; 2011).

2.33.2 Contrasting effects of type 2 and type 1 diabetes on plasma RBP4 levels: the significance of transthyretin.

Retinol-binding protein 4 (RBP4) is the principle carrier of retinol in the human plasma, which circulates as a complex with transthyretin (TTR), a homotetrameric thyroxine transport protein. Although this complex formation is thought to prevent glomerular filtration of RBP4, it also stabilizes the quaternary structure of TTR. Recent studies indicate elevated plasma levels of RBP4 in type 2 diabetes (T2D). In contrast, reduced RBP4 levels were observed in type 1 diabetes (T1D). Herein, we critically examine the probable mechanisms involved in the regulation of RBP4 and TTR levels during T2D and T1D. The available evidences point to the involvement of pancreatic factors in regulating the expression of both RBP4 and TTR. It appears that during T1D, TTR levels are reduced and it exists predominantly as a monomer that may interfere its interaction with RBP4 resulting in its loss through glomerular filtration. However, plasma TTR levels remain high under T2D conditions and thus reducing glomerular filtration of RBP4. Therefore, the plasma TTR levels appear to be an important determinant of plasma RBP4 levels in these two diabetic conditions. (Pullakhandam, R., and etal 2012).

2.34 Transthyretin and type-2 diabetes.

2.34.1 Transthyretin and amyloid in the islets of Langerhans in type-2 diabetes.

Transthyretin (TTR) is a major amyloid fibril protein in certain systemic forms of amyloidosis. It is a plasma protein, mainly synthesized by the liver but expression occurs also at certain minor locations, including the endocrine cells in the islets of Langerhans. With the use of immunohistochemistry and in situ hybridization, we have studied the distribution of transthyretin-containing cells in islets of Langerhans in type-2 diabetic and nondiabetic individuals. TTR expression was particularly seen in alpha (glucagon) cells. Islets from type-2 diabetic patients had proportionally more transthyretin-reactive islet cells, including beta cells. A weak transthyretin immunoreaction in IAPP-derived amyloid occurred in some specimens. In seeding experiments in vitro, we found that TTR fibrils did not seed IAPP while IAPP fibrils

seeded TTR. It is suggested that islet expression of transthyretin may be altered in type-2 diabetes. (Westermarck, G.T. and Westermarck, P; 2008).

2.34.2 Microalbuminuria is a major determinant of elevated plasma retinol-binding protein 4 in type 2 diabetic patients

Plasma retinol-binding protein 4 (RBP4) may be a new adipokine linked to obesity-induced insulin resistance and type 2 diabetes. The impact of diabetic nephropathy on plasma RBP4 levels, however, is not known. We tested the hypothesis that microalbuminuria is associated with elevated plasma concentrations of RBP4 in type 2 diabetic subjects. Retinol, its binding protein and transthyretin (TTR) were measured in the plasma and urine of 62 type 2 diabetic subjects, 26 of whom had microalbuminuria. The results were compared to 35 healthy control subjects. Despite no differences in plasma retinol, concentrations of the RBP4 were significantly elevated in plasma of diabetic patients and significantly higher in those with microalbuminuria. The higher plasma levels of the binding protein in subjects with microalbuminuria were accompanied by both significantly elevated plasma TTR and increased urinary levels of RBP4. There were no correlations of plasma-binding protein levels and parameters of insulin resistance. Our study suggests that plasma RBP4 levels in type 2 diabetic patients are affected by incipient nephropathy. Therefore, further studies evaluating RBP4 as a regulator of systemic insulin resistance and type 2 diabetes will need to take renal function into consideration. (Raila, J., and etal; 2007).

Chapter Three Materials and Methods

3.1 Area of study:

This study was conducted at JAZC Khartoum 2 around Katrrena Street in the north and east .the center has been opened in 21 of august 1998 in Khartoum state. The center receives more than 200 patients daily. The numbers of registered patients about 50000 until now, there are three consultants of surgery and five consultants of medicine and ophthalmologist, dermatologist, obstetrician and vascular surgeon.

3.2 Study design:

Retrospective-crossectional study to evaluate of diabetes mellitus type 2 patients' nutritional status and control by transthyretin test in Jaber Abu Alzi Diabetic Center (JAZC)

3.3 Study period:

This study was carried out between “February, 2013 to November 2018”.

3.4 Study population:

The study population included 385 volunteers, classified according to gender, age, status of disease, duration, microalbuminuria, treatment plan.

3.5 Sample size:

$$n = Z^2 * (p) * (1-p) / d^2$$

Where:

Z = Z value (e.g. 1.96 for 95% confidence level)

p = prevalence or % of disease

(0.5 used for sample size needed)

d = confidence interval, expressed as decimal

$$N = (1.96)^2 * 0.5 / (0.05)^2 = 385.$$

3.6 Data collection Techniques:

Interview.

Blood sample from patient.

Records review.

3.7 Data collection Tools:

Questionnaire paper.

Hospital records.

Recorder, paper and pen.

3.8 Non-response, non-completions, termination:

If it proves impossible to complete a once it has begun, and it cannot be complete; this case will be removed from study sample and replaced with another person according to diagnosis and eligibility.

3.9 Data management and analysis:

Data was reviewed within the field and cleaning of all questionnaires and consistency was done, the data was analyzed using SPSS version 22[®] and Excel 2007. Analysis was done by using significant test *p* value less than .05 significant tests, chi square, and T test (independent), and correlation was used, and simple and multiple correlation regression test.

3.10 Data entry and storage:

Full questionnaires was entered in the dataset in SPSS.

Data storage was password protected.

Full questionnaire was stored in locked space.

Incomplete questionnaires was excluded.

3.11 Criteria to select samples:

Volunteers who were selected in this study, volunteers who were age more than 21 years old, volunteers who were known diabetes type 2 (differentiate Between DM Type 1 and 2 done by guide in JAZC hospital by, family history of type 2, mode of presentation [ketoacidosis, severity of symptoms], HbA1c, Response to treatment [oral hypoglycemic agent]), and not under medication or diseases affect the study.

3.12 Criteria to reject samples:

Volunteers who were refused to participate in the study, volunteers less than 21 years old, patients with both renal, and liver disease was not included in this study. Also in this study, the samples were not collected from patients under medication or disease affect transthyretin test.

3.13 Study Group:

The study included 385 subjects those with diabetes type 2.

3.14 Sampling:

Informed consent was taken from each subject individually, before taking samples, and medical history was taken from the physician and patient files, after explaining to the volunteer the procedure, the questionnaire was filled, 5ml of blood in SST gel tube, and another 5 ml in EDTA₃ tube was taken from the volunteer initially after asking the patient if he is fasting, write ID number in the questionnaire paper, Blood samples were obtained via standard venipuncture techniques in SST gel separated serum tubes; following collection, samples were centrifuged after clotting (10 min at 15000 RCF) and immediately transferred the serum to labeled Eppendorf 1½ ml tube with cap, and analyzed the FBS using the remain serum immediately. Samples was kept in a laboratory refrigerator (2 - 8 °C), then transported by icebox to assembly point in freezer (< - 10 °C) till the time of analysis.

Urine sample obtained in clean 120 ml urine container for random fresh urine, urine samples were tested for microalbumin immediately.

3.15 Statistical analysis:

The statistical analyses was performed using statistical package for social sciences (SPSS) version 22 and excel 2007.

3.16 Ethical clearance:

An ethical clearance of this study will be approved by the ethical committee of University of Gezira. Informed consent will obtained from each participant before taking the samples.

3.17 Method of Estimation:

3.17.1 Chemicals and Reagents:

In this studied chemicals and reagents used for estimation were obtained from Biosystems Company Spain, **Boditech MED INC company South Korea**, Human company Germany, and Bioassay Technology Laboratory Company China.

3.17.2 Spectrophotometer Biosystems BTS-310

System Function:

Semi-automated, discrete, random access, STAT sample priority, 75 Non-volatile memory locations, Low voltage halogen lamp. Silicon Photodiode. Photometric range from - 0.2 to 2.2 Abs. Spectral range from 340 to 700 nm. Filter wheel of 9 positions (7 filters).

Throughput:

Manually.

Measuring principles:

Absorbance photometry, Turbidimetry.

Methodology:

End point, Fixed-time, Kinetic.

Single/Dual reagents chemistries.

Monochromatic/Dichromatic.

Linear/Nonlinear multipoint calibration.

Programming:

Open system with calculation chemistries.

Reagent volume:

Reagent volume, and sample volume as manufacturer instruction.

Reaction system:

Cuvette: Optional 5 mm.

Reaction volume: As manufacturer instruction.

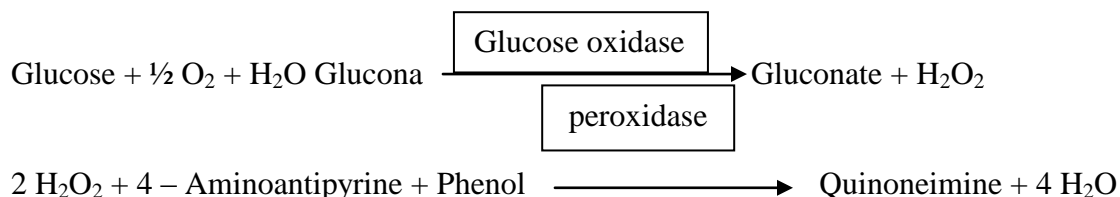
Reaction temperature: 37 °C.

Temperature fluctuation: ± 0.1 °C.

Probe: Dependent mixing, with wash intervals.

Method of Glucose estimation:**Glucose test Principle:**

Glucose in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry.



3.17.3 *i*-CHROMA™:

***i*-CHROMA™ Principle:**

The *i*-CHROMA™ Reader is a compact Point-of-Care test system for fluorescence detection to determine different analysis in whole blood, plasma, serum or urine.

The portable high-performance immunoassay system consists of the *i*-CHROMA™ laser fluorescence reader and the test cartridge customized to the particular parameters including the associated reagent. The Point-of-Care analysis system is characterized by its comprehensive parameter menu and provides skilled personnel with high-quality medical information with most easy handling.

The *i*-CHROMA™ system is based on the fluorescence immunoassay technology. This technology allows for quantization of individual or several analytics at a time by measuring of a laser induced epi-fluorescence. The test determination is realized on a particular, parameter related test cartridge.

***i*-CHROMA™ Specifications:**

Measuring Channels: 1.

Measuring Method: fluorescence detection with Laser (2.5 mW).

Sample Type: Whole Blood, Plasma, Serum and Urine.

Sample volume: 5 - 30 µl, depend on Test Parameter.

Reagent: closed System.

Calibration: automatically with Chip card.

Display: illuminated four-line display, each with 16 character.

Method of Hb A_{1C} estimation:

Hb A_{1C} Principle:

Glycated protein is formed post-translationally through the slow, nonenzymatic reaction between glucose and amino groups on proteins. HbA_{1c} is a clinically useful index of mean glycemia during the preceding 120 days, the average life span of erythrocytes. Carefully controlled studies have documented a close relationship between the concentrations of HbA_{1c} and mean glycemia. HbA_{1c} is considered as a more reliable parameter in monitoring glycemia over the glycemic reading with the conventional glucometer.

***i*-CHROMA™ HbA_{1c}** is based on the fluorescence immunoassay technology, specifically the competition immune-detection method. Whole blood is added to the mixture of hemolysis buffer and detection buffer, which results in hemolysis of red blood cells. The mixture containing HbA_{1c} from the hemolyzed red blood cells and fluorescence-labeled HbA_{1c} peptides from detection buffer is loaded onto the sample well of the Cartridge. The mixture then migrates through the nitrocellulose matrix of the test strip by capillary action. HbA_{1c} from the blood competes with fluorescence-labeled HbA_{1c} peptides for binding sites on HbA_{1c} antibodies fixed on the nitrocellulose matrix. As a result, the higher concentration of HbA_{1c} produces a lower fluorescence signal from HbA_{1c}-peptides. The signal is interpreted and the result displayed on *i*-CHROMA™ Reader in units of percentage.

Method of Microalbumin estimation:

Microalbumin test principles:

***i*-CHROMA™ Microalbumin** is based on fluorescence immunoassay technology. *i*-CHROMA™ Microalbumin uses a sandwich immunodetection method, such that by mixing detector buffer with urine specimen in test vial, the fluorescence-labeled detector anti-albumin antibody in buffer binds to albumin antigen in urine specimen. As the sample mixture is loaded onto the sample well of the test device and migrates the nitrocellulose matrix of test strip by capillary action, the complexes of detector antibody and albumin are captured to anti-albumin sandwich pair antibody that has been immobilized on test strip.

Thus the more albumin antigen is in urine specimen, the more complexes are accumulated on test strip. Signal intensity of fluorescence of detector antibody reflects amount of albumin captured and is microprocessed from *i*-CHROMA™ Reader to show albumin concentration in urine specimen. The default result unit of *i*-CHROMA™ Microalbumin is displayed as an mg/L from *i*-CHROMA™ Reader. The working range of *i*-CHROMA™ Microalbumin system is 2 - 300 mg/L.

3.17.4 ELISA:

ELISA Principle:

Enzyme-linked Immunosorbent Assays (ELISAs) combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily-assayed enzyme. ELISAs can provide a useful measurement of antigen or antibody concentration. There are two main variations on this method: The ELISA can be used to detect

the presence of antigens that are recognized by an antibody or it can be used to test for antibodies that recognize an antigen.

A general ELISA is a five-step procedure: 1) coat the microtiter plate wells with antigen; 2) block all unbound sites to prevent false positive results; 3) add primary antibody (e.g. rabbit monoclonal antibody) to the wells; 4) add secondary antibody conjugated to an enzyme (e.g. anti-mouse IgG); 5) reaction of a substrate with the enzyme to produce a colored product, thus indicating a positive reaction. There are many different types of ELISAs. One of the most common types of ELISA is "sandwich ELISA".

Method of Transthyretin estimation:

Principles:

This immunoassay allows for the in vitro rapid detection of Human TTR concentrations in serum, plasma and other biological fluids. This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for transthyretin (TTR) has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any transthyretin (TTR) present is bound by the immobilized antibody.

After removing any unbound substances, a biotin-conjugated antibody specific for transthyretin (TTR) is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of transthyretin (TTR) bound in the initial step. The color development is stopped and the intensity of the color is measure

Chapter Four

4.1 Results

Subjects were divided into 6 groups, according to gender, age, hypertension, microalbuminuria, duration, type of treatment, as shown in figure (4 -1).

Blood samples were collected from volunteers via venipuncture in fasting 12 hrs, and random urine samples, and mean levels were compared as shown in table (٤- 1), and figure (٤- 8).

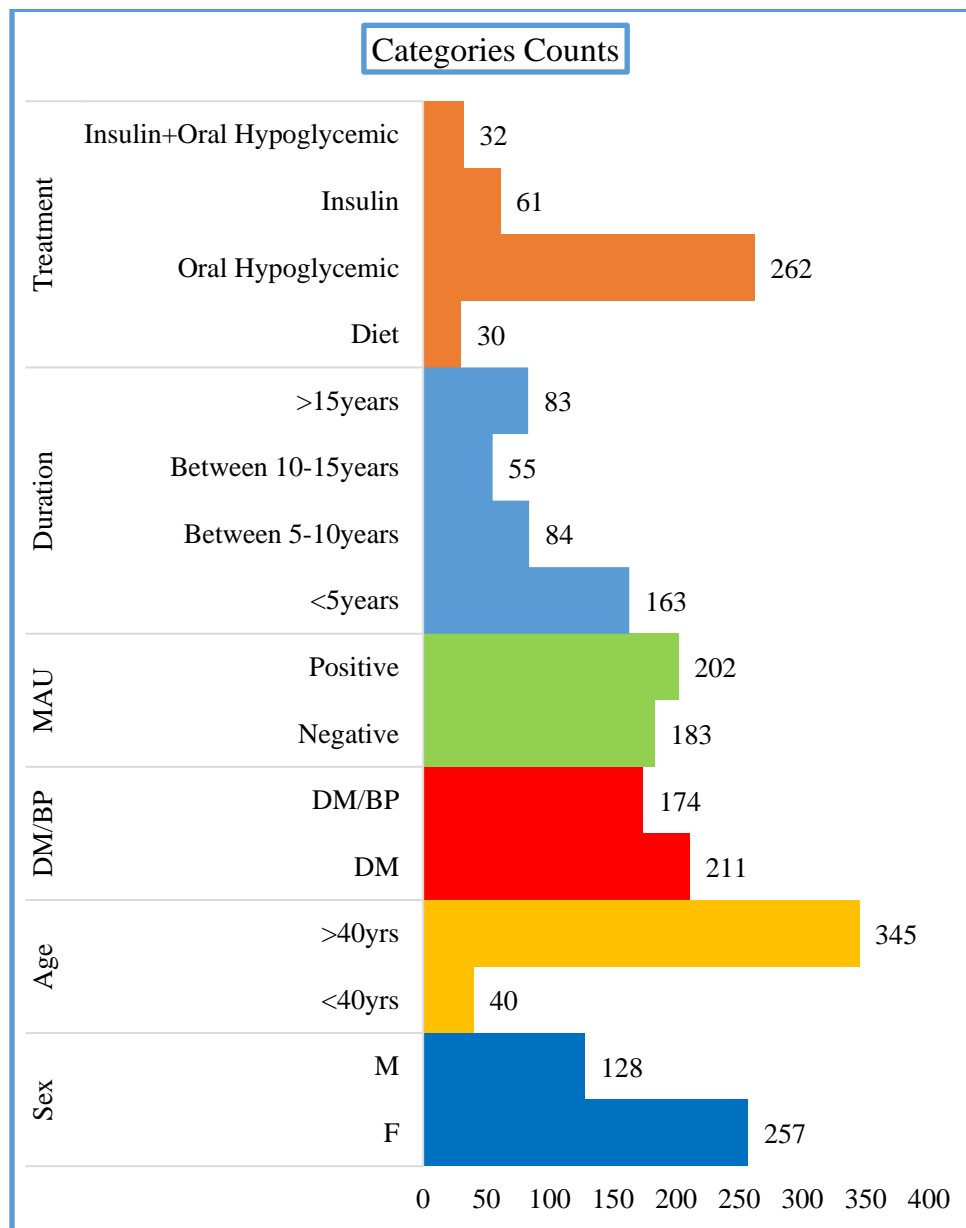


Figure 4 – 1: Categories distribution

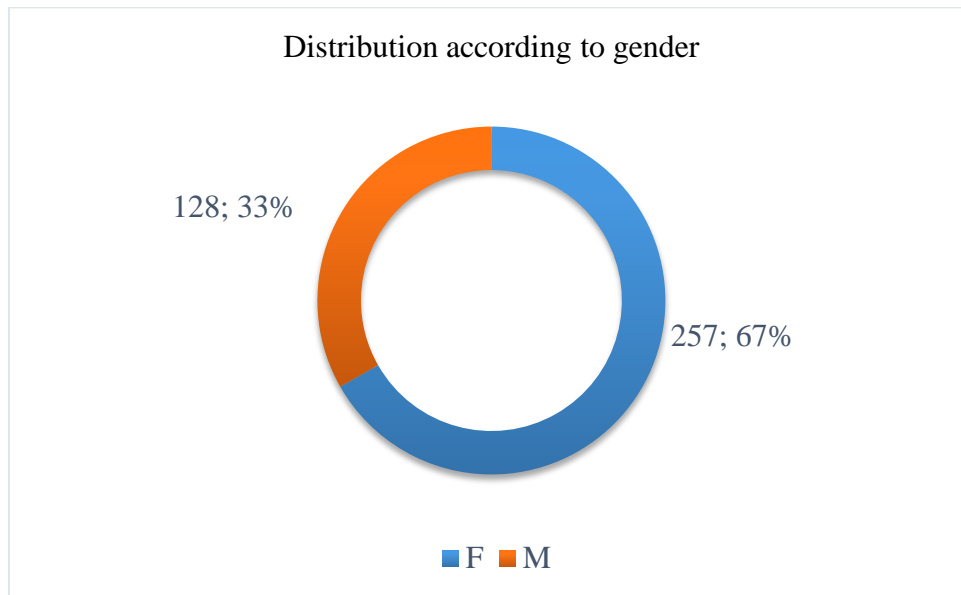


Figure 4 – 2: Distribution according to gender

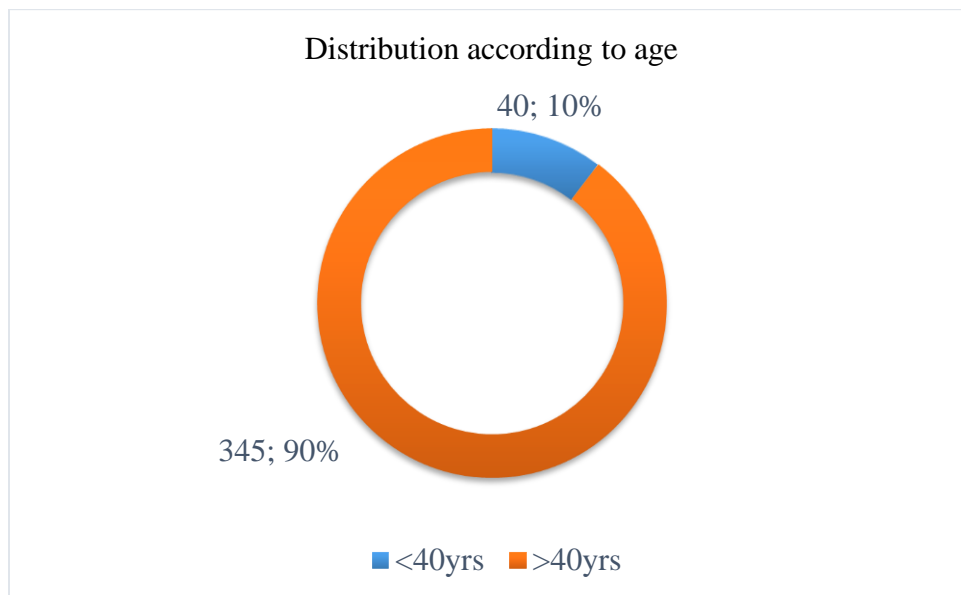


Figure 4– 3: Distribution according to age

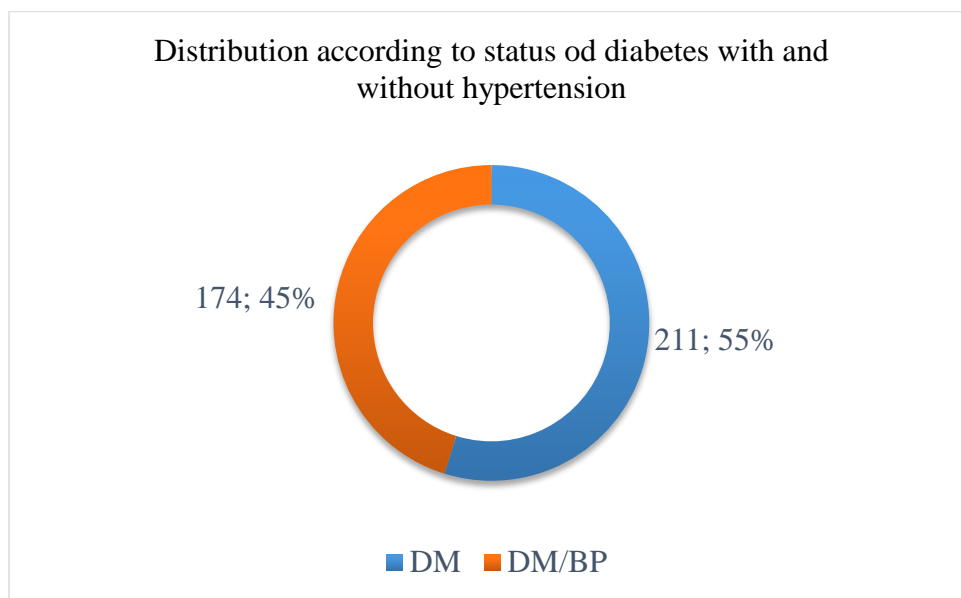


Figure 4 – 4: Distribution according to status of diabetes with or without hypertension

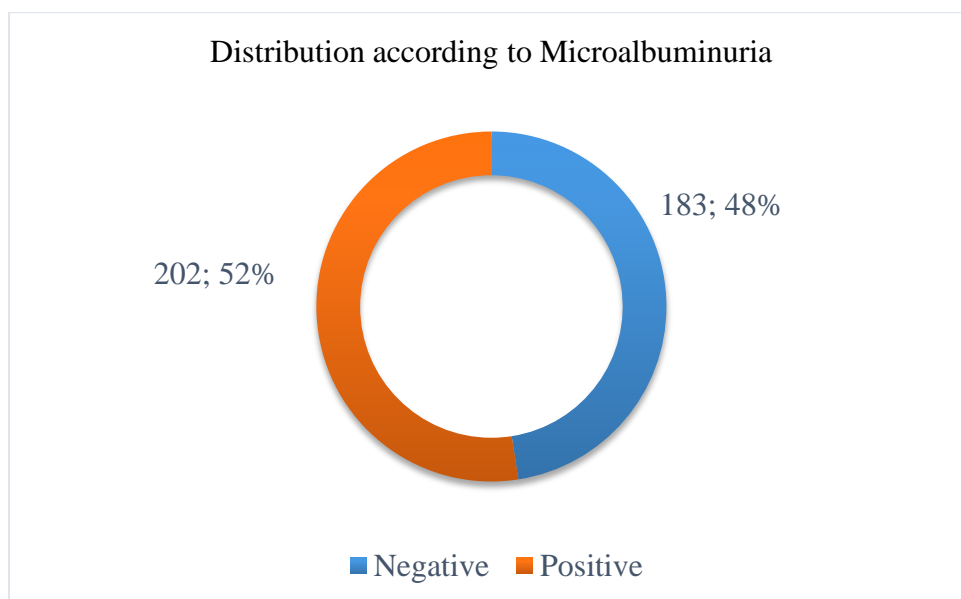


Figure 4 – 5: Distribution according to Microalbuminuria

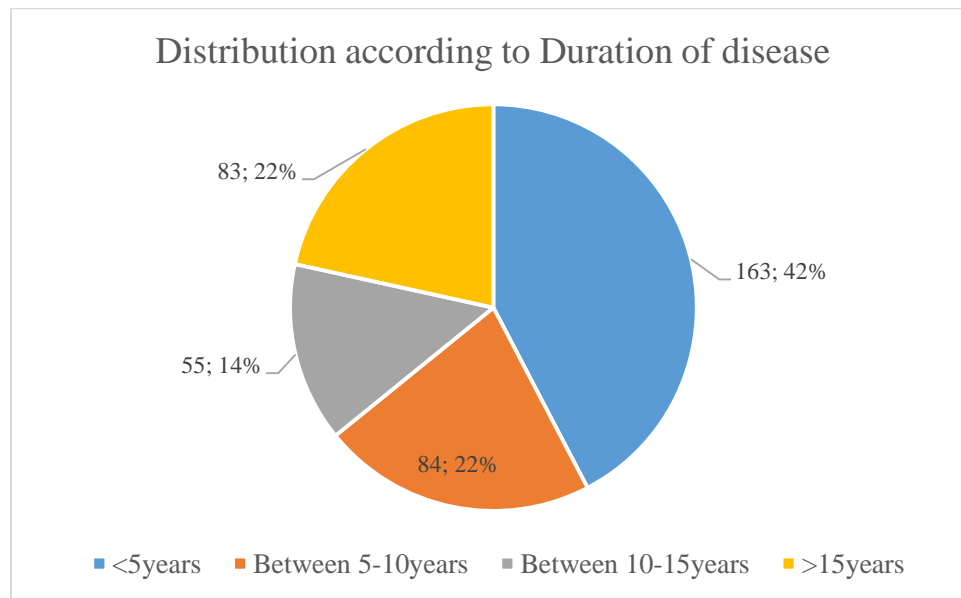


Figure 4 – 6: Distribution according to the duration

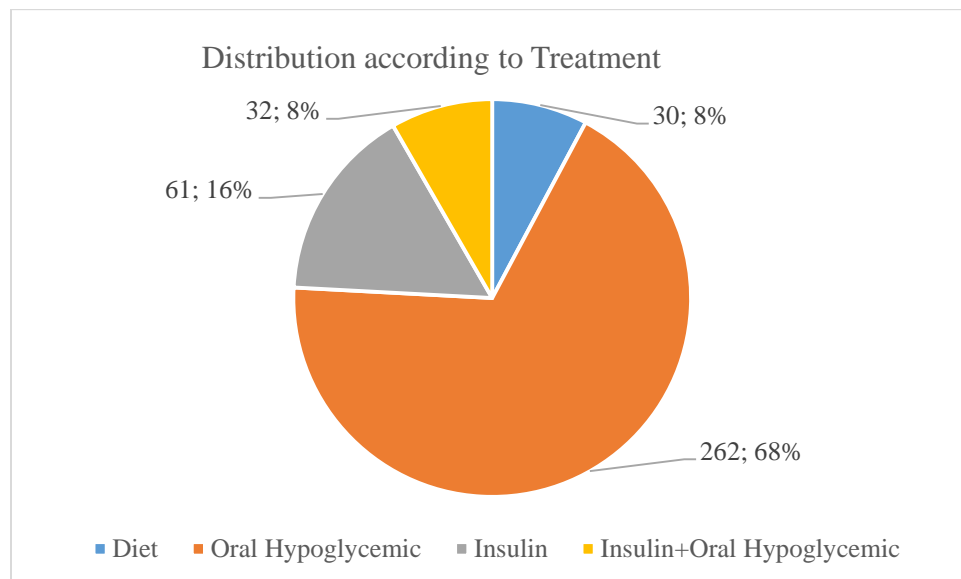


Figure 4 – 7: Distribution according to treatment

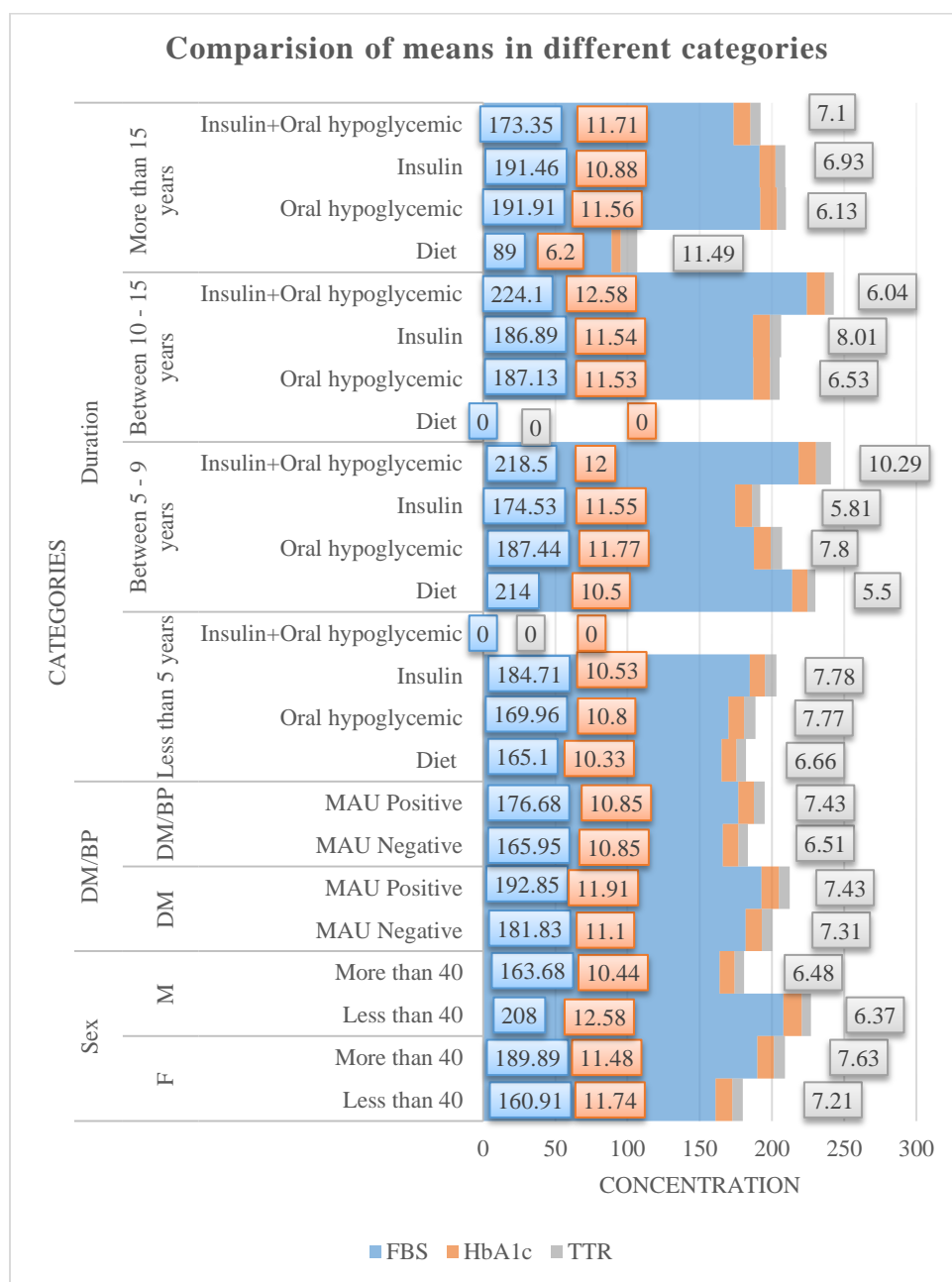


Figure 4 - 8: Distribution of mean levels according to subcategories

Table 4- 1: Levels of mean and frequencies

Groups	Categories	Frequency,%	Mean		
			TTR	FBS	HbA1c
Sex	F	257, 66.8%	7.59	187.29	11.51
	M	128, 33.2%	6.48	166.45	10.57
Age	Less than 40 yrs	40, 10.4%	7.00	173.07	11.96
	More than 40 yrs	345, 89.6%	7.24	181.00	11.13
DM/BP	DM	211, 54.8%	7.37	186.81	11.46
	DM/BP	174, 45.2%	7.04	172.54	10.88
MAU	Negative	183, 47.5%	6.98	175.32	10.98
	Positive	202, 52.5%	7.43	184.93	11.39
Duration	Less than 5yrs	163, 42.3%	7.57	169.78	10.71
	Between 5 – 9 yrs	84, 21.8%	7.33	186.41	11.73
	Between 10 - 15 yrs	55, 14.3%	6.63	194.65	11.60
	More than 15 yrs	83, 21.6%	6.84	184.79	11.32
Treatment	Diet	30, 7.8%	6.71	167.43	10.21
	Oral hypoglycemic	262, 68.1%	7.38	179.02	11.23
	Insulin	61, 15.8%	6.88	185.30	11.13
	Insulin+Oral hypoglycemic	32, 8.3%	7.02	194.52	12.00

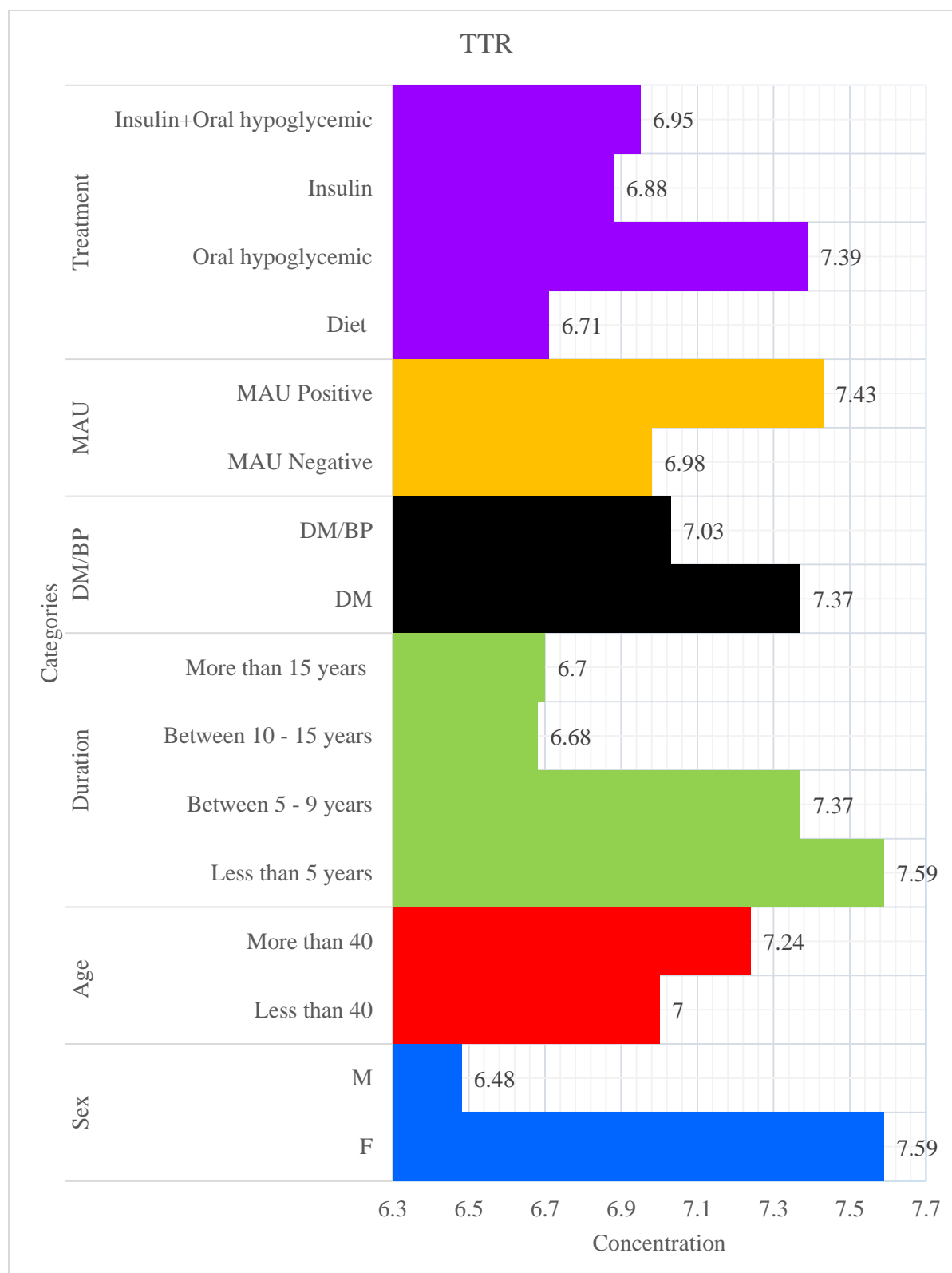


Figure 4 - 9: Distribution of TTR mean levels according to categories

The means serum TTR showed significant difference between female and male, ($p = 0.001$) as shown in table (٤- 2), and Figure (٤- 9).

Table 4 - 2: TTR Sex mean, and significance

TTR		Mean	sig
Sex	F	7.59	.001
	M	6.48	

The means of serum TTR in patients with negative MAU compared to positive MAU were significant difference, ($p = 0.006$) as shown in table (٤- 3), and Figure (٤- 10).

Table 4 - 3: TTR MAU Frequencies', Percentage and significance

TTR		Frequencies', Percentage	sig
MAU	Negative	183, 47.5%	.006
	Positive	202, 52.5%	

The means of FBS in different duration of the diabetes were significantly different, compared between groups, ($p = 0.035$), table (4 - 6) and Figure (٤- 10).

FBS Duration < 5 years mean vs between 5 – 9 years means were significantly different, ($p = 0.036$), table (4 - 4) and Figure (٤- 10).

FBS Duration < 5 years vs between 10 – 15 years were significantly different, ($p = 0.014$), table (4 - 4) and Figure (4 - 10).

FBS Duration < 5 years vs >15 years were significantly different, ($p = 0.027$), table (4 - 4) and Figure (4 - 10).

Correlation of FBS to HbA1c, were significantly different, ($p = 0.000$) as shown in table (4 - 6) and Figure (4 - 10).

Correlation of FBS to sex, were significantly different ($p = 0.000$) as shown in table (4 - 7) and Figure (4 - 10).

Correlation of FBS to DMBP were significantly different, ($p = 0.007$) as shown in table (4 - 7) and Figure (4 - 10).

Table 4 - 4: FBS Duration mean, SEM and significance

FBS		Mean	Std. Error	sig
Duration	Less than 5 yrs	169.82	7.822	.036
	Between 5 – 9 yrs	186.25		
	Less than 5 yrs	169.82	9.082	.014
	Between 10 – 15 yrs	192.20		
	Less than 5 yrs	169.82	7.854	.027
	More than 15 yrs	187.27		

Table 4 - 6: Correlation Pearson Coefficient and significance

Correlations					
		FBS	HbA _{1c}	TTR	Duration
FBS	Pearson Correlation	1	.523 ^{**}	-.003	.123 [*]
	Sig. (2-tailed)		.000	.950	.015
	N	385	385	385	385
HbA _{1c}	Pearson Correlation	.523 ^{**}	1	.028	.106 [*]
	Sig. (2-tailed)	.000		.588	.038
	N	385	385	385	385
TTR	Pearson Correlation	-.003	.028	1	-.075
	Sig. (2-tailed)	.950	.588		.144
	N	385	385	385	385
Duration	Pearson Correlation	.123 [*]	.106 [*]	-.075	1
	Sig. (2-tailed)	.015	.038	.144	
	N	385	385	385	385
**. Correlation is significant at the 0.01 level (2-tailed).					
*. Correlation is significant at the 0.05 level (2-tailed).					

Table 4 – 7: Correlation Kendall's tau_b Coefficient and significance

Kendall's tau_b	Correlations								
			Sex	Age	MAU	DMBP	FBS	Duration	HbA1c
Sex	Correlation Coefficient		1.000	.078	.020	-.109*	-.150**		-.141**
	Sig. (2-tailed)		.	.128	.690	.033	.000		.001
Age	Correlation Coefficient		.078	1.000	.034	.207**	.020		-.080
	Sig. (2-tailed)		.128	.	.507	.000	.634		.057
MAU	Correlation Coefficient		.020	.034	1.000	.081	.070		.074
	Sig. (2-tailed)		.690	.507	.	.115	.093		.079
DMBP	Correlation Coefficient		-.109*	.207**	.081	1.000	-.112**		-.105*
	Sig. (2-tailed)		.033	.000	.115	.	.007		.013
FBS	Correlation Coefficient		-.150**	.020	.070	-.112**	1.000		.417**
	Sig. (2-tailed)		.000	.634	.093	.007	.		.000
HbA1c	Correlation Coefficient		-.141**	-.080	.074	-.105*	.417**		1.000
	Sig. (2-tailed)		.001	.057	.079	.013	.000		.
TTR	Correlation Coefficient		-.079	-.023	.005	-.042	.004		.031
	Sig. (2-tailed)		.059	.576	.904	.312	.908		.364
Duration	Correlation Coefficient						.081*		.106**
	Sig. (2-tailed)						.035		.006
Treatment	Correlation Coefficient							.461**	.090*
	Sig. (2-tailed)							.000	.026
*. Correlation is significant at the 0.05 level (2-tailed).									
**. Correlation is significant at the 0.01 level (2-tailed).									

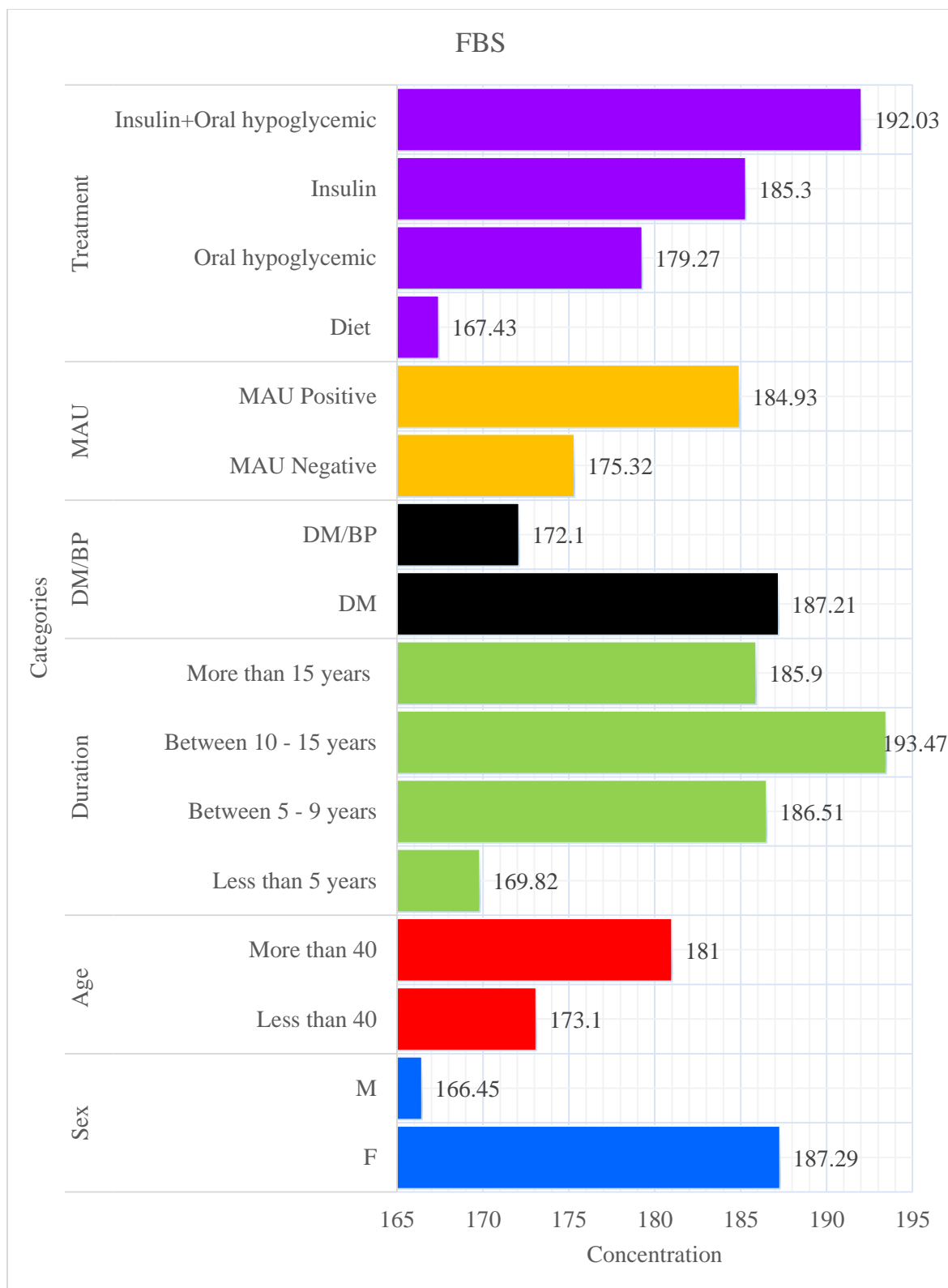


Figure 4 - 10: Distribution of FBS mean levels according to categories

HbA1c means in the duration of diabetes were significantly different, ($p = 0.006$), table (4 - 6).

HbA1c Duration < 5 years vs between 5 – 10 years were significantly different, ($p = 0.005$), table (4 - 5) and Figure (4 - 11).

HbA1c Duration < 5 years vs between 10 – 15 years were significantly different, ($p = 0.014$), table (4 - 5) and Figure (4 - 11).

HbA1c Treatment were significantly different, ($p = 0.026$), table (4 - 7) and Figure (4 - 11).

HbA1c Treatment Diet vs oral hypoglycemia were significantly different, ($p = 0.039$), table (4 - 5) and Figure (4 - 11).

HbA1c Treatment Diet vs Insulin+Oral hypoglycemia were significantly different, ($p = 0.006$), table (4 - 5) and Figure (4 - 11).

Correlation of HbA1c to sex, were significantly different, ($p = 0.001$) as shown in table (4 - 7) and Figure (4 - 11).

Correlation of HbA1c to DMBP, were significantly different, ($p = 0.013$) as shown in table (4 - 7) and Figure (4 - 11).

Table 4 - 5: HbA1c mean, SEM and significance

HbA1c			Mean	Std. Error	sig
Duration		Less than 5yrs	10.71	.34123	.005
		Between 5-10yrs	11.73		
		Less than 5yrs	10.71	.39617	.014
		Between 10-15yrs	11.60		
Treatment		Diet	10.21	.49184	.039
		Oral hypoglycemic	11.23		
		Diet	10.21	.64849	.006
		Insulin+Oral hypoglycemic	12.00		

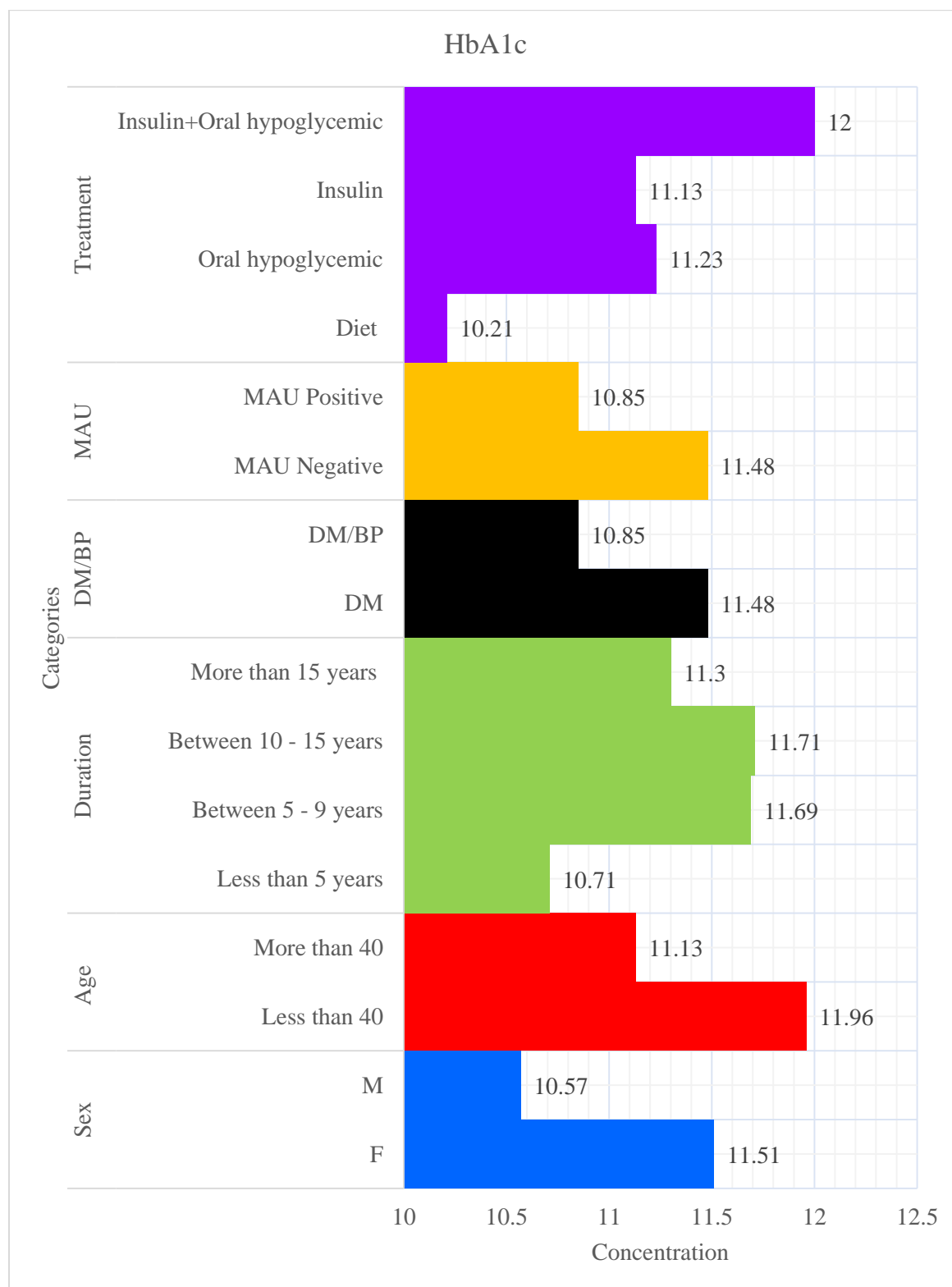


Figure 4 - 11: Distribution of HbA1c mean levels according to categories

Treatment vs Duration were significantly different, ($p = 0.000$) as shown in table (4 - 7).

Correlation of DMBP to sex, were significantly different, ($p = 0.033$) as shown in table (4 - 7).

Correlation of DMBP to age, were significantly different, ($p = 0.000$) as shown in table (4 - 7).

4.2 Discussion:

To determine the evaluation of Transthyretin test in diabetes mellitus type 2, Transthyretin test have been studied in human serum and compared with gender, age, FBS, HbA_{1c}, status of the diabetes with or without blood hypertension, Microalbuminuria, duration of the disease, and treatment plan. 385 subject were included in this study according to sex female were 257 out of 385 (66.75%) and male were 128 out of 385 (33.25%), Figure (4 - 2). According to age 31 out of 385 (8.1%) were less than 40 years old, and 354 out of 385 (91.9%) were more than 40 years old Figure (4 - 3). According to status of diabetic and diabetic hypertensive patients 211 out of 385 (54.81%) were diabetes mellitus only, and 174 out of 385 (45.19%) were diabetic with blood hypertension Figure (4 - 4). According to microalbuminuria 183 out of 385 (47.53%) were negative test and 202 out of 385 (52.47%) were positive Figure (4 - 5). According to the duration of diabetic 163 out of 385 (42.34%) subjects were less than 5 years, 83 out of 385 (21.56%) were between 5 to 10 years, 58 out of 385 (15.1%) were between 10 to 15 years, and 81 (21.0%) were more than 15 years Figure (4 - 6). According to treatment plans 30 out of 385 (7.8%) subjects were diet, 262 out of 385 (68.1%) were oral hypoglycemic, 61 out of 385 (15.8%) subjects were insulin, and 32 out of 385 (8.3%) were Insulin+Oral hypoglycemic treatment Figure (4 - 7).

In this study, the TTR in all sample, according to sex, when female compared to male - as shown in table (4 - 2), and Figure (4 - 1), that demonstrate the distribution of mean levels of female and male of all samples – gave a significant increased level by 14.6% (p value = 0.001), which in agreement with [Houston-Bolze MS](#), [Downing MT](#), and etal.(Houston-Bolze, M.S., and etal; 1996).

In the other hand TTR in all sample, according to microalbuminuria, when negative test results compared to positive test results - as shown in table (4 - 3), and Figure (4 - 9), which showed the comparisons of mean levels of negative test results, and positive test results were significantly decreased by 6.45% (p value = 0.006). These findings were agreed with other reports of J Raila, A Henze and etal (Raila, J., and etal; 2007), and also agree with Luísa Lobato, Idalina Beirão, and etal (Lobato, L., and etal; 2003).

In the study, the FBS in all sample, according to the duration, the comparison of the different types of durations gave a significant different, compared between mean groups, (p =

0.035), table (4 - 6) and Figure (4 - 10), which agreed with [Meena Verma](#), [Sangeeta Paneri](#), and etal. (Verma, M., and etal; 2006).

The comparison FBS Duration < 5 years vs FBS Duration between 5 – 10 years table (4 - 4) and Figure (4 - 10). < 5 years decreased from between 5 – 10 years by 9.7% ($p = 0.036$), which explain the comparisons of mean levels of FBS duration of disease in figure (4 - 1), which agreed with [Meena Verma](#), [Sangeeta Paneri](#), and etal (Verma, M., and etal; 2006)

The comparison of FBS Duration < 5 years vs FBS Duration between 10 – 15 years table (4 - 4) and Figure (4 - 10). < 5 years decreased from between 10 – 15 years by 13.2%, ($p = 0.014$), gave a significantly different, compared between mean groups, ($p = 0.035$), table (4 - 6)., which explain the comparisons of mean levels of FBS duration of disease in figure (4 - 1) , which agreed with [Meena Verma](#), [Sangeeta Paneri](#), and etal (Verma, M., and etal; 2006).

The comparison FBS Duration < 5 year's vs FBS Duration >15 years table (4 - 4) and Figure (4 - 4). < 5 years decreased from >15 years by 10.3 % ($p = 0.027$), gave a significantly different, compared between mean groups, ($p = 0.035$), table (4 - 6)., which explain the comparisons of mean levels of levels of FBS duration of disease in figure (4 - 1) which agreed with [Meena Verma](#), [Sangeeta Paneri](#), and etal (Verma, M., and etal; 2006).

The Correlation of FBS to HbA1c, in all sample were gave significantly different, ($p = 0.000$) as shown in table (4 - 6).) which agreed with [Ezra Belay Ketema](#) and Kelemu Tilahun (Ketema, E.B. and Kibret, K.T; 2015).

In this study according to the sex Correlation of FBS in female and male, were significantly different that showed comparisons of mean levels of gave increase in female compare to male by 11.13% ($p = 0.000$) as shown in table (4 - 7) and Figure (4 - 10), which agreed with Emmanuel M. Musenge, Alexey Manankov, and etal (Musenge, E.M., and etal; 2016), disagreed with [Edo AE](#), [Akhuemokhan K](#), and etal. (Edo, A.E. and Akhuemokhan, K; 2012), and disagreed with [Faerch K](#), [Borch-Johnsen K](#), and etal (Faerch, K., and etal; 2010).

In the other way, according to the status of diabetes with or without hypertension Correlation of FBS in diabetic patient to FBS in diabetic with hypertension patients were significantly different gave increased diabetes patient compare to diabetes with hypertension by 8.1 %, ($p = 0.007$) as shown in table (4 - 7). Which agreed with Yashoda Mittal (Mittal, Y; 2014).

This study showed that HbA1c Duration were significantly different, ($p = 0.006$), table (4 - 6). Which agreed with Chinwe O. Ewenighi, and etal (Ewenighi, C.O., and etal; 2012).

HbA1c Duration < 5 years vs between 5 – 10 years were significantly different, ($p = 0.005$), table (4 - 5) and Figure (4 - 11). Which agreed with Chinwe O. Ewenighi¹, and etal (Ewenighi, C.O., and etal; 2012).

HbA1c Duration < 5 years vs between 10 – 15 years were significantly different, ($p = 0.014$), table (4 - 5) and Figure (4 - 11). Which agreed with Chinwe O. Ewenighi¹, and etal (Ewenighi, C.O., and etal; 2012).

But in the HbA1c Treatment were significantly different, ($p = 0.026$), table (4 - 7) and Figure (4 - 11). Which agreed with Nathan DM, McKitrick C, and etal (Nathan, D.M., and etal; 1996).

In this study according to the HbA1c Treatment Diet vs oral hypoglycemia were significantly different, ($p = 0.039$), table (4 - 5) and Figure (4 - 11). Which agreed with Nathan DM, McKitrick C, and etal (Nathan, D.M., and etal; 1996).

The study showed that HbA1c Treatment Diet vs Insulin+Oral hypoglycemia were significantly different, ($p = 0.006$), table (4 - 5) and Figure (4 - 11). Which agreed with Nathan DM, McKitrick C, and etal (Nathan, D.M., and etal; 1996).

The study of Correlation of HbA1c to sex, were significantly different, ($p = 0.001$) as shown in table (4 - 7) and Figure (4 - 11). Which agreed with Chinwe O. Ewenighi¹, and etal (Ewenighi, C.O., and etal; 2012), and Lisa Arnetz, Neda Rajamand Ekberg, and etal (Arnetz, L., and etal; 2014).

In the study of Correlation of HbA1c to DMBP, were significantly different, ($p = 0.013$) as shown in table (4 - 7) and Figure (4 - 11). Which agreed with Pedro Cabrales, Miguel A Salazar Vázquez, and etal (Cabrales, P., and etal; 2008).

This study showed according to Treatment vs Duration were significantly different, ($p = 0.000$) as shown in table (4 - 7). Which agreed with Manal M. Hassan, Steven A. Curley, and etal (Hassan, M.M., and etal; 2010).

In this study according to the Correlation of DMBP to sex, were significantly different, ($p = 0.033$) as shown in table (4 - 7). Which agreed with N. K. Chowta, P. Pant, and M. N. Chowta (Chowta, N.K., and etal; 2009).

In this study of Correlation of DMBP to age, were significantly different, ($p = 0.000$) as shown in table (4 - 7). Which agreed with N. K. Chowta, P. Pant, and M. N. Chowta (Chowta, N.K., and etal; 2009).

Chapter Five

5.1 Conclusion:

The results of this study revealed that TTR is act as management, control and nutritional biomarker test for type2 DM patients, TTR is verifying and accurate for managing and controlling type 2 diabetes mellitus

5.2 Recommendation:

This study is done in diabetic type 2, and diabetic type2 with high blood hypertension patients, should be carried in other abnormal individuals.

This study is done in diabetic type 2, should be carried in type 1.

Other study should be done to evaluate TTR in urine measurement.

In this study TTR tested with FBS, MAU, and HbA1c, more research should be done using other influencing with insulin, glucagon, C-peptide, systatin C, T₄, & T₃.

This study was carried in Khartoum state; other study should be carried all the country.

This study was carried in adult Sudanese, need other study included other age groups.

Other study should be done to evaluate the effects of anemia and malnutrition laboratory tests with Transthyretin.

Other study should be carried to evaluate the effects of Evaluation treatment plan of DM after short time with TTR instead of 2 to 3 months HbA_{1c}.

Other study should be carried TTR to evaluate the effects of brain damage with hypoglycemic coma.

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