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Evaluation of Blood Glucose and Thyroid Function In Sudanese Diabetic Patients

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DEDICATION

To my dear parents: my father and my mother for all their helps.

To my husband Omer for his extreme support and patience.

My sister Molhima: for her care of my lovely kids:Rabah ,Rawan, Rami.

Especially to: Ammar Mohamed ELamin. Who taught me the meaning of friendship and a lot of things.

To all of them I dedicate this work with real love and respect.

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ABSTRACT

This study composes of two parts. The first one is a survey of thyroid abnormalities. and the second one is experiment to estimate the level of thyroid hormones (T_4, T_3, TSH) among diabetic population and the relationship between the level of glucose, thyroid hormones and lipid profile (TC, LDL, TG, HDL) and comparison of the results with non diabetic group. The survey part of the study to determine the thyroid abnormalities, hypothyroidism or hyperthyroidism clinical or subclinical. Also the study group was divided in to two groups according to insulin requirement. Type1 is insulin-dependent diabetes mellitus and type II is non insulin-dependent diabetes mellitus. The study subjects selected for this study consist of one hundred Sudanese diabetic patients, they had mean age of 46.51±10.672 years, a mean height of 162.06± 10.77 cm and a mean weight of 73.47±14.91 kg. Fifty healthy non- diabetic people without endocrine disease were chosen as controls. Glucose, and thyroid hormones, thyroxine and triiodothyronine (Total T_4 and T_3) and thyroid stimulating hormone (TSH) were measured. In addition hemoglobin (Hb), total cholesterol (TC), triglyceride (TG), high-density lipoproteins (HDL) were also measured in both groups. Low- density lipoprotein (LDL) was calculated for each sample. Physical examinations such as hight, weight, and history of diabetes, family history, treatment were recorded in both groups. Determination of serum hormones concentration was carried out using highly sensitive RIA technique. While determination of blood glucose, hemoglobin, and lipid profile was carried out using enzymatic colorimetric method. The results of this study showed that:

13 patients of the population screened had thyroid disease. The commonest diagnosis was sub clinical hyperthyroidism (6%), followed by sub clinical hypothyroidism (5%) and hyperthyroidism (2%). Female patients with diabetes had the highest annual risk of developing thyroid disease but all patient groups had a higher incidence of thyroid dysfunction, compared with control group.

Experimental part: the present study found highly significant (P=0.028) low level of thyroid stimulating hormone. And significant negative (r=- 0.272 ; P= 0.001) correlation was observed between glucose level and thyroid stimulating hormone. And highly significant (P=0.03) low level of triiodothyronine (T_3) of diabetic patients compared with non diabetic group. No significant variation was found at level of

tetraiodothyronine (T_4). Highly significant (p=0.001) elevated level of blood glucose was found among diabetic patients compared to non diabetic group.

The study found a significant (p=0.001, p=0.009, p=0.001 respectively) elevated level of some serum lipids (TC, LDL, TG) of diabetic patients compared to non diabetic group. And significant (p=0.001, p= 0.023, p=0.001 respectively) positive correlation (r=0.307, r=0.296, r=0.186 respectively) was found between glucose and some serum lipids (TC, LDL, TG). No variation was found at the level of HDL and Hb.

This study conclude that regular screening for thyroid hormones levels especially thyroid stimulating hormone in all diabetic patients will allow early treatment of sub clinical thyroid dysfunction which affects glucose level and development of cardiovascular disease.

الخـــــلاصـــة

هــذه الدراســة مكونة مــن جزئين مســح احصائين والاخر تجريبي.لمعرفة العلاقــة بــين مــرض السكــري ومستوي هرمونات الغــدة الدرقية. وثأثيــر ذ لــك علــي مستـــــوي الدهنيات(TC, LDL ,TG, HDL) .

في هذه الدراسة تم الحصول علي عينات دم من مائة فرد من مختلف الأعمار رجال ونســاء مصابين بداء السكري بنوعيه الأول والثاني(داء الســكري المعتمــد علــي الأنســولين)و(داء السكري غيرالمعتمدعلي الأنسولين) . كما تم الحصول علي عينــات دم مــن خمســين فــردا اصحاء غيرمصابين بداء السكري أو أي نوع من أمراض الغــدة الدرقيــة. بــنفس الأعمــار السابقة كمجموعة تحكم. تم قياس هرموني الغدة الدرقية الكلية (الثيروكسين والثيرونين ثلاثــي اليود).كماتم أيضا قياس الهرمون المحفــز للغــدة الدرقيــة ومســتوى بعــض اجــزاء مــن الدهون(TC,LDL,TG,HDL) وخضاب الدم . تم استخدام الطريقة العالية الحساسية والدقة و هي طريقة المقايسة المناعية الإشعاعية لقياس هذه الهرمونات. كما تم قيــاس الجلوكــوز و مستوى الدهون(TC,LDL,TG,HDL) في السيرم وخضاب الدم باستخدام قياس الطيــف الضوئي. تم تشخيص ثلاثة عشر فرد لديهم خلل في مستوي هرمونات الغدة الدرقيــة اوخلــل تحت سريرى من جملة المائة شخص المصابين بداء السكري(13%)

أظهرت هذه الدراسة:انخفاضا ملحوظاذامعني(P=0.028) في مستوي الهرمون المحفز للغــدة الدرقية مقارنة مع مستوي الهرمون المحفزللغدة الدرقية للاصحاء. كما وجدت علاقة معنويــة عكسية قوية(r= -0.272, p=0.001) بين مستوي الجلكوز في الــدم ومســتوي الهرمــون المحفزللغدة الدرقية (TSH). كما ان هنالك انخفاض معنوي(p=0.03) في مستوي الثيرونين ثلاثي اليود لدي مرضي السكري مقارنة بالاصحاء. بينما لم يتاثر مستوي الثيروكســين لــدي هولاء المرضي مقارنة بالاصحاءكما ان هنالك اختلاف معنــوي(p=0.001) واضــح فــي مستوى الجلكوز في الدم لدي هولاء المرضي مقارنة بالاصحاء.

كما اظهرت هذه الدراسة ذيادة معنوية معتبرة احصائيا (p=0.001, P=0.009, P=0.001 respectively) في مستوي الدهنيات (TC, LDL, TG) لدي هولاء المرضــي مقارنــة بالاصحاء بينما لا يوجد اختلاف في مستوي HDL ومستوي Hb في الدم. كمــا وجــدت علاقة معنوية طردية قوية r=0.307p=0.001, r=0.186 p=0.023, r=0.296 p=0.001 بين مستوي الجلكوز في الدم والدهنيات (TC, LDL, TG). بينما لم توجد اي فروق معنويــة في مستوي خضاب الدم ومستوي (HDL) بين المرضي والاصحاء.

توصلت هده الدراسة الي ان هنالك ضرورة لعمل فحص دوري لمستوي هرمونـــات الغـــدة الدرقية لمرضي السكري وخصوصا مستوي الهرمون المحفز للغدة الدرقيـــة لتحديـــد الخلـــل التحت سريري لهرمونات الغدة الدرقية، لما في ذلك من أثر علي مستوي الجلكوز في الـــدم بالاضافة إلى مستوى بعض اجزاء الدهون (TC,LDL,TG,HDL) في مصل الدم وتاثيرها علي امراض القلب والشرايين.

TABLE OF CONTENTS

DEDICATION ……………………………………………………………………….….…I
ACKNOWLEDGEMENT………………………………………………………….…II
ABSTRACT………………………………………………………………………….III
ARABIC ABSTRACT……………………………………………………………..V
TABLE OF CONTENTS……………………………………………..……..……VII
LIST OF TABLES……………………………………………………...……..…..XI
LIST OF FIGURES……………………………………………….………..….……XII
ABBREVIATIONS……………………………………………………………...XIII
INTRODUCTION ………………………………………………………….........1
Objectives...2
CHAPTER ONE.…………………………..……………………………......3
LITERATURE REVIEW ………………………………………….…..……..3
1.1. Diabetes mellitus…………………………………………………………...…...3
1.1.1. Diagnosis of Diabetes mellitus……………………………….………...4
1.1.2. Classification of diabetes mellitus…………………………………….….5
1.1.2.1. Insulin-dependent diabetes mellitus……………………………..………..…6
1.1.2.2 .Non-insulin-dependent diabetes mellitus………………………..……...…6
1.1.3. Diabetes mellitus and thyroid disease…………………………….……..…7
1.1.4. Prevalence of thyroid dysfunction.………………………………………….…9
1.1.5. Screening of thyroid dysfunction.…………………………………...………9
1.1.6. Type1 diabetes mellitus and thyroid dysfunction………………………..........9
1.1.7 .Type II diabetes mellitus and thyroid dysfunction……………………..………10
1.1.8. Diabetes mellitus in Sudan………………………………………….......11
1.2. The Thyroid gland…………………………………………………........…13
1.2.1. Thyroid hormones……………………………………………..……..…13
1.2.2. Synthesis of thyroid hormones……………………………........…......13
1.2.3. Secretion of T_3 and T_4…………………………………………………..14
1.2.4. Transport of thyroid hormones……………………………………….…....14
1.2.5. Control of thyroid function…………………………………....…..…14
1.2.6. Metabolism of thyroid hormones……………………………………….…....15
1.2.7. Thyroid hormones’ action…………………………………………..…..15
1.2.8. Effects of thyroid hormones……………………………...…..……15

1.2.9. Iodine and thyroid function……………………………..……….……….…...…16
1.2.10 .Thyroid gland disorders…………………………………..….……….....…16
1.2.10.1. Hypothyroidism...16
1.2.10.2. Subclinical hypothyroidism...17
1.2.10.3. Hypothyroidism and glycemic status……………….…………………..17
1.2.10.4. Hyperthyroidism...17
1.2.10.5.Subclinical hyperthyroidism………………………….…..…….…….…..17
1.2.10.6. Hyperthyroidism and glycemic Status………………….………………......17
1.3. Diabetes mellitus and dyslipidaemia……………………………………………...18
1.3.1. Cholesterol and diabetes mellitus……………………….……………...…...19
1.3.2. Storage of triglyceride……………………………………………………...20
1.4. Effects of thyroid on lipids metabolism…………………….…………….……….20
CHAPTER TWO…………………………………………….……….……………...22
MATERIALS AND METHODS…………………………….……………...…....22
2.1. Subjects…………………………………………………….……………….…...22
2.2 .Materials………………………………………..……………………………..22
2.2.1. Equipment and disposables…………………………………………..….22
2.3. Reagents for radioimmunoassay……………………….………………...…....23
2.3.1. TSH Kit…………………………………...……….……………………….....23
2.3.2. T_4 and T_3 Kits………………………………...…………….……………………24
2.4. Lipid, glucose and hemoglobin reagents…………………………………...…...24
2.4.1 .Triglyceride.………………………………………………………………..…24
2.4.2. Cholesterol…………………………...………………………….…………....24
2.4.3. HDL…………………………………………………………………………..24
2.4.4. LDL……………………………….…………………………………………...24
2.4.5.Blood glucose………………………………………………….…….…...…...25
2.4.6. Hemoglobin…………………………………………………….......….......25
2.5. Methodology and principle………………………………………….……..….25
2.5.1. Blood samples…………………………………………………..………...…25
2.5.2.Principle of radioimmunoassay (RIA)………………………………..………..25
2.5.2.1. Determination of total serum thyroxine (T4)……………………..…......….26
2.5.2.2. Determination of total serum triiodothyronine (T_3)…………….……..…….26

2.5.3. Principle of immunoradiometric assay (IRMA)…………….……………......…26
2.5.3.1. Determination of thyroid stimulating hormone (TSH)………………………..27
2.5.4. General principle of colorimetric method……………………..……........……27
2.5.4.1. Principle for determination of triglyceride…………………...……......…..…27
2.5.4.2. Principle for determination of total cholesterol……………………………….28
2.5.4.3. Principle for determination of HDL – cholesterol………………….……..….29
2.5.4.4. Determination of blood glucose……………………………………............…30
2.5.4.5. Determination of hemoglobin (Hb) concentration……………….……....…..30
2.6. Statistical analysis……………………………………………………..………...31
CHAPTER THREE……………………………………………………….…..…..…32
RESULTS……………………………………………………………………….……...32
3.1. Preamble of results……………………………………………………….……..…32
3.2 .Prevalence of thyroid dysfunction…………………………………………….……...33
3.3.Serum total thyroid hormones (T_4 and T_3)……………………….……….....……35
3.4.Serum thyroid stimulating hormone (TSH)…………………….……………..35
3.5 . Blood glucose level…………..……………………………………….………..35
3.6. Hemoglobin concentration (Hb)………...………………………………………35
3.7 .Serum total cholesterol concentration (TC)…………………………….……..41
3.8. Serum triglyceride concentration (TG)…....………………………….……….…..41
3.9 .Serum low- density lipoprotein concentration (LDL)……………...……….….…41
3.10 .Serum high – density lipoprotein concentration (HDL)…...……………...……...41
3.11. Correlation between glucose and total cholesterol (TC)…………...………...…..51
3.12.Correlation between glucose and triglyceride (TG)……………………….……...51
3.13.Correlation between glucose and LDL-cholesterol (LDL)……………………......51
3.14. Correlation between glucose and thyroid stimulating hormone…...……….…...…51
CHAPTER FOUR...………………………………………………………….……...…56
DISCUSSION……………………………………………………………………..……56
4.1. Prevalence of thyroid dysfunction……...…………………………….………...…56
4.2.Thyroid status……………………………………………………….……….……..58
4.2.1.Serum thyroid stimulating hormone (TSH)…………………….……………..…..58
4.2.2 .Serum total thyroid hormones (T_4 and T_3)…………………….………………...59
4.2.2.1.Triiodothyronine (T_3)…………………………………………………………..59
4.2.2.2.Tetraiodothyronine (T_4)…………………………………...…………………60

4.3. Glucose level……………………………………….……………………….…..60
4.4. Lipid profile……………………………………...……………………..…..60
4.5. Lipid profile and thyroid…………………………..…...………………..…..……….61
CONCLUSIONS AND RECOMMENDATIONS……..………………….……..…….62
Conclusions……………………………………….…………………………....62
Recommendations………………………………..……………………...……..62
References…………………………………………………………………..…..63
Appendix 1………………………………………………………………….….73
Thyroid hormones, blood glucose, hemoglobin in diabetic patients.............................73
Appendix 2 ……………………………………………………………...……..74
Thyroid hormones, blood glucose, hemoglobin in diabetic patients………..………….74
Appendix 3...75
Thyroid hormones, blood glucose, hemoglobin in non diabetic subject……………….75
Appendix 4: Lipid Profile (TC, HDL, TG, LDL) in diabetic patients……………….….76
Appendix 5: Lipid Profile (TC, HDL, TG, LDL) in diabetic patients………………...77
Appendix 6: Lipid Profile (TC, HDL, TG, LDL) in non diabetic subject……..…...….78
Appendix7: Study Questionnaire ………………………………………….…...….79
Appendix 8: Normal Range of different measurments in human……………..…….80

List of Tables

Table **3. 1**: Classification of clinical status of thyroid.... **Error! Bookmark not defined.**

Table **3. 2:** level of total thyroid hormones ($T_4$and T_3), thyroid stimulating hormone (TSH), blood glucose and hemoglobin, in diabetic and non- diabetic groups........ **Error! Bookmark not defined.**

Table **3. 3: l**evels of total cholesterol, triglyceride, high- density lipoprotein, low-density lipoprotein in diabetic and non- diabetic subjects.......... **Error! Bookmark not defined.**

Table **3. 4**: levels of total ($T_4$and T_3), thyroid stimulating hormone (TSH) glucose and hemoglobin, in diabetic (Type I and TypeII) and non- diabetic ... **Error! Bookmark not defined.**

Table **3. 5:** Total cholesterol, triglyceride, low- density lipoprotein, high- density lipoprotein of diabetic (Type1 and TypeII) and non diabetic groups...**Error! Bookmark not defined.**

LIST OF FIGURES

Figure **3. 1:** Prevalence of thyroid dysfunction in diabetic and non-diabetic subjects… .... **Error! Bookmark not defined.**
Figure **3. 2:** Mean values of glucose for diabetic and non- diabetic subjects......... **Error! Bookmark not defined.**
Figure **3. 3:** Mean values of T_4 (nmole/L) for diabetic and non- diabetic subjects. **Error! Bookmark not defined.**
Figure **3. 4:** Mean values of T_3 (nmole/L) for diabetic and non- diabetic subjects. .... **Error! Bookmark not defined.**
Figure **3. 5:** Mean values of TSH (mlU/L) for diabetic and non- diabetic subjects.**Error! Bookmark not defined.**
Figure **3. 6:** Level of lipid (TC, TG, LDL, HDL mg/dl) for diabetic and non- diabetic subjects.. **Error! Bookmark not defined.**
Figure **3. 7:** Mean values of blood glucose (mg/dl) for diabetic (Type1 and TypeII) and non diabetic subjects.. **Error! Bookmark not defined.**
Figure **3. 8:** Mean values of thyroxin (T4 nmol/l) for diabetic (Type I and Type II) and non diabetic subjects.. **Error! Bookmark not defined.**
Figure **3. 9:** Mean values of Triiodothyronine (T3 nmol/l) for diabetic (Type1 and TypeII) and non diabetic subjects................................. **Error! Bookmark not defined.**
Figure **3. 10:** Mean values of thyroid stimulating hormone (TSH mlU/L) for diabetic (Type1 and TypeII) and non diabetic subjects. .... **Error! Bookmark not defined.**
Figure **3. 11:** Lipid Profile (TC, TG, HDL, LDL mg/dl) for diabetic (Type1 and TypeII) and non-diabetic subjects. .... **Error! Bookmark not defined.**

Figure **3. 12:** Correlation between glucose and total cholesterol (TC).**Error! Bookmark not defined.**
Figure **3. 13:** Correlation between glucose and triglyceride (TG) **Error! Bookmark not defined.**
Figure **3. 14:** Correlation between glucose and LDL-cholesterol. **Error! Bookmark not defined.**
Figure **3. 15:** Correlation between glucose and TSH. .... **Error! Bookmark not defined.**

ABBREVIATIONS

Abbreviation	Name
4 – AA	4 – aminoantipyrine
Ab	Antibody
Ag	Antigen
APO	Apolipoprotein
ATP	Adenosine Triphosphate
BMI	Body mass index
CE	Cholesterol esterase
CHD	Coronary heart disease
CM	Chylomicrons
CO	Cholesterol oxidase
DHAP	Dihydroxy acetone phosphate
FFA	Free Fatty Acid
G-3-P	Glycerol -3-phospate
GOD	Glucose oxidase
GPO	Glycerol phosphate oxidase
HDL	High density lipoprotein
IDDM	Insulin dependant diabetes mellitus
IRMA	Immunoradiometric assay
LDL	Low density lipoprotein

LPL	Lipoprotein lipase
NIDDM	Non – insulin dependant diabetes mellitus
POD	Peroxidase
RIA	Radioimmunoassay
rT_3	Reverse Triiodothyronine
(Tgab)	Thyroglobulin antibodies
(TPOab)	Thyroid peroxidase antibodies
T_4	Thyroxine
TBG	Thyroxine Binding globulin
TBPA	Thyroxine Binding prealbumin
TG	Triacylglycerol (Triglyceride)
TSH	Thyroid stimulating hormone
VLDL	Very low-density lipoprotein

INTRODUCTION

Diabetes mellitus in Sudan as in many developing countries constitutes a growing health problem with a major impact. Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia and predisposes to chronic complications affecting the eyes, blood vessels, nerves, kidneys and endocrine glands (Abdelgader *et al*., 2006). It is generally believed that both thyroid disorders and diabetes mellitus are common in Sudan. Thyroid disorders such as goiter, nodules, and autoimmune thyroid disease and thyroid dysfunction have rarely been investigated in diabetic patients. A number of symptoms and signs are well-established manifestations of thyroid dysfunction. Additional findings in patients, personal and family histories indicate increase risk of developing thyroid dysfunction, diabetes mellitus represent risk factor of developing thyroid dysfunction (Paul *et al*., 2000).

The prevalence of thyroid dysfunctions in diabetes mellitus population has not received sufficient attention yet. Currently, there are no data on the relation between diabetes mellitus and thyroid dysfunction there is a lack of data concerning this problem. For all these reasons we studied the prevalence of thyroid disorder, lipid abnormalities among Sudanese diabetic patients. Estimation of lipid profile for each patient because of their relationship to cardiovascular disease. The analysis of serum lipids has become an important health measurement. Abnormalities of plasma lipids and lipoproteins have been documented in untreated and treated diabetic population by different groups. The hyperlipidemia and the hyperlipoproteinemias have been implicated as risk factors in diabetic population (Retnam *et al*., 1983). Hence knowledge of the various aspects of the lipid profile and the significance of each of the parameters is vital and is essential part of management of coronary heart disease (CHD) and people at risk of CHD.

Diabetic subjects had been diagnosed under supervision of consultant physician and endocrinologist their age 25–75 year. classified in two groups, type I(insulin-dependent diabetes mellitus) and type II (non-insulin dependent diabetes mellitus) were randomly selected from those attending the outpatient clinic of the Endocrine and Internal Medicine Units of Mulazmin Medical Center. The patients underwent a clinical and laboratory evaluation and answered a standardized questionnaire to collect information using pre-tested questionnaires. Initially information, on age, family and diabetes history, medication used and residence, blood pressure, weight (kg) and height (cm) were measured and body mass index (BMI, kg/m2) was calculated. A blood sample was

taken after12–14 h overnight fasting period. The laboratory assessment included fasting plasma glucose, hemoglobin, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides. Thyroid function test, (TSH, T_4, T_3) were done for all samples using a highly sensitive radioimmunoassay. Fasting blood sugar (FBS) was measured on the day of blood collection by enzymatic colorimetric method using glucose oxidase. For lipid measurements total cholesterol and triglyceride were assayed using enzymatic colorimetric tests with cholesterol esterase and cholesterol oxidase, and glycerol phosphate oxidase, respectively. HDL-C was measured after precipitation of the apolipoprotein B containing lipoproteins with phosphotungistic acid. All study samples and control people were collected from Khartoum, Khartoum North, and Omdurman area. Samples were stored at - 20 C° in Central Laboratory for Medical Analysis and Research at Sudan Atomic Energy Commission until analyzed. Some blood analyses were done at the day of blood collection. All measurements were done at radioimmunoassay laboratory, Institute of Radiobiology, Sudan Atomic Energy Commission.

Our aim was to study the spectrum of thyroid disorders in diabetic subjects with previously undiagnosed thyroid dysfunction, and compare them with results obtained from a sample of the normal adult population and the relation between diabetes and thyroid dysfunction. This study suggests that thyroid function should be screened annually in diabetic patients to detect asymptomatic thyroid dysfunction which is increased in frequency in diabetic patients, since progression from subclinical disease to clinical disease has been observed in longitudinal studies. Authors suggest that clinically unrecognized thyroid failure could predispose to ischemic heart disease in the diabetic population. Thus thyroid function tests should be measured in patients with an otherwise inexplicably worsening dyslipidemia.

Objectives:

1. To determine the prevalence of undiagnosed thyroid disease in diabetic patients not previously suspected having thyroid disease.
2. To investigate the relationship between diabetes mellitus and thyroid function.
3. To explore the relationship of plasma lipid levels to the degree of blood glucose control.
4. To investigate if there is any correlation between lipid profile and thyroid function in diabetic patients.

CHAPTER ONE

LITERATURE REVIEW

1.1. Diabetes mellitus :

Diabetes mellitus is a major public health problem affecting many people. It is the leading cause of adult blindness and amputation, and a major cause of renal failure, heart attack and stroke. Diabetes is not a one disease but rather is a heterogeneous group of syndromes characterized by an elevation of fasting blood glucose caused by a relative or absolute deficiency in insulin, metabolic alterations is caused by inadequate release of insulin are aggravated by an excess of glucagons (Abdelgader *et al*., 2006 Pamela *et al.,*2000). Hyperglycaemia has an important role in the pathogenesis of long-term complications. Diabetic patients with poor blood glucose control are particularly at risk. Furthermore, complications appear to affect organs where cells do not require insulin for glucose uptake, such as those of the nervous system, heart, kidneys and small blood vessels. Consequently, these cells have high concentrations of intracellular glucose during hyperglycaemia (Nessar *et al.,* 2004; Elbagir *et al* 1998).

Diabetes is characterized by polyuria, polydipsia, and weight loss in spite of polyphagia hyperglycemia, glycosuria, ketosis, acidosis, and coma. There are wide spread biochemical abnormalities but the fundamental defects to which most of the abnormalities can be traced are reduced entry of glucose into various peripheral tissues and increased liberation of glucose into the circulation from the liver. There is therefore an extra cellular glucose excess and in many cells, an intracellular glucose deficiency, there is also decrease in the entry of amino acids into muscle and an increase in lipolysis (Ganong, 2003).

Diabetes mellitus and thyroid diseases are the two common endocriniopathies seen in the adult population. With insulin and thyroid hormones being intimately involved in cellular metabolism and thus excess or deficiency of either of these hormones could result in the functional derangement of the other. When thyroid disease occurs in some one with diabetes it can make blood glucose control more difficult (Sathish and Mohan, 2003).

In the past thirty years, numerous goiter surveys has been conducted in Sudan, a study done in Darfur compared with Khartoum reveling that goiter prevalence was 57% in Darfur and 12.6% in Khartoum (Kambal*,* 1969). The study was extended and updated to study goiter prevalence in Sudan and reported that, the prevalence of goiter was 87.1%

in Darfur region and 17.5% in Khartoum (Eltom,1984) another survey was a comparison between simple goiter in Omdurman and the effects of thyroxin versus iodine in the treatment of simple goiter (Hassan*,* 1993). Study done concerned iodine supplementation in a goiter endemic area (Babikir, 1996). Recently, another research that has been done on maternal thyroid hormones levels in normal Sudanese pregnant women to evaluate suspected thyroid abnormalities (Hanadi, 2004). Both diabetes and thyroid disorder involve a dysfunction of the endocrine system, which regulates the body metabolism. Diabetes and thyroid disorder tend to appear jointly in patients. Almost one – third of the patients with type I diabetes have been found to have common thyroid disease. This is because type I diabetes and common thyroid disorders have autoimmune origin. People with one autoimmune disease are more likely to develop another autoimmune disease than the general population. Thyroid disorders are also common in type II diabetes because both of these diseases tend to occur more frequently as people grow old (Yasmin *et al.,* 2006 Patricia*,* 2000; Hansen *et al* 1999).

Because of the link between diabetes and thyroid diseases, the American Diabetic Association has recommended that people with diabetes should be tested for thyroid disorder. The thyroid -stimulating hormone (TSH) blood test is the best to determine thyroid function (Yasmin *et al.,* 2006).

Diabetic patients have a higher prevalence of thyroid disorders compared with the normal population (Jennal *et al.*, 2002; Patricia, 2000). Thyroid disorders are more common in females(Sukkar *et al*.,2000) it is not surprising that up to 30 % of female type I diabetic patient have thyroid disease. A number of reports have also indicated a higher than normal prevalence of thyroid disorders in type II diabetic patients, with hypothyroidism being the most common disorder (Patricia., 2000).

1.1.1. Diagnosis of Diabetes Mellitus:

Diabetes occurs either because of a lack of insulin or because of the presence of factors that oppose the action of insulin. The result of insufficient action of insulin is an increase in blood glucose concentration (hyperglycaemia). Many other metabolic abnormalities occur, notably an increase in ketone bodies in the blood when there is a severe lack of insulin (Abdelgader, 2006). The diagnosis of diabetes must always be established by measuring blood glucose, although glycosuria nearly always indicates diabetes. Fasting blood glucose concentrations greater than 140mg/dL and random blood glucose concentrations greater than 200 mg/dl are clearly diagnostic of diabetes

and fasting values less than 110mg/dL or random levels less than140 mg/dl exclude diabetes. Glycosuria usually occurs when blood glucose values are greater than 180 mg/dl but this threshold varies considerably between individuals and increases with age. Only if blood glucose concentrations are borderline and there is cause for a doubt about the presence of diabetes glucose tolerance test should be performed (Abdelgader *et al*., 2006; Elbagir *et al*., 1995, 1999; Elmahadi *et al., 1989*; Peter, 1982)

For reliable results a glucose tolerance test should be performed in the morning after an overnight fast with the patient sitting quietly. It is also important that he should have had normal meals for the last three days and should not have been dieting. False results may also occur if the patient has been ill recently or has had prolonged bed rest. Blood glucose concentrations are measured fasting and then every half an hour (For two hours) after a drink of 75 g of glucose in 250-350 ml water (in children 1-75 g/kg to a maximum of 75 g), Urine tests should be performed before the glucose drink and at one and two hours. (Tietz *etal*., 2000; Peter, 1982). In diabetes, glucose piles up in the bloodstream, especially after meals. If glucose load is given to a diabetic, the plasma glucose rises higher and returns to the base line more slowly than it dose in normal individuals. The response to a standard oral test dose of glucose, the oral glucose tolerance test, is used in the clinical diagnosis of diabetes. Impaired glucose tolerance in diabetes is due in part to reduced entry of glucose in to cells (decreased peripheral utilization) In the absence of insulin, the entry of glucose in to skeletal, cardiac, and smooth muscle and other tissues is decreased (Ganong , 2003).

1.1.2. Classification of diabetes mellitus:

According to the World Health Organization, diabetes mellitus is defined as a metabolic disorder of multiple aetiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Abdelgader, 2006).

Although all types of diabetes have hyperglycaemia in common, the causes vary, as does treatment and long-term outlook. Diabetes can be classified into two groups: Insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) based on their requirement for insulin (Peter, 1982; Abdelgader, 2006). This division is important clinically in assessing the need for treatment and in understanding the causes of diabetes, which are different in the two groups. Nevertheless, although most patients can be clearly distinguished as having one type of

diabetes or the other, some non-insulin-dependent diabetics come to need insulin for good health, if not survival (El-shazly *et al*., 2000; Peter, 1982).

1.1.2.1. Insulin-dependent diabetes mellitus (IDDM) :

The disease is characterized by an absolute deficiency of insulin caused by massive autoimmune attack in the B-cells of the pancreas. This destruction requires a stimulus from the environment (such as a viral infection) and genetic determinant that allows the B-cells to be recognized as non-self .The islet of Langerhans become infiltrated with activated T-lymphocytes leading to condition called insulitis over a period of years, this autoimmune attack leads to a gradual depletion of B-cell population. Insulin-dependent diabetes is due to damage to and eventual loss of the B cells of the pancreatic islets of Langerhans with resulting loss of insulin production (Pamela *et al* 2000; Peter, 1982).

However, symptoms appear abruptly when 80% to90% of the B-cells have been destroyed. At this point the pancreas fails to respond adequately to ingestion of glucose, and insulin therapy is required to restore metabolic control. Patients with IDDM can usually be recognized by abrupt appearance of polyuria (frequent urination). Polydipsia (excessive thirst) and polyphagia (excessive hunger).Symptoms are usually accompanied by fatigue, weight loss, and weakness. The diagnosis is confirmed by fasting blood glucose greater than 140mg/dl. Commonly accompanied by ketoacidosis, which can be life-threatening (Pamela *et al*., 2000). Type I (insulin-dependent diabetes mellitus) is an autoimmune and heterogeneous disease. Thyroid disease is the most common autoimmune disease in type I diabetes mellitus (Trimarchi *et al*.,1984; Riley *et al* 1981).

1.1.2.2. Non-insulin-dependent diabetes mellitus :

Non-insulin-Dependent Diabetes (NIDDM) or type II diabetes is the most common form of the disease. It develops gradually without obvious symptoms. The disease is often detected by routine screening tests. Non-insulin-dependent diabetics secrete insulin, and their serum insulin concentrations may be diminished, normal, or even increased. The cause of the diabetes is unknown; the best-known association is with obesity (Frazade *et al*., 2006; Peter, 1982). Type II Diabetes Mellitus (DM) is linked with behavioral, environmental, and social factors such as overweight, physical activity, sedentary behavior, and unhealthy dietary habits (Knowler *et al,* 1995).

However many individuals have symptoms of polyuria and polydipsia of several weeks duration. Polyphagia may be present, but is less common. Patients with NIDDM have

functional B-cells and do not require insulin to sustain life, although insulin may be required controlling hyperglycemia in some patients. Diagnosis is based most commonly on the presence of hyperglycemia that is blood glucose concentration greater than 140mg/dl. The disease does not involve viruses or autoimmune antibodies. The metabolic alternation observed in NIDDM are milder than those described for the insulin-dependent diabetes mellitus form of the disease, and are thought to be due to a combination of two factors dysfuncational B-cells and insulin resistance (Pamela *et al* .,2005;Peter,1982).The incidence and prevalence of type II diabetes mellitus are rapidly increasing worldwide in both developing and developed nations (King *et al.*, 1998; Amos *et al*., 1997).

1.1.3. Diabetes mellitus and thyroid disease:

The adverse effect of diabetes on the circulatory, visual, renal, and endocrine function are commonly recognized and have been extensively studied. The effects of decreased insulin secretion or resistance to insulin action on endocrine glands have not been carefully documented. Both clinical and animal research has demonstrated that diabetes mellitus is commonly associated with altered thyroid. The thyroid hormones, 3, 5, 3'-triiodothyronine, T_3 and thyroxine, T_4 are usually suppressed in both human and experimental animals with diabetes, this effects appears to involve change in hypothalamic thyrotropin–releasing hormone (TRH) secretion as well as change in pituitary thyrotropin, thyroid stimulating hormone, (TSH) and direct effects at the level of thyroid glands (Steger and Rabe 1997).

Diabetes mellitus appears to influence thyroid function in at least two sites, one at the level of hypothalamic control of thyroid stimulating hormone (TSH) release and the other at the conversion of thyroxine (T_4) to 3,5,3'-triiodothyronine (T_3) in the peripheral tissue (Engin *et a*l.,1999;Castells,1984;Shah,1983). Alterations in thyroid hormones indicate the characteristics of low T_3 syndrome. Marked hyperglycemia decreases the activity and concentration of hepatic T_4 -5' deiodinase. The characteristic findings include low serum concentrations of T_3 elevated levels of reverse T_3 (rT_3) and low, normal, or high levels of T_4. The values return to normal level after correction of hyperglycemia (Gilani, 1984). Low serum T_3 is due to reduced peripheral conversion of thyroxine (T_4) to tri-iodothyronine (T_3) via 5' monodeiodination reaction and low serum T_4 due to decreased protein binding and an inappropriately low serum TSH concentration. Many studies indicate that it may be the long-term diabetic control that

determines the plasma T_3 levels. Poorly controlled diabetes may also result in impaired TSH response to TRH or loss of normal nocturnal TSH peak. TSH responses and "low T_3 state" may normalize with improvement in glycemic status. but even with good diabetes control (Sathish and Mohan*,* 2003; Patricia, 2000).

The diagnosis of thyroid dysfunction in diabetic patients based solely on clinical manifestation can be difficult. Underlying thyroid disorders may go undiagnosed because the signs and symptoms are similar to those of diabetes. Symptoms of hypothyroidism are common in patients with type II diabetes such as severe diabetic nephropathy can be mistaken for hypothyroidism because patients with this condition may have odema, fatigue, and weight gain. Symptoms of hyperthyroidism may be attributed to poor diabetic control in patients with type1 diabetes as such as weigh loss, despite increased appetite and fatigue (Yasmin *et al*., 2006; Patricia, 2000*)*.

Thyroid disorders can have a major impact on glucose control and untreated thyroid disorder can affect the management of diabetes, hypothyroidism can decrease the insulin requirement in patients with diabetes while hyperthyroidism worsens glucose tolerance or control and increase insulin requirement (Yasmin *et al*., 2006; Patricia, 2000).

In euthyroid individuals with diabetes mellitus, the serum T_3 levels, basal TSH levels and TSH response to thyrotropin releasing hormone (TRH) may all be strongly influenced by the glycemic status (Sathish and Mohan *2003*). When hyperthyroidism is also present in a patient with poorly controlled diabetes, the total and even free T_4 and T_3 concentrations may be inappropriately normal, in which case the diagnosis would be difficult. A suppressed serum basal TSH or an absolutely flat response to TRH would support the diagnosis. In the cases of this type that have been described, serum thyroid hormones rose to hyperthyroid levels with treatment of the diabetes, and the diagnosis became clear (Cavalieri ,1999).

So, the presence of hyperthyroidism should be suspected in diabetic patients with or without thyroid enlargement in the presence of unexplained weight loss, supraventricular tachycardia, increased body warmth, heat intolerance, and tremor, unexplained increase in insulin requirement, ketoacidosis, and instability of the diabetes or prior symptoms of hyperthyroidism (Kozak and Coopan*,*1985).

1.1.4. Prevalence of thyroid dysfunction:

The prevalence of undiagnosed thyroid disease in diabetic patients receiving community diabetes care was 5.5% (9.5% of female patients), and the prevalence of thyroid disease in the entire population of diabetic patients registered in the general practice was 10.8%. These findings suggest that screening for thyroid disease should be considered in patients receiving diabetes care in the community (Smithson,1998).

Among a diabetic clinic population of 5,000 there were 113 patients (1.1%) with concurrent clinical thyroid dysfunction (56 hyperthyroid, 57 hypothyroid). Seventy-one (62.8%) of these patients were insulin-dependent and diabetes preceded thyroid disease in 85 (75.2%). The value of screening diabetic patients for evidence of thyroid dysfunction is important (Sugrue *et al.,* 1982).

The Whickham survey found that thyroid dysfunction affected 6.6% of adults. There is a recognized association between thyroid disease and diabetes mellitus (Tunbridge, *etal*.,1997). The prevalence rate for thyroid disease was found to be 6.6% in the general population and 10.8% in diabetic patients (Patricia, 2000).

A variety of thyroid abnormalities may co-exist and interact with diabetes mellitus. The reported frequency of hyperthyroidism and hypothyroidism in patients with diabetes has varied from 3.2 % to 4.6 % and 0.7 % to 4.0 % respectively (Sugrue *et al.,* 1999).

High prevalence of hypothyroidism particular in those with uncontrolled diabetes has been reported in Iranian patients (Hamid *et al*., 1998). The combination of diabetes mellitus and primary hypothyroidism is not as rare as generally believed. Nine hypothyroid patients were found among 530 diabetics patients a prevalence of 1.7%. In 5 patients the diabetes occur first (Arthur and Herbert, 1968).

1.1.5. Screening of thyroid dysfunction:

A recent Royal College of Physicians consensus statement does not support routine screening of asymptomatic individuals for thyroid disease in the UK (Vanderpump *et al* 1995). The American Thyroid Association, however, has suggested that screening men and women aged ≥35 years at intervals of 5 years is cost-effective (Ladenson *et al* 2000). Whilst there is debate as to whom in the general population to screen, and how often, there is more agreement, that screening is justified in populations at increased risk of thyroid disease, including people with diabetes (Cooper, 2001).

Different reviewed of international diabetes and thyroid association guidelines for policies on thyroid screening. There was a striking lack of consensus between these

guidelines as to whether and when to screen for thyroid disease in diabetic patients. Indeed, of the 11 guidelines reviewed, four did not mention thyroid function testing (TFT), two recommended periodic testing in high-risk groups, and one suggested there was insufficient evidence for or against testing. This lack of consensus is probably due to the lack of robust data in diabetic patients (Badman and Chowdhury, 2002).

1.1.6. Type1 diabetes mellitus and thyroid dysfunction:

Type I diabetes is associated with a number of polyglandular autoimmune syndromes (Trence *et al.,* 1984). Commonly affected endocrine organs are the thyroid, parathyroid, adrenals and gonads (Betterle *et al.,* 1984). Even without progression to clinical disease, increased levels of organ-specific auto antibodies are detectable in people with Type I diabetes and their first-degree relatives (Grey *and Clarke*,1978).

Early studies reported a prevalence of 5–8% of hypo- or hyperthyroidism in patients with Type I diabetes (Grey *et al.*,1980; Nabarro *et al.,* 1979) similar to the 6.6% prevalence in the general population found in the Whickham survey.

Gray and his group studied the prevalence of previously undetected thyroid failure, as defined by elevated TSH, in 605 patients with Type I diabetes managed in secondary care. They found an overall prevalence of subclinical and clinical hypothyroidism of 12%. The prevalence was much higher in women (17%) than men (6.1%) and increased in both women and men aged ≥50 years (24% and 8%, respectively).

A later survey at the same centre randomly selected 1310 patients with either Type I or Type II diabetes, but no previously diagnosed thyroid pathology, the prevalence of thyroid dysfunction (sub- or clinical hypo- or hyperthyroidism) was 31.4% in women and 12.4% in men. The annual incidence of clinical thyroid dysfunction in women was 3.2% approximately six times that calculated in the Whickham survey, whilst in men the figure of 1.6% was more than 10 times the corresponding calculated value (Perros *et al.,* 1995).

1.1.7. Type II diabetes mellitus and thyroid dysfunction:

Smithson reported an analysis of 206 patients with diabetes managed in primary care. Screening revealed 11 with previously undiagnosed thyroid dysfunction in addition to eight with previously known disease, making an overall prevalence of thyroid dysfunction of 10.8%, and an undiagnosed disease prevalence of 5.5% (9.5% in women). Those findings led the author to advocate screening for people with community managed diabetes (Smithson, 2004).

A larger study in secondary care included 904 patients with Type II diabetes. The prevalence of thyroid dysfunction was 10.9% in women and 6.9% in men. The incidence of clinically significant disease in women was 1.1%, twice that calculated from the Whickham survey. In men, the annual incidence was 0.8%, eight times that in the general population (Perros *et al,* 1995).

One important caveat is that poor diabetic control may interfere with the thyroid axis. Evidence for this came from a study of 290 patients with Type II diabetes who were hospitalized due to poor diabetic control. A high prevalence of abnormal thyroid function tests was noted in both women (40.9%) and men (19.8%). However, TSH concentrations fell as control of diabetes improved, as judged by decreasing HbA1c values (Celani *et al.*, 1994; Mouradian and Abourizk, 1983). This led the authors to suggest that diagnosis of thyroid dysfunction should be delayed until glycemic control has been optimized and thyroid function tests at diagnosis of Type II diabetes and further testing if these values are abnormal. The case for routine screening in Type II diabetes is less clear than for Type I, although advocated by some authors.

1.1.8. Diabetes mellitus in Sudan:

In Africa, at the start of the past century, DM was seen as a rare medical condition. Albert Cook in 1960 reported, "Diabetes is rather uncommon and very fatal. Since the early 1960s, a number of epidemiological studies have been carried out to elucidate the prevalence and nature of diabetes in the African population (King *et al.*, 1998) The prevalence of diabetes in African communities is increasing with ageing of the population and life style changes (Sobngwi *et al.*, 2001)

The prevalence of diabetes among African patients is 70-90% as type II and 25% type I (Motala *et al.*, 2003; Papoz *et al.,* 1998; Elbagir *et al.*, 1998).

Diabetes mellitus in Sudan, as in many developing countries, constitutes a growing health problem with a major impact. It can be estimated from hospital record that the number of diabetic patients is increasing in all socio-economic classes.Recently population-based epidemiological studies were conducted among the adult population of northern Sudan. Most of the knowledge about diabetes mellitus comes from a few based of studies (Elbagir *et al.*, 1996).

In 1985, WHO estimated that 30 million people had diabetes; today the estimate is around 100 million and projections are that this prevalence will double to 200 million in the next 15 years (Laakso 1999; King *et al.,*1998; Klein,1995). The number of diabetic

patients is also increasing in Sudan. A recent national survey showed increased prevalence of diabetes (Elmahadi *et al.,* 1989). These patients are at a significantly higher risk of diabetic complications if adequate medical care and patient education are not provided. Non-insulin-dependent diabetes mellitus accounted for 75% of all diabetic patients attending diabetic clinic in Khartoum (Elmahadi *et al., 1989*). In a more recent study 95% of all diabetic patients attending a diabetes clinic in the out central state were classified as having NIDDM, however 75% were treated with insulin (Bani and Anokute,1994).

In both studies, the classification of insulin dependence was based on early onset of diabetes (<15 years) and previous history of diabetic ketoacidosis. Obesity was a feature characterizing 39-49% of all the diabetic patients, and strong association with a family history of diabetes has been reported *(*Bani and Anokute1994; Elmahadi *et al.,* 1989) Insulin-dependent diabetes mellitus among children 7-14 years of age, is not rare in Sudan, and showed a steady rise in the incidence rate over 4-years period (Elamin *et al., 1989;* 1992). IDDM in Sudan consist primarily of autoimmune diabetes, which is characterized by a high prevalence of islet-cell antibodies .In the previous studies it was found that in Sudan, NIDDM is a common disease with a sever clinical course, and that most patient are poorly controlled and exhibit a high prevalence of acute and chronic complications (Elmahadi *et al., 1989).* The prevalence of micro-and macro-vascular complications was more common among the older patients and in patients with poor metabolic control. Chronic renal failure was present in almost one-third of the patients with nephropathy. In another study, among adult patient with established chronic renal failure, 9% had diabetic renal disease (Abboud *et al.,*2000).

Among children and adolescents, almost all these with diabetes were poorly controlled, and received a minimum of diabetes care. Both acute and long-term complications were common and associated with a high mortality rates among these children (Elamin *et al*., 1992). The poor metabolic control of Sudanese diabetic patients was attributed to the poor compliance and poor knowledge of diabetes .and to problem associated with injection and drug availability (Elmahadi *et al.*, 1989).The ability of patients with diabetes to understand and manger their diseases in ordinary daily life is a most important for successful therapy. Despite the above studies, there is still a paucity of information on diabetes in Sudan; available knowledge has prompted us to conduct the current diabetes research, with the goal of contributing to the overall improvement of diabetes care in Sudan.

1.2. The thyroid gland:

The thyroid is butterfly shaped gland, which wraps around the front part of the windpipe just below the Adam's apple. The thyroid is a largest single endocrine gland, its weight 20- 25g, but it varies with age, sex and physiological condition. It is only endocrine gland that does not store its hormones with in the cell, but in follicular cavities surrounded by the cell (Sukkar *et al.,* 2000). It produces hormones that regulate metabolism and organ functions.

1.2.1.Thyroid hormones:

The thyroid gland produces two iodoaminoacid hormones: 3,5,3' –triiodothyronine (T_3) and 3,5,3',5' tetraiodothyronine (thyroxine T_4), which have long been recognized for their importance in regulation of general metabolism (Robert *et al.,*2000) such as effects on carbohydrate metabolism, lipid metabolism and protein metabolism. In addition thyroid hormones increase heat production and oxygen consumption (Robert *et al*.,2000).The thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) are synthesized and secreted by the follicular cells. Iodine is the most important element in the production of these hormones (Ladenson *et al*., 2000).

1.2.2. Synthesis of thyroid hormones:

Iodine is absorbed from the diet or drinking water, transported in the blood to the thyroid gland, where it is very efficiently transferred to iodide ion. Once in the gland, the iodide ion is oxidized to free iodine, the reaction being catalyzed by peroxidase enzyme. The free iodine first iodinates position 3 of the tyrosine residues of the protein thyroglobulin to form monoiodotyrosine (MIT) and subsequently iodinates position 5 to form diiodothyronine (DIT),). Thyroglobulin is a complex protein, and is precursor of T_4 and T_3. It is a large, iodinated, glycoprotein with a molecular weight of 660 Kda. It contains 123 tyrosine residues, each of which is a potential site of iodination. Thyroglobulin is synthesized in the thyroid cells and secreted in to the colloid by exocytosis of granules that also contain thyroid peroxidase, the enzyme that catalyze the oxidation of I and it is binding. MIT and DIT can be coupled to form T_4 or T_3 (Ganong, 2003). The iodotyrosines are also deiodonated in the thyroid gland by deiodonase enzymes. The free iodine is reutilized for thyroid hormone synthesis, a process known as the intrathyroidal iodine cycle, which is maximally utilized in situation of iodine deficiency (Ganong 2003). Thyroglobulin is unique among body proteins in its content of iodinated amino acids (Sukkar,*et al*.,2000).

1.2.3. Secretion of T_4 and T_3:

Stimulation by Thyroid Stimulating Hormone (TSH) leads to pinocytic ingestion of the protein by phagocytic action of the microvilli. The thyroid cells ingest colloid by endocytosis.The droplets of thyroglobulin fuse with primary lysosomes where protolytic digestion of the protein occurs to release T_4 and T_3, DIT, MIT, were librated in to the cytoplasm (Helfand *etal.,*2004). T_3 is more efficient biologically, and indeed conversion of T_4 in to T_3 by the deiodinase occurs in several tissues including the liver and adipose tissue (Robert *et al.*,2000). T_4 and T_3 are released into the circulation, while DIT and MIT are deiodinated by intrathyrodial deiodinase and the I^- is conserved and mostly reutilized for hormone synthesis (Ganong, 2003).

1.2.4.Transport of thyroid hormones:

Thyroid hormones are transported in serum bound to carrier proteins. There are three major thyroid hormones transport proteins. Thyroxin-binding globulin (TBG) thyroxin binding prealbumin (TBPA), and albumin. T_4 and T_3 have high affinity for TBG which allow it to carry about 70% of the circulating thyroid hormones. Most of thyroid hormones in the plasma are associated with proteins and less than 10 % is free. The free hormones are in equilibrium with the bound form, the free fraction is conducting the functions at the tissue level (Radatii *et al.,* 1985).

1.2.5. Control of thyroid function:

The activity of thyroid gland is controlled by thyroid – stimulating hormone (TSH) from the anterior pituitary gland. The secretion of this is controlled by thyrotrophin releasing hormone (TRH) from the hypothalamus. Thyroid hormones (T_4 and T_3) suppress TSH secretion, negative feedback (Brook and Marshal, 2001). TSH influences the thyroid functions in different ways. TSH stimulates the steps involved in the synthesis and release of thyroid hormones, from the process of iodide trapping to the activation of proteases which liberate thyroid hormones from their attachment to the thyroglobulin molecule into the colloid (Sukkar *et al.,* 2000).

1.2.6. Metabolism of thyroid hormones:

The normal secretion from the thyroid gland contains approximately 80 % thyroxine T_4 and 20% triiodothyronine T_3. The biological active form of thyroid hormone is T_3 and it's derived from T_4 (Brent, 1994). T_4 and T_3 are deiodinated in the liver, the Kidneys, and many other tissues. One third of the circulating T_4 is normally converted to T_3 in adult humans, and 45 % is converted to rT_3. Thus much more T_3 and rT_3 are formed by deiodination than are secreted by the thyroid. Two different enzymes are involved 5-deiodinase catalyzing the formation of T_3 and 5 – deiodinase catalyzing the formation of rT_3. Because T_3 act more rabidly than T_4 and is 3-5 times as potent on a molar basis, T_4 is believed to be metabolically inert until it is converted to T_3. In the liver T_4 and T_3 are conjugated to form sulfates and glucuronides. These conjugates enter the bile and pass to intestine (Ganong, 2003).

1.2.7. Thyroid hormones' action:

Although the thyroid gland produces more T_4 than T_3, the latter hormone is more potent and acts much faster than the former. T_3 enters the cell more easily than the T_4. The latter is also transformed to T_3 in the cytoplasm. T_3 binds avidly to receptors on the cell nucleus and promotes messenger ribonucleic acid (m RNA) and ribosomal RNA (rRNA) synthesis. In most of its actions, T_3 acts more rapidly and is three to five times more potent than T_4 this is because it is less tightly bound to plasma proteins but binds more avidly to thyroid hormone receptors. RT_3 is inert. The nuclear receptors for thyroid hormones are considered as nuclear transcription factors. One such receptor (TRβ2) is found in the brain cells only. Other receptors are found in almost all other body tissues (Sukkar *et al.,* 2000). It is likely that all cells in the body are targets for thyroid hormones (Hardman and Limbird, 2001).

1.2.8. Effects of thyroid hormones:

Thyroid gland regulates body metabolism and the process energy utilization and storage, Thyroid hormones: increase the rate of absorption of carbohydrate from the gastrointestinal tract, therefore, the blood glucose level rises rabidly after a carbohydrate meal, are essential for normal growth, skeletal maturation and the development of the fetal and neonatal. In absence of thyroid hormones, growth hormone secretion may also

be depressed, and thyroid hormones potentiate the effect of growth hormone on the tissues (Ganong, 2003). Thyroid hormones lead to alterations in mental state. Too little thyroid hormone and the individual tend to feel mentally sluggish, while too much induces anxiety and nervousness (Ganong, *2003*).

Increased thyroid hormone levels stimulate fat mobilization, leading to increased concentration of fatty acids in plasma. They also enhance oxidation of fatty acids in many tissues. Finally, plasma concentrations of cholesterol and triglycerides were inversely correlated with thyroid hormone levels. One diagnostic indication of hypothyroidism is increased blood cholesterol concentration (Brent, 1994).

1.2.9. Iodine and thyroid function:

Iodine forms an integral part of the thyroxine and triiodothyronine molecules. Iodine is taken in food and in drinking water. A minimum daily intake of about 100 – 150 μg (in the form of potassium or sodium iodide) is required for normal thyroid function. The iodine is easily absorbed in the intestine to circulate in the plasma in a concentration of about 0.3 μg/100 ml; 30-50 of iodide circulating in the blood is taken up by the thyroid while the rest is lost in the urine. The thyroid can therefore remove iodide from the blood against a concentration gradient ranging from about 40 times in a normal gland to several hundred times in an over active gland. This selective uptake of iodine by the thyroid gland, or iodine trapping, can be achieved only by active transport (iodine pump). The trapped iodide ions are oxidized into iodine in thyroid cell by means of peroxidase enzyme. The oxidized form is capable of combining with tyrosine (Sukkar *et al.,* 2000).

1.2.10. Thyroid gland disorders:

All thyroid disturbances are more in females than in males (Sukkar *et al.,* 2000). Disorders of the thyroid are common. They consist of two general presentations: changes in the size or shape of the gland or changes in secretion of hormones from the gland (Hardman and Limbird, 2001;Williams,2001). Goiter refers to any enlargement of thyroid gland, with or without disturbance of thyroid function (Sukkar *et al*., 2000). Hypothyroidism is a clinical syndrome resulting from deficiency thyroid hormones, which in turn results in generalization slowing down of metabolic processes (Greenspan and Gardner, 2004). Hyperthyroidism, which is characterized by high blood levels of thyroid hormones, can occur at an early age or it may occur later in life (Sukkar *et al., 2000*).

1.2.10.1. Hypothyroidism :

Hypothyroidism is a condition in which the thyroid gland does not make sufficient thyroid hormones to meet the body's requirements. Hypothyroidism is often referred to as an under-active thyroid gland caused in almost all cases by autoimmune disease (Brook and Marshal*, 2001*).

1.2.10.2. Sub clinical hypothyroidism:

Is defined as asymptomatic state characterized by normal serum concentration of T_4 and T_3 and elevated serum concentration of thyroid stimulating hormone (Greenspan and Gardner,2004).

1.2.10.3. Hypothyroidism and glycemic status:

In hypothyroidism, the synthesis and release of insulin is decreased. The rate of hepatic glucose output is decreased probably due to reduced gluconeogenesis. In patients utilizing exogenous insulin they may be decrease in insulin requirements from reduced insulin degradation. The net effect is an increased risk of recurrent hypoglycemia in a diabetic individual. (Mohn *et al.,* 2001)

1.2.10.4. Hyperthyroidism:

Over-activity of thyroid gland is a clinical syndrome produced by sustained high plasma concentrations of thyroid hormones, may be easy to diagnose clinically or may remain unsuspected for a long time (Vanderpump *et al*.,1996; Longmore *et al,* 2001). It results from over secretion of thyroid hormones. In most species, this condition is less common than hypothyroidism. In humans the most common form of hyperthyroidism is Graves's disease, an immune disease in which auto antibodies bind to and activate the thyroid stimulating hormone receptors leading to conditional stimulation of thyroid hormone synthesis. Another interesting, but rare cause of hyperthyroidism is so called thyrotoxicosis (Ganong*, 2003*).

1.2.10.5. Sub clinical hyperthyroidism:

Subclinical hyperthyroidism is defined as persistently suppressed serum TSH with normal thyroxine and triiodothyronine in patients who do not have symptoms (Kek *et al,* 2003).While the diagnostic criteria and treatment modalities for overt hyperthyroidism are well known.

1.2.10.6.Hyperthyroidism and glycemic status:

Graves' disease is the commonest cause of hyperthyroidism. While Graves's disease may be associated with type I diabetes in polyglandular autoimmune syndrome, thyrotoxicosis by itself is diabetogenic. Variable glucose intolerance is seen in up to 50% of patients with Graves and frank diabetes occurs in 2-3%, when hyperthyroidism develops in normal individuals. In known diabetic patients, the diabetic control deteriorates (Donckier, *2003*). Varied metabolic changes may occur as a result of hyperthyroidism and contribute to the deterioration of glycemic control status (Sathish and Mohan*., 2003*).

1.3. Diabetes mellitus and dyslipidaemia:

Both diabetes and thyroid dysfunction are associated with dyslipidaemia. Thyroxine replacement reduces both total and LDL cholesterol in people with sub clinical hypothyroidism (Franklyn *et al*., 1993). Although this area is controversial, cross-sectional studies have shown rather conflicting results, with some suggesting higher total and LDL cholesterol levels in sub clinical hypothyroidism compared with euthyroid patients (Elder *et al*., 1990).

Gray and his group studied 49 patients with diabetes and primary thyroid failure and found their mean baseline cholesterol significantly elevated in comparison with age, sex and weight-matched diabetic controls. Treatment of those with low T_4 concentrations led to a significant reduction in mean plasma cholesterol. Although no significant differences were noted in triglyceride metabolism, those results prompted the authors to suggest that clinically unrecognized thyroid failure could predispose to ischaemic heart disease in the diabetic population. Thus thyroid function tests should be measured in patients with an otherwise inexplicably worsening dyslipidaemia (Retnam *etal.,* 1983; Walden *et al* 1984)

Many people with diabetes remain uncontrolled for dyslipidemia. Population – based studies have consistently demonstrated that patients with diabetes have an increased risk of cardiovascular disease (CVD) and low density lipoprotein cholesterol (LDL-C) has been noted to be the strongest predicator of coronary heart disease (CHD) events. Clinical trials have provided evidence that treatment of dyslipidemia reduces mortality and prevents or delays the incidence of micro vascular and macro vascular complications in persons with diabetes *(*Dzien *et al.,* 1991).

Many reports showed that less than half of adult with diabetes had total cholesterol levels < 200 mg/dl. (LDL-C) which forms the bases for treatment as well as HDL – C and triglycerides, which are frequently abnormal in persons with diabetes (Jacobs et al ., 2005) Premature atherosclerosis is often found in patients with diabetes mellitus (DM) type1 and alteration in lipid metabolism seems to play an important role in the development of this complication. The accumulation of triglyceride – rich lipoprotein particles like very low density lipoproteins (VLDL) in insulin – deficient patient occurs because lipoprotein lipase activity is depressed with out sufficient insulin for adequate tissue levels. (Dzien *et al.,* 1991).

Jacobs and his colleagues (2005) report and placing those with both cardiovascular disease and diabetes, in to a very high risk category where treatment of the LDL-cholesterol to a goal of <70 mg/dl is a therapeutic option, there will be more intensified efforts to treat these individuals. Type II diabetes mellitus (DM) is a well known risk factor for the development of cardiovascular disease due to alteration in lipid and lipoprotein profile (Hayden & Reaven *2000*). Lipid profile in typeII diabetes is characterized by an elevation in both postprandial and fasting plasma triglyceride (TG) and low level of HDL cholesterol (Deman *et al.,* 1996).

Significantly increased levels of LDL- cholesterol and high Triglyceride (TG) associated with low HDL – cholesterol was found in diabetic subjects. In fact typeII diabetes is characterized not only by alteration in the glucose – insulin axis but marked features described as the diabetic dysilipidemia (Kreisberg , 1998).The most frequent alternations of lipid and lipoprotein profiles were the combination of elevated TG (VLDL- TG), decrease clearance of TG- rich lipoproteins, and decreased high – density lipoprotein (HDL) (Smaoui *et al.,* 2004) most likely due to a low lipoprotein lipase activity well known in diabetic patients (Taskinen,2002).

1.3.1. Cholesterol and diabetes mellitus:

In diabetes the plasma cholesterol level is usually elevated and this is play a role in the accelerated development of the atherosclerotic vascular disease that is a major long – term complication of diabetes in humans. The rise in plasma cholesterol level is due to an increase in the plasma concentration of VLDL or decrease removal of VLDL and LDL from the circula5tion (Ganong *2003).*

1.3.2. Storage of triglyceride :

Because triacylglycerols are only slightly soluble in water and can not form stable micelles by themselves, they coalesce within adipocytes to form oily droplets that are nearly anhydrous. These lipid droplets are the major energy reserve of the body. In liver little triacylglycerol is stored, instead most is exported , packaged with cholesterol and cholesteryl esters, phospholipids, and protein (apoB – 100) to form lipoprotein particles called very law density lipoprotein (VLDL). These are secreted in to the blood and deliver the newly synthesized lipids to the peripheral tissues (Robert *et al.,* 2000).

1.4. Effects of thyroid on lipids metabolism:

Alterations of the lipid profile are well known phenomena in thyroid dysfunction. Thyroid hormones regulate lipid metabolism through various mechanisms. Increased thyroid hormone levels stimulate fat mobilization, leading to increased concentrations of fatty acids in plasma. They also enhance oxidation of fatty acids in many tissues. Finally, plasma concentrations of cholesterol and triglycerides are inversely correlated with thyroid hormone levels (Danese *et al*.,2000; Spandrio *et al.,* 1993).

It has been known for over fifty years that an increase in thyroid activity reduces the level of cholesterol in blood where as a decrease in thyroid activity increases it. The blood cholesterol level represents the balance between ingestion and formation in one hand and excretion and utilization on the other hand. The action of the thyroid gland on cholesterol metabolism is complex, It stimulates the formation of cholesterol by the liver, but has greater effect on increasing it is excretion in the bile, and increasing it is entry into cells. The net result is that the blood level falls. Also it was clearly approved that the decrease in plasma cholesterol concentration is due to increased formation of LDL receptors in the liver, resulting in increased hepatic removal of cholesterol from the circulation (Ganong, 2003).

A study carried out by Duntas and Leonidas, (2002) showed that hyperthyroidism exhibits an enhanced excretion of cholesterol and increased turn over of LDL resulting in a decreased total and LDL-cholesterol, whereas HDL is decreased or not affected. Also, the composition and the transport of lipoproteins are seriously disturbed in thyroid diseases. Rassul, *et al*., (1988) postulated that clinical manifestation of hypothyroidism lead to changes of plasma lipoproteins, which are characterized by elevated LDL-cholesterol, an increase of the ratio of LDL-cholesterol/ HDL-cholesterol.(Rassul *et al*.,

1988). Hypothyroidism is accompanied by a variety of abnormalities in plasma lipid metabolism, including elevated triglyceride and low–density lipoprotein cholesterol concentrations. Even sub-clinical hypothyroidism can exacerbate the coexisting dyslipidemia commonly found in typeII diabetes and further increase the risk of cardiovascular disease (Hyden and Reaven, 2000). Subclinical hypothyroidism can elevate LDL cholesterol and worsen pre-existing dyslipidemia. Since diabetic patients are at high risk for cardiovascular disease, the diagnosis and treatment of sub clinical thyroid disease is important (Brook and Marshal, 2001; Patricia, 2000).

CHAPTER TWO

MATERIALS AND METHODS

2.1. Subjects :

One hundred Sudanese diabetic patients were randomly selected from Mulazmin Medical Center. They had a mean age of (46.51± 10.6) years, a mean height of (162 ±10.7) cm and a mean weight of (73.4±14.9) kg.

Full information was taken from each subject about, type, treatment, history of diabetes, family history about diabetes and other diseases and complications. All subjects were under treatment or diet control.

Risk factor information for, blood pressure, body mass index (BMI) was also available.

Fifty apparently healthy non- diabetic people were chosen as controls. Their mean age 35.92±9.4 year, mean height 167.3± 6.6cm and mean weight 70.9± 6.4 kg. All of them were not suffering from any endocrine diseases and those with evidence of abnormality were excluded before sample collection.

Blood glucose level and hemoglobin concentration ,total cholesterol, triglyceride, low-density lipoprotein cholesterol, high –density lipoprotein cholesterol and total thyroid hormones and thyroid stimulating hormone were measured for each sample in all diabetic and non- diabetic subjects. Samples from patients and controls were taken under supervision of consultant physician and endocrinologist.

2.2. Materials:

2.2.1. Equipment and disposables:

The following list of equipment and consumables were used to perform different experiments and measurements.

1. Multi detector gamma counter. PG-RIA.MAS, Stratec Biochemical System AG, SN 2486000052.
2. Colorimeter 253. Germany: scientific technical supplies. capable of measuring absorbance at (500 – 550 ± 10 – 20) nm
3. Vortex mixer. Vortex-2 genie, Scientific Industries, INC. Model G-560E, Serial No.2-84J.
4. Magnetic separator, Multimix Major, Luckam, Serial No. 424, Model No MF.
5. Multi-tube vortexer ,ALPHA laboratories, Serial No. 093647 Cat. No 2601.

6. Centrifuge .EBA 20 from LEEe Ltd. England
7. Centrifuge EBA 20-D- 7853L. Tubes (13 × 100 m/m).
8. Water distill (Double distiller), J. Bibby Science Products Limited, Model A4D, Serial No. 89/5/1595.
9. Pipettes (10 µL – 1000 µL) and disposable tips from Eppendorf Gmbt, Itamurge 65, fed Rep. Germany
10. Adjustable repeating syringe (10 µL - 2mL per shot) from eppendorf Gmbt, Itamurge 65, Fed, Rep.Germany.
11. Serum container (Eppendorf) Tubes: polystyrene assay tubes code NO(5001) round bottomed tubes with a capacity of 5 ml.
12. Syringes: Sterile 5 ml.
13. Absorbent cotton.
14. Well equipped RIA Lab.
15. Storage tubes:

2.3. Reagents for radioimmunoassay :

Thyroid hormones and thyroid stimulating hormone were measured using ready made radioimmunoassay kits.

2.3.1. TSH Kit :

All radioimmunoassay specific reagents for the measurement of thyroid and thyroid related hormones were supplied by the Department of Isotopes from China Institute for Atomic Energy (CIAE).

Human TSH in serum was determined quantitatively with magnetic separation reagents include ^{125}I– anti – TSH (monoclonal) solution (red), magnetic TSH antibody (Polyclonal) suspension, TSH set of standards (A,B,C,D,E,F,G) with the following concentrations, (0.00 , 0.28 , 1.10, 2.90 , 10.00 , 20.00 , 90.00) mlU/L in lyophilized form. These standards were calibrated according to the international reference preparation of TSH code MRRC 80, 1558. The kit also contains concentrated wash buffer (phosphate buffer) and locally prepared control samples.

2.3.2. T_4 and T_3 Kits:

Serum T_4 and T_3 were measured by RIA kits, the components for each kit are as follow radiolabeled antigen (Tracer) ^{125}I -T_4 or ^{125}I – T_3, solutions, 55 ml, red solution in baritone buffer with bovine serum albumin and ANS (8 – anillino -1- naphthalene sulfonic acid). T_4 or T_3 - antibody suspension with anti microbial agent (Sodium azide) 6 vials T_4 standard human serum with concentration (0.0 – 20 – 40 – 80 – 160 - 240 – nmol/L). or 6 vial T_3 standard human serum with anti microbial agent with concentration (0.0 , 0.5 , 1.6 , 2.0 , 4.0 , 8.0 nmol / L) and magnatizable separating agent.

2.4. Lipid, glucose and hemoglobin reagents :

Lipid profile (TC, TG, HDL) , hemoglobin (Hb) and glucose was measured using reagents and standard obtained from LINEAR chemicals (cromatest) Barcelona – Spain.

2.4.1. Triglycerides :

Monoregent (R1): Buffer 50 m mol / L, PH 6.8

LPL ≥ 12 ku/L , Gk ≥ 1 ku/L, GPO ≥ 10 ku/L, ATP 2.0 m mol, Mg^{2+} 40 m mol ,

POD ≥ 2.5 ku/L, 4-AA 0.5 m mol/L, Phenol 3 m mol.

Triglycerides standard: Glycerol 200 mg/dl

2.4.2. Cholesterol :

Monoreagnent (R1): consists of sodium cholate 1mmol/L, cholesterol esterase > 250 U/L, cholesterol Oxidase > 250 U/L. Cholesterol peroxidase > 1 KU / L, 4-aminoantipyrine 0.33 m mol / L , ADPS, 0.4 m mol/l.

Cholesterol standard: 200 mg/dL

2.4.3. HDL :

Precipitating reagent consists of :(R1): Phosphotungstic acid 0.63 m mol/L, magnesium chloride 25 m mol/L stabilizers.

HDL – cholesterol standard: cholesterol 50 mg/dl.

2.4.4. LDL – cholesterol :

LDL- cholesterol values were estimated by friedewald equation.

LDL = TC – (HDL + TG/ 5). LDL: low –density lipoprotein, HDL: high- density lipoprotein, TC: total cholesterol, TG: triglyceride.

2.4.5. Blood glucose:

Monoreagent, phosphate buffer 100mmol/L., PH 7.5, glucose oxidase ›10KU/L, peroxidase›2KU/L,4−aminoantipyrine 0.5mmol / L , phenol5mmol/L.

glucose standard: 100mg/dl.

2.4.6. Hemoglobin :

Drabkin's solution composed of cyanide-ferricyanide solution. Which prepared by addition of 200 mg potassium cyanide, 200 mg potassium ferricyanide and 1000 mg sodium bicarbonate and completed to one liter distilled water. The diluent should be clear and pale yellow in colour and when measured against water as blank at a wave length of 540 nm absorbance must be zero. Cyanthaemoglobin standard :12g/dl

2.5. Methodology and principle :

2.5.1. Blood samples : -

After obtaining informed consent from each subject five ml of venous blood in dry syringe were collected after 12 hours overnight fasting period from each subject. The blood was divided into three tubes.1 ml was collected in EDTA tube for hemoglobin measurement. 1 ml was collected in floride oxalate for glucose measurement and 3 ml were allowed to clot and immediately centrifuged at 2000 r.p.m. for 10 minutes and sera was stored at -20 c^{o} until analyzed. All serum samples were stored in refrigerator, tightly closed, at Sudan atomic energy commission, radioisotopes laboratory until analyzed. Glucose and hemoglobin were measured immediately.

The level of TSH was estimated by immunoradiometric assay, thyroid hormones total T_4 and T_3 were estimated by Radioimmunoassay (RIA). While commercial enzymatic methods were used for determination of hemoglobin (Hb), blood glucose, total cholesterol, triglycerides, and HDL- cholesterol. HDL was measured after precipitation of apolipoprotien B-containing particles.

2.5.2. Principle of radioimmunoassay (RIA):

2.5.2.1. Determination of total serum thyroxin (T4):

The T_4 and T_3 radioimmunoassay method depend on the competition between ^{125}I labeled T_4 or T_3 and T_4 or T_3 contained in standard or specimens to be assayed for affixed and limited number of T_4 or T_3 antibody binding sites. After the incubation, the amount of ^{125}I labeled T_4 or T_3 bound to the antibody is inversely related to the amount of T_4 or T_3 present in the sample. By measuring the proportion of I^{125} labeled T_4 or T_3 bound in the presence of reference standards containing various known amount of T_4 or

T_3 the concentration of T_4 or T_3 present in unknown sample can be interpolated. The steps of the procedure can be summarized as follows. Sufficient test tubes used in assay were labeled in duplicates and arranged in assay rack, using the following protocol:

- 50 µl sample or standard were pipetted in to labeled tubes.
- 500 µL (^{125}I-T_4) solution were added in to each tubes..
- 500 µL antibody suspensions were dispensed in to labeled tubes.

All tubes were vortexed and mixed thoroughly and incubated at 37 ˚C for 45 minutes then the test tubes rack was placed on magnetic separator for 10 minutes, the supernatant was decanted, tubes placed on pad up absorbent paper to drain for 5min, finally all tubes were counted with gamma counter for 60 seconds.

2.5.2.2. Determination of total serum triiodothyronine (T_3):

Serum T_3 was determined using the following Assay Protocol:

Tubes used in assay were labeled in duplicate and arranged in assay rack, using the following protocol:

- 50 µL were pipetted in to the labeled tubes (standards, samples and Qcs)
- 500µL (^{125} I – T_3) solution (red) were added in to all tubes.
- 500µL T_3 antibody suspension was added to all tubes.

All tubes were vortexed and mixed thoroughly and incubated at 37 ˚C for 60 minutes then the test tubes rack was placed on magnetic separator for 10 minutes, then the supernatant was decanted ,tubes placed on pad up absorbent paper to drain for 5min. Finally all tubes were counted with gamma counter for 60 seconds.

2.5.3. Principles of immunoradiometric assay (IRMA):

2.5.3.1. Determination of thyroid stimulating hormone (TSH):

IRMA is a radio labeled antibody immunoradiometric assay, is best example of excess reagent method (Edward). Measurement of TSH in human serum involves the reaction of TSH present in serum with monoclonal and polyclonal antibody. the monoclonal antibody is labeled with^{125}I as tracer and the polyclonal antibody is coupled to magnetic iron oxide particles. Labeled antibody (Ab*) was allowed to react with antigen (Ag), the complex formed was washed using buffer. Polyclonal antibody was added to the medium to form a sandwich complex Ab*-Ag-Ab. complex was washed twice to remove excess labeled antibody and read in gamma counter for 60 seconds. The concentration of Ag reacted with the antibodies is determined using standard of Ag . The following protocol was used to determine TSH.

Tubes used in assay were labeled and arranged in assay rack.

- 200μL of standards, controls, and unknown were pipetted in to the labeled tubes.
- 50μL of (^{125} I-anti –TSH) solution was added, incubated 1 hour at 37 ˚C
- 500μL well mixed antibody suspension was added, and mixed well, tubes were vortexed gently, and incubated 1 hour at room temperature.

the test tubes rack was placed on magnetic separator for 10 minutes, then supernatant was decanted , the precipitant was washed using 1.0 ml wash buffer , the tubes were vortexed and replaced on magnetic separator allow to stand for 10 min . Washing step was repeated, supernatant was decanted and drained thoroughly on absorbent paper, and tubes were counted with gamma counter for 60.

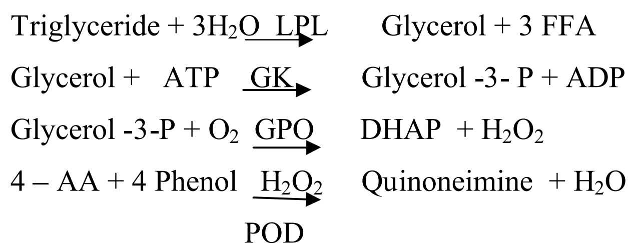
2.5.4. General principle of colorimetric method:

Beer's law: The fraction of monochromatic radiant energy absorbed on passing through a solution is directly proportional to the concentration of the absorber. It is a relationship between the light absorptive capacity and the concentration of the absorber in solution (Sharma. 2001). According to the following equation concentration of sample can be calculated.

A (absorbance) of sample/ A (absorbance) of standard ×concentration of standard mg/dl

2.5.4.1. Principle for determination of triglyceride:

The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to Glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL). The Glycerol phosphorylated by adenosine triphosphate (ATP) in the presence of glycerolkinase (GK) to form glycerol -3- phosphate (G-3-P) and adenosine diphosphate. G -3—P: is oxidized by glycerol phosphate Oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide. A red chromogen is produced by the peroxidase (POD) catalyzed coupling of 4- aminoantipyrine ,and phenol with hydrogen peroxide (H_2O_2) proportional to the concentration of triglyceride in the sample .



Triglyceride was determined using the following Assay Protocol:

- All reagent and samples was brought to room temperature.
- In to labeled test tubes the following were pipetted.

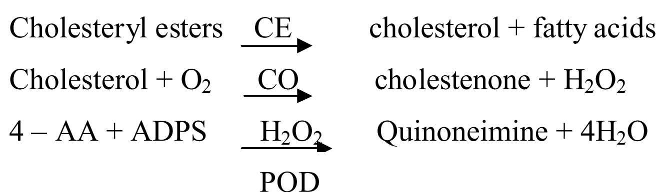
Tubes	Blank	Sample	Standard
Mono reagent	1.0 ml	1.0 ml	1.0 ml
Sample	-	10 µL	-
Standard	-	-	10µL

- the tubes were mixed and incubated for 15 min at room temperature. The absorbance (A) of the standard and the samples were measured at 500 mm against the blank.

Triglyceride = A sample / A standard × concentration of standard (200mg/dl)

2.5.4.2. Principle for determination of total cholesterol :

The method for the measurement of total cholesterol in serum involves the use of three enzyme: cholesterol esterase (CE) ,Cholesterol oxidase (CO) and peroxidase (POD). in the presence of the former the mixture of ADPS and 4 – aminoantipyrine (4 – AA) are condensed by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of cholesterol in the sample .



Cholesterol was determined using the following Assay Protocol:

- All reagent and samples was brought to room temperature.
- In to labeled test tubes the following were pipetted.

Tube	Blank	sample	standard
Monoreagent	1.0ml	1.0ml	1.0ml
Sample	-	10µL	-
Standard	-	-	10µL

- The tubes were mixed and incubated for 10 min at room temperature.
- The absorbance (A) of the standard and the samples were measured at 550 mm

Total cholesterol = A sample/ A standard × concentration of standard (200mg/dl)

2.5.4.3. Principle for determination of HDL – cholesterol:

This technique uses a separation method based on the selective precipitation of apoliprotein B – containing lipoproteins (VLDL, HDL) by phosphotungstic acid/$MgCl_2$, sedimentation of the precipitant by centrifugation, and subsequent enzymatic analysis of high-density lipoproteins (HDL) as residual cholesterol remaining in the clear supernatant. High density lipoprotein was determined using the following Assay Protocol:

precipitation method:

All reagents were brought to room temperature.

- In to labeled test tubes the following were pipetted

Sample	0.2 ml
Precipitating reagent	0.1 ml

- The tubes were let to stand for 10 minutes at room temperature.
- Centrifuged at minimum of 4000 r. p. m for 10 minutes.
- The supernatant was collected carefully.

Colorimetric method:

- In to labeled test tubes the following were pipetted:

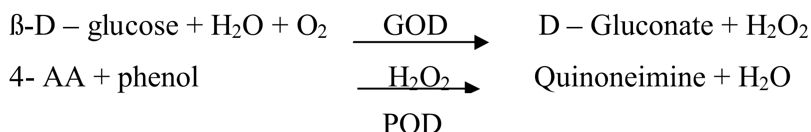
Tubes	**Blank**	**Sample supernat**	**Standard supernat**
Monoreagent	1.0 ml	1.0 ml	1.0 ml
Supernat	-	50 µL	-
Standard	-	-	50 µL

- The tubes were mixed and incubated for 10 min at room temperature.
- The absorbance (A) of the samples and the standard were measured at 550 mm against the blank.

HDL- cholesterol = A sample / A standard × concentration of standard (50mg/dl)

2.5.4.4. Determination of blood glucose:

The glucose is oxidized to D – gluconate by the glucose oxidase (GOD) with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of phenol and aminoantipyrine (4-AA) is oxidized by hydrogen peroxide, to form a red quinoneimine dye proportional to the concentration of glucose in the sample.



$$\beta\text{-D – glucose} + H_2O + O_2 \xrightarrow{GOD} \text{D – Gluconate} + H_2O_2$$

$$\text{4- AA + phenol} \xrightarrow[POD]{H_2O_2} \text{Quinoneimine} + H_2O$$

Glucose was determined using the following Assay Protocol:

- All reagent and samples was brought to room temperature.
- In to labeled test tubes the following were pipetted.

Tubes	Blank	Sample	Standard
Monoreagent	1.0 ml	1.0 ml	1.0 ml
Sample	-	10 µL	-
Stand	-	-	10 µL

- The tubes were mixed and incubated for 10 min at room temperature.
- The absorbance (A) of the samples and the standard were measured at 500 mm against the blank.

Glucose mg/dl = A sample/ A standard × concentration of standard (100mg/dl)

2.5.4.5. Determination of hemoglobin (Hb) concentration:

Hemoglobin was determined using the following Assay Protocol:

- 5ml of Drabkin solution was pipetted in to test tubes(sample, standard, blank)
- 20 µL of whole blood was added to sample tube.
- 20 µL of standard to standard tube.
- The tube was mixed well and incubated for 5min at room temperature.
- The absorbance was measured at 540nm against reagent blank.

Hb mg/dl = A sample/ A standard × concentration of standard. (12mg/dl)

2.6. Statistical analysis:

SPSS (Statistical Package for Social Sciences) software (SPSS, Version 13.0; SPSS Inc., Chicago,IL) was used for the statistical analysis. Prevalence was carried out using Microsoft Excel program.

One sample Kolmogorov–Smirnov test was used to evaluate the distribution characteristics of variables. The differences between the groups were tested for significance by Student's t-test, one-way ANOVA test (analysis of continuous variables with normal distribution) chi-square test and Kruskall–Wallis test. Mann–Whitney U-test, were used (for variables with non-normal distribution).

Some variables were log-transformed when they were not normally distributed.

The relationship between variables was analyzed by Pearson's correlation or Spearman's.rho correlation tests. Differences and correlations were considered significant at $p < 0.05$. Results are reported as the mean ± SD

CHAPTER THREE

RESULTS

3.1. Preamble of results:

This study was a pilot study. Case- control study to compare blood glucose level and thyroid hormones, lipid profile, hemoglobin concentration in diabetic and non diabetic subjects. To perform this, comparison analysis was done to see if there is any difference between control and the study group. An attempt was done to correlate between all parameters. The study group was further subdivided into two groups insulin-dependent diabetes mellitus Type I and non-insulin dependent diabetes mellitus Type II to find if there is any difference between subgroups.

The result of this study covers one hundred Sudanese diabetic patients and fifty healthy non –diabetic subjects as controls. An informed consent was obtained from all cases. All study cases were chosen from Mulazmin Medical Center; 61.0% of the patients were females while the males were found to represent 39.0%. However, for the control group each sex was found to represent 50%. Their mean ages were found to be (46.51± 10.6) and (35.92±9.42) years for the patients and control groups respectively ranged between 25-65 years. All of the participating subjects are from Omdurman, Khartoum and Khartoum north.

Data collected from diabetic patients include type of diabetes mellitus, type I represent 41% and type II which was found to be 59%. 63% of the patients were found to have past family history. The mean weight was found to 73.47 ± 14.00 Kg and 70.92 ± 6.40 Kg for the patients and control groups respectively. 162.06 ± 0.06 and 167.28 ± 6.68 cm was found to be the mean of the high in the study and control groups respectively. With regard to the history of blood pressure 57% of the patients were found to have hypertension. Body Mass Index (BMI) was calculated for each diabetic subject to determine obese patient. 62% of patients were obese.

Serum hormone level were analyzed: total thyroid hormones (T_4 and T_3) thyroid stimulating hormone (TSH) and also serum total cholesterol (TC), triglyceride (TG) low- density lipoproteins cholesterol (LDL), high – density lipoproteins cholesterol (HDL), hemoglobin (Hb) and blood glucose levels, the mean and SD of all parameters were calculated

3.2. Prevalence of thyroid dysfunction:

Hundred diabetic patients were examined and the association of diabetes mellitus and thyroid dysfunction was found in 13 patients (11 females, 2 male). Screening for thyroid disease, by estimating serum TSH, total T_4 andT_3 concentration.

For the study purposes, thyroid status has been suggested to be classified in to five categories:

Table 3. 1: Classification of clinical status of thyroid.

Tyroid Status	T_4nmol/L	T_3nmol/L	TSH mlU/L
Euthyroid	50-150	0.8-3.0	0.4-4.0
Hyperthyroidism	> 150	>3.0	<0.4
Subhyperthyroidism	Normal	Normal	<0.4
Hypothyroidism	<50	<0.3	>4.0
Subhypothyroidism	Normal	Normal	>4.0

New thyroid disease was diagnosed in 13 patients (11 females, 2 males) 5 with subclinical hypothyroidism, 2 with hyperthyroidism and 6 with subclinical hyperthyroidism. Thus the prevalence of undiagnosed thyroid disease in diabetic patients receiving community diabetes care was 13%.

The overall prevalence of thyroid disease was found to be 13%, as shown in figure (3.1).and table (3.1), was highest in diabetic females (11%). The commonest diagnosis was subclinical hyperthyroidism (6%), followed by subclinical hypothyroidism (5%) and hyperthyroidism (2%). Female patients with diabetes had the highest annual risk of developing thyroid disease but all patient groups had a higher incidence of thyroid dysfunction, compared with control group.

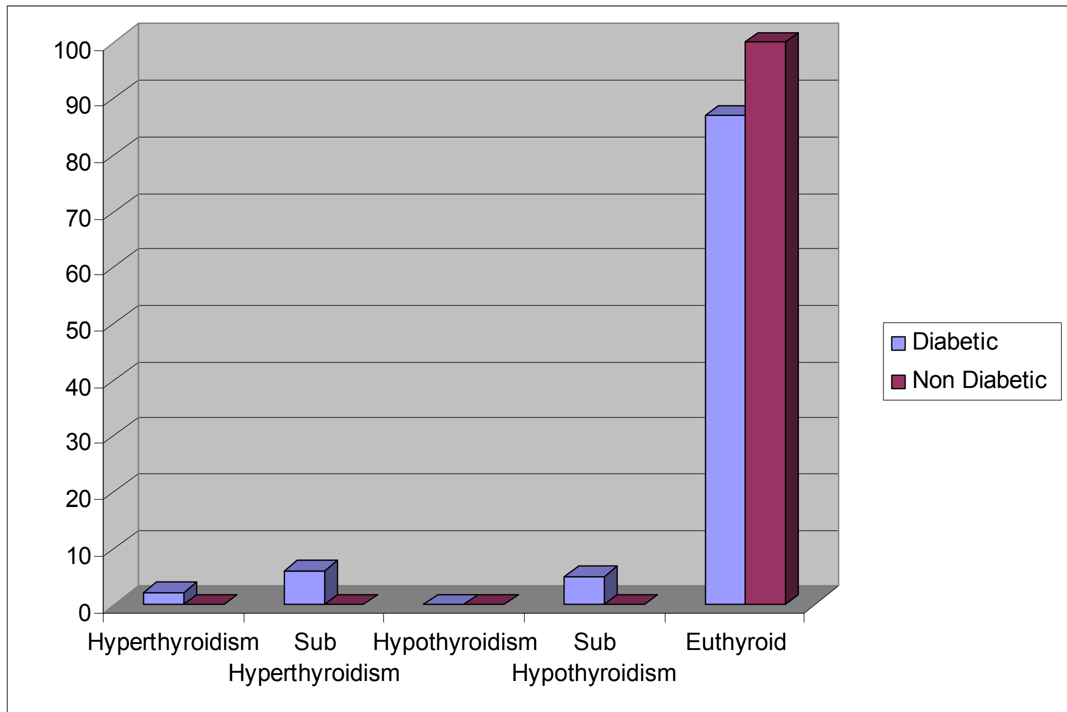


Figure 3.1: Prevalence of thyroid dysfunction in diabetic and non-diabetic subjects.

3.3. Serum total thyroid hormones (T_4 and T_3):

Mean serum concentration of total thyroid hormones (T_4 and T_3) in diabetic patients and non- diabetic subjects (control) as shown in table (3.2) and figure (3.3 and 3.4).

The mean of total triiodothyronine (T_3) in diabetic patients (1.923±0.62) was lower than the mean of non- diabetic subjects (2.114±0.53). Significant ($p=0.03$) difference was observed between them.

No variation was found when we compared the mean serum of total thyroxin (T_4) in diabetic patients (102.75 ±22.0) and the mean serum of non diabetic subject (99.76±17.1). There was no significant difference ($p=0.481$) between the means.

3.4. Serum thyroid stimulating hormone (TSH):

The mean (1.113±0.937) of thyroid stimulating hormone (TSH) of diabetic patients was significantly ($p=0.028$) lower than the mean (1.440±0.639) of non diabetic subjects as shown in table (3.2) and figure (3.5).

3.5. Blood glucose level:

The mean glucose level in blood (199.46±65.9)of diabetic patients was significantly ($p=0.001$) higher than the mean (87.1±14.00) of non- diabetic subjects as shown in table (3.2) and figure (3.2).

3.6. Hemoglobin concentration (Hb):

There was no significant difference ($p=0.424$) between the mean (82.5±10.0) of hemoglobin concentration of diabetic patient compared with the mean (80.8±14.3) concentration of non diabetic subjects (control) as shown in table (3.2).

- Same results were found when we divided diabetic pool in two types (typ1 and typeII) when we compared levels of T_4, T_3, TSH, glucose and hemoglobin as shown in figures (3.7) (3.8) (3.9) (3.10) and table (3.4). Slight difference was observed.

Table 3. 2: level of total thyroid hormones ($T_4$and T_3), thyroid stimulating hormone (TSH), blood glucose and hemoglobin, in diabetic and non- diabetic subjects.

Measurement	diabetic Subject	Control Group	p- values
T4 nmol/l	102 .75 ± 22.084	99.7 6 ± 17.145	0.481
T3 nmol/l	1.923 ± 0.6195	2.114 ± 0.5318	0.03
TSH mlU/l	1.113 ± 0.9369	1.440 ± 0.6386	0.028
Glucose mg/dl	199.46± 65.949	87.12± 14.001	0.001
Hb %	82.50 ± 10.089	80.88 ± 14.359	0.424

Values mean ± SD.

Figure 3.2: Mean values of glucose for diabetic and non- diabetic subjects

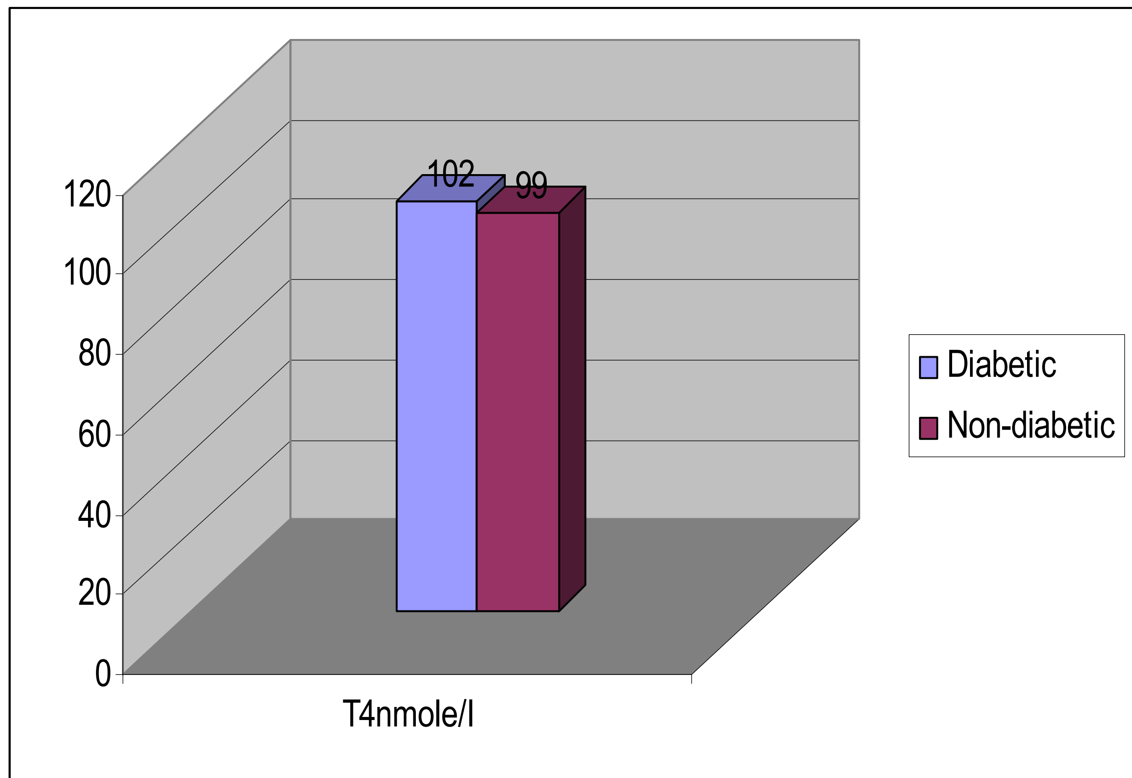


Figure 3.3: Mean values of T_4 (nmole/L) for diabetic and non- diabetic subjects.

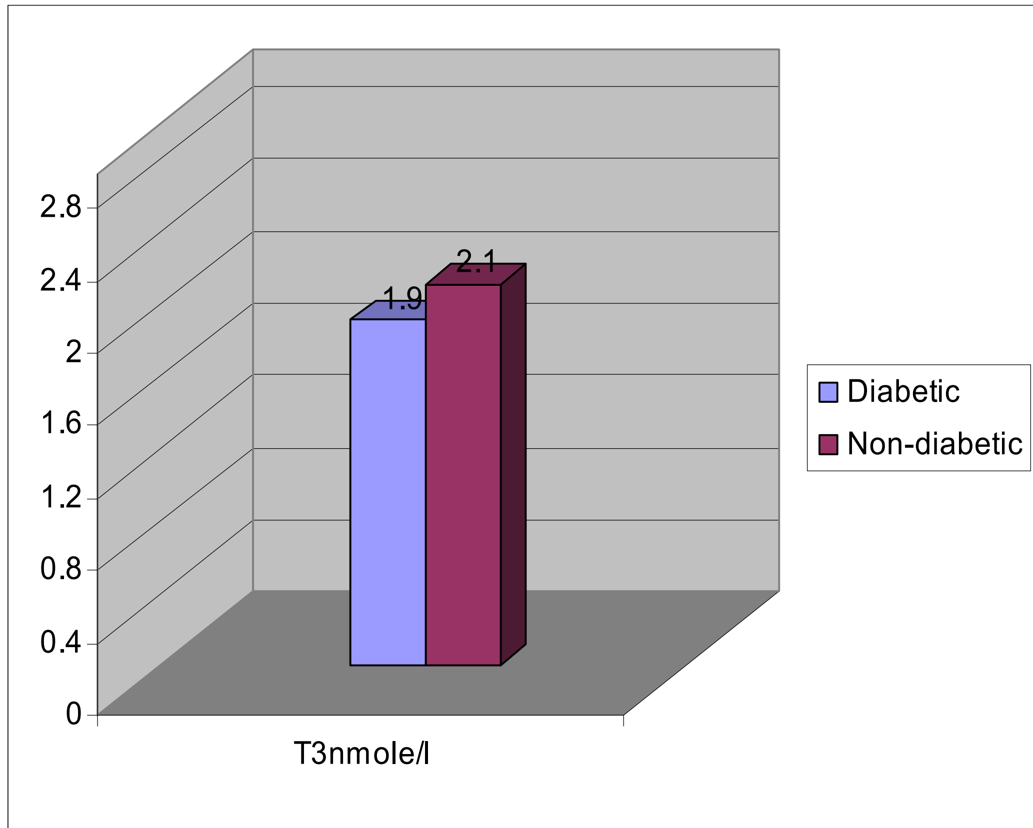


Figure 3. 4: Mean values of T_3 (nmole/L) for diabetic and non- diabetic subjects.

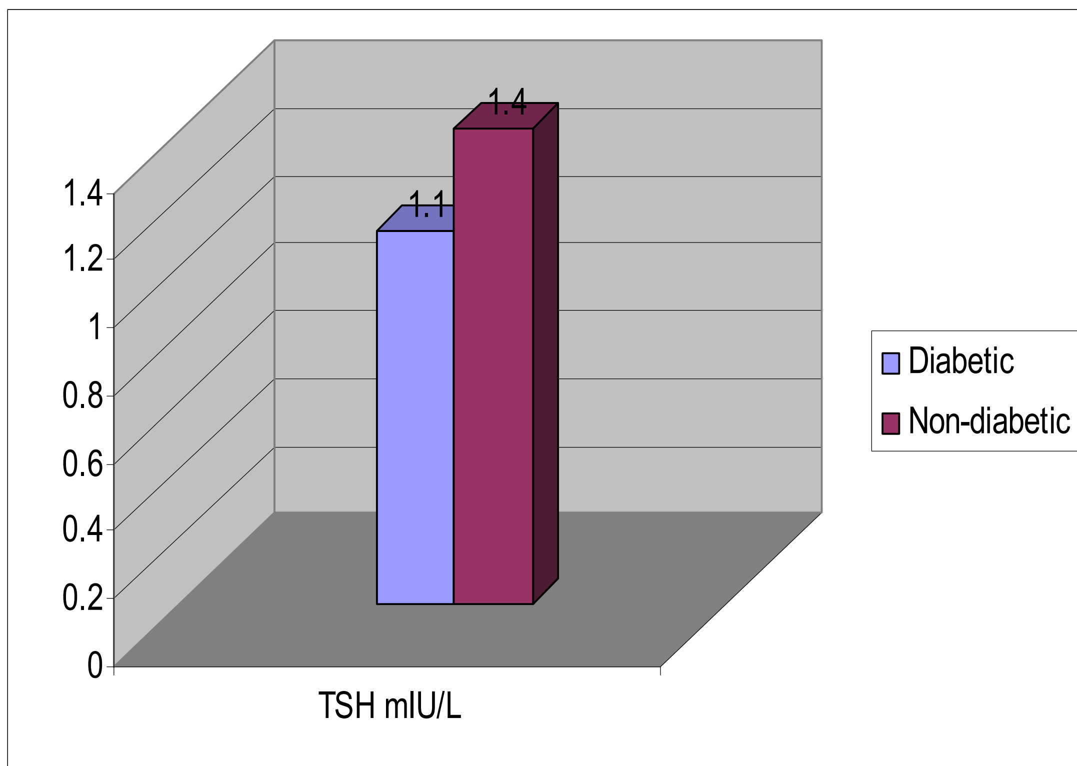


Figure 3.5: Mean values of TSH (mlU/L) for diabetic and non- diabetic subjects.

Results of lipid profile:

3.7. Serum total cholesterol concentration (TC):

The mean (189.6±44.5) serum of total cholesterol level of diabetic patients was significantly (p=.001) higher than the mean (161.8±35.70) serum of non-diabetic (control) subjects as shown in table (3.3) and figure (3.6).

3.8. Serum triglycerides concentration (TG):

The mean (149.25±93.65) of triglyceride in diabetic patients was higher than the mean (107.18± 54.71) of non- diabetic subjects and statistically significant (p= 0.001) as shown in table (3.3) and figure (3.6).

3.9. Serum low- density lipoprotein concentration (LDL):

The mean (114.17 ± 42.45) concentration of serum low –density lipoprotein of diabetic patients was significantly (p=0.009) higher than the mean (92.8±33.54) of non-diabetic subjects (control) as shown in table (3.3) and figure (3.6).

3.10. Serum high – density lipoprotein concentration (HDL):

The mean (44.33 ± 16.40) of high – density lipoprotein in diabetic patients was lower than the mean (46.98 ±19.71) of non-diabetic subjects as shown in table (3.3) and figure (3.6). And this difference was statistically not significant (p=0.376).

- When we compared lipid profile (TC, TG, LDL, HDL) same results were found when we divided diabetic pool in two types (typ1 and typeII) as shown in figure (3.11) and table (3.5).

Table 3. 3: levels of total cholesterol, triglyceride, high- density lipoprotein, low-density lipoprotein in diabetic and non- diabetic subjects

Measurement	diabetic Subject	Non-diabetic	p- values
TC mg/dl	189.6 1 ± 44.51	161.88 ± 35.73	0.001
TG mg/dl	149.25 ± 93.65	107.18 ± 54.71	0.001
LDL mg/dl	114.17 ± 42.45	92.80 ± 33.54	0.009
HDL mg/dl	44.33 ± 16.40	46.98 ± 19.71	0.376

Values mean ± SD.

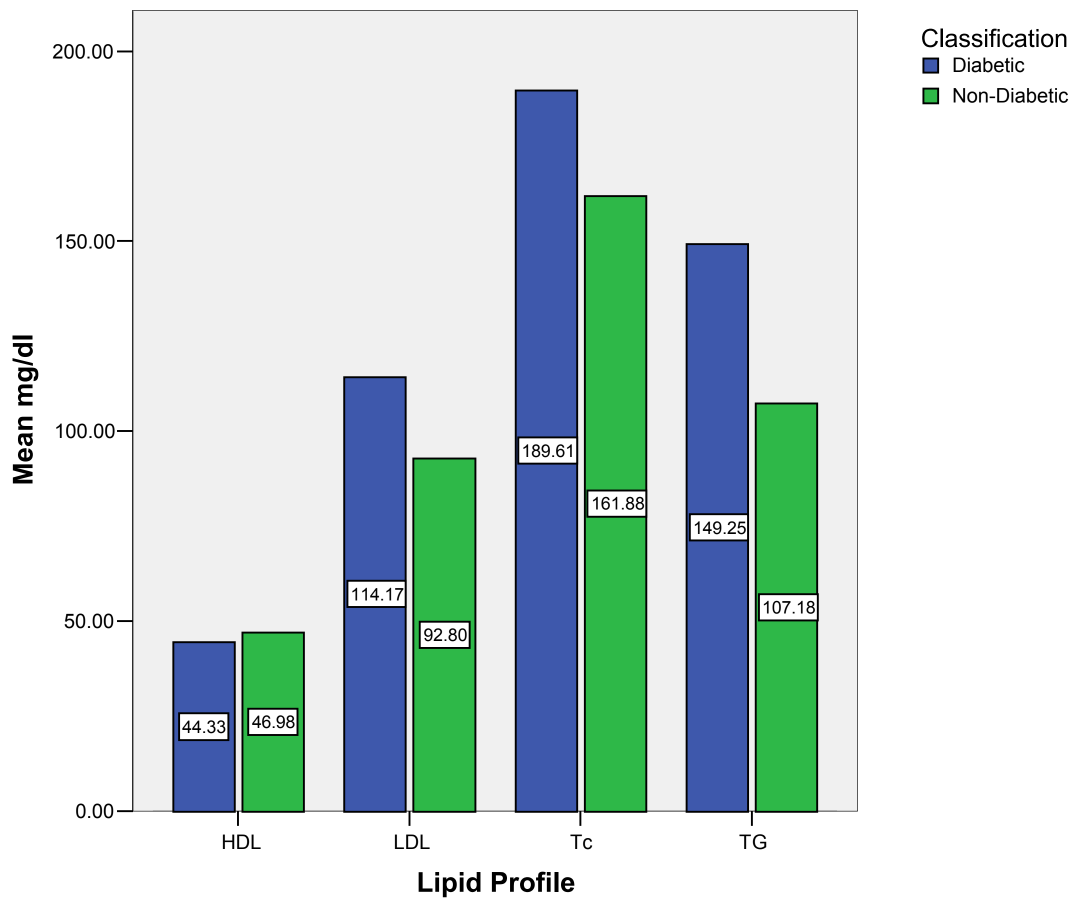


Figure 3.6: Level of lipid (TC, TG, LDL, HDL mg/dl) for diabetic and non- diabetic subjects

Table 3. 4: levels of total ($T_4$and T_3), thyroid stimulating hormone (TSH) glucose and hemoglobin, in diabetic (Type I and TypeII) and non- diabetic subjects.

Measurements	Type I	TypeII	Non-diabetic	p-value
T4 nmol/l	103.73± 22.80	101.95± 21.66	99.76 ± 17.15	0.64
T3 nmol/l	2.15± 0.50	1.73± 0.65	2.11± 0.60	0.000
TSH mlU/l	1.48 ± 1.58	0.97± 0.49	1.44 ± 0.64	0.001
Glu mg/dl	217.38± 60.61	184.80 ± 67.04	87.12 ± 14.00	0.001
Hb %	83.04 ± 11.28	82.05± 9.09	80.88 ± 14.36	0.67

Values mean ± SD.

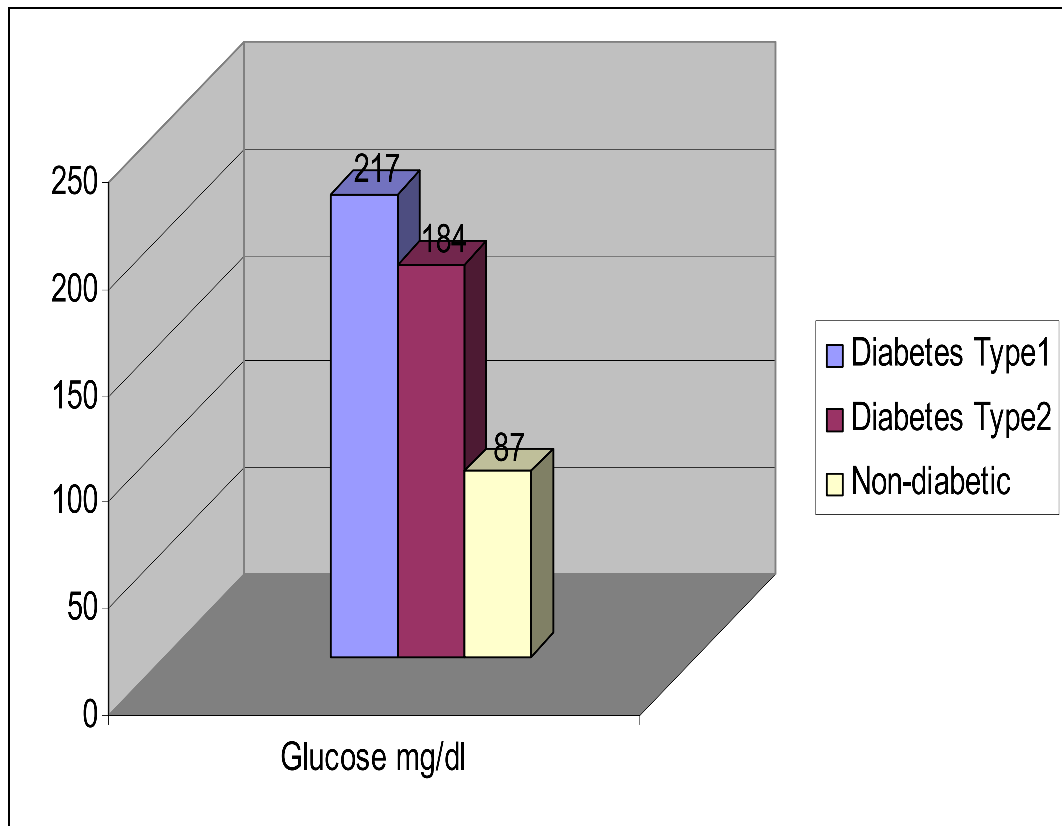


Figure 3.7: Mean values of blood glucose (mg/dl) for diabetic (Type1 and TypeII) and non diabetic subjects.

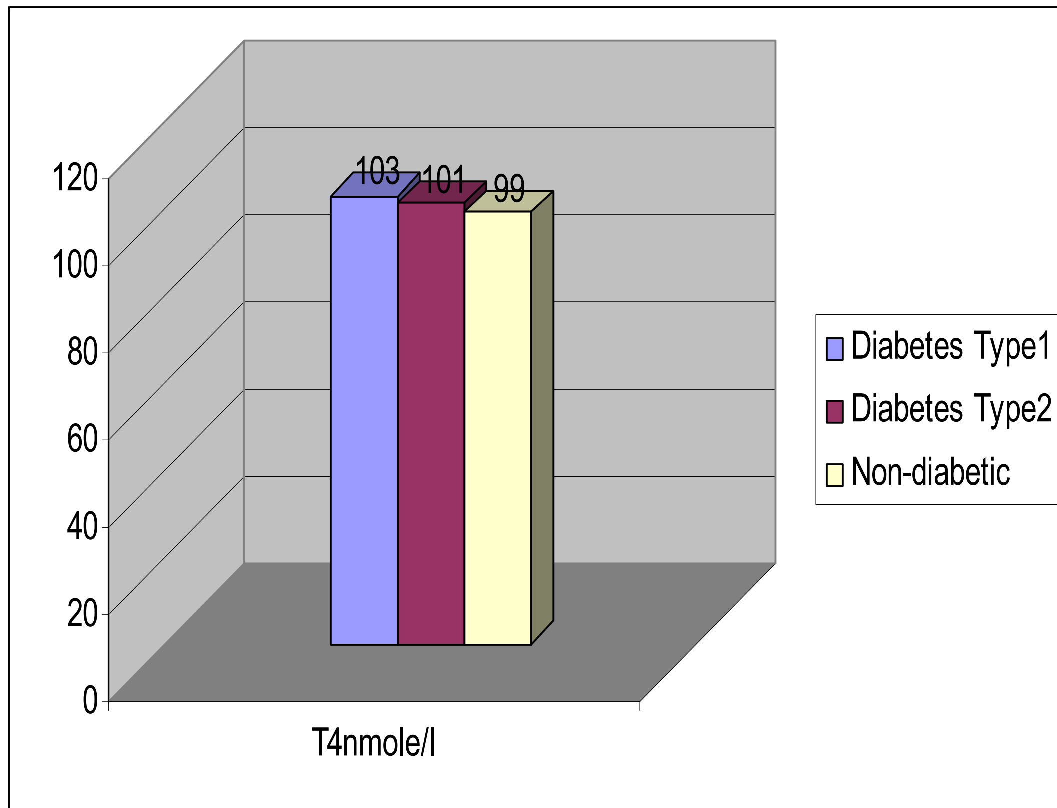


Figure 3.8: Mean values of thyroxin (T4 nmol/l) for diabetic (Type I and Type II) and non diabetic subjects.

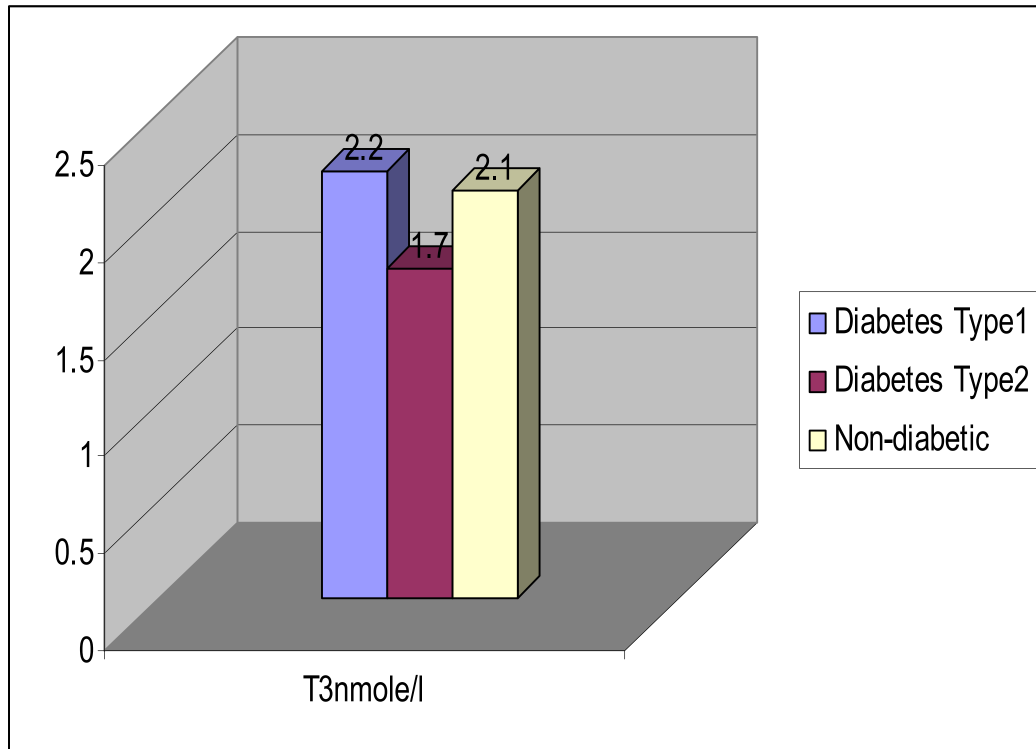


Figure 3.9: Mean values of Triiodothyronine (T3 nmol/l) for diabetic (Type1 and TypeII) and non diabetic subjects.

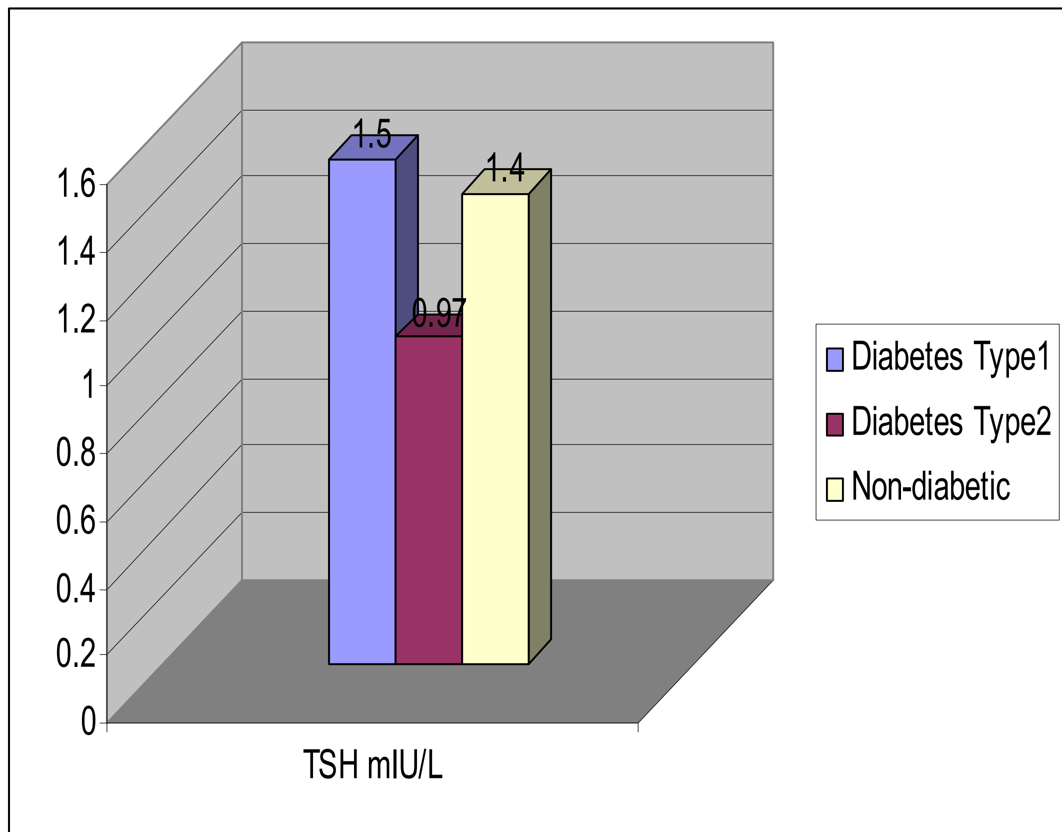


Figure 3.10: Mean values of thyroid stimulating hormone (TSH mlU/L) for diabetic (Type1 and TypeII) and non diabetic subjects.

Table 3. 5: Total cholesterol, triglyceride, low- density lipoprotein, high- density lipoprotein of diabetic (Type1 and TypeII) and non diabetic subjects.

Measurements	Type I	TypeII	Non-diabetic	p-value
TC mg/dl	187.71± 42.43	191.16± 46.47	161.88 ± 35.73	0.001
TG mg/dl	151.42±116.65	147.47±70.60	107.18±54.71	0.005
LDL mg/dl	110.82± 41.71	116.91± 43.23	92.80 ± 33.54	0.005
HDL mg/dl	46.58± 18.80	42.49 ± 14.05	46.98 ± 19.71	0.53

Values mean±SD

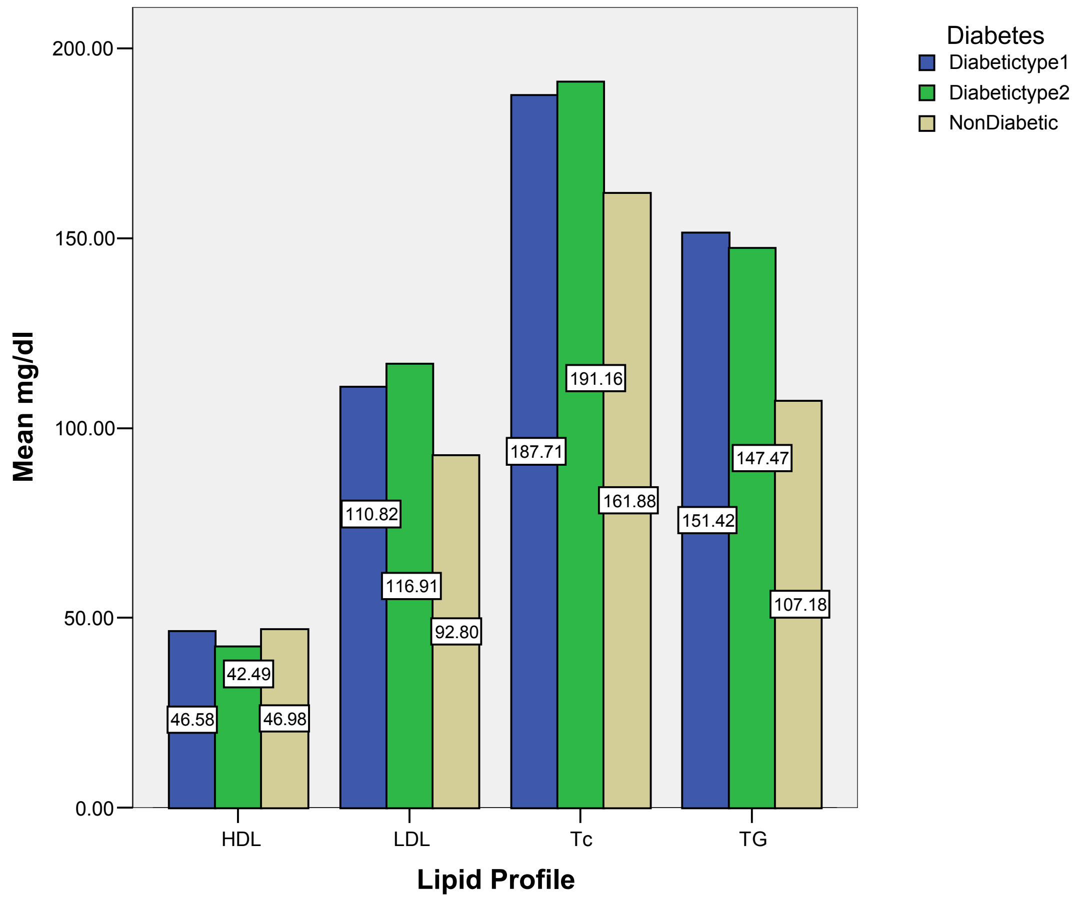


Figure 3.11: Lipid Profile (TC, TG, HDL, LDL mg/dl) of diabetic (Type1 and TypeII) and non-diabetic subjects.

3.11. Correlation between glucose and total cholesterol (TC):

Glucose was significantly correlated (positive) with total cholesterol as shown in figure (3.12) ($r=0.307$, $p=0.001$).

3.12. Correlation between glucose and triglyceride (TG):

Significant correlation ($P=0.001$, $r=0.296$) was found between glucose and triglyceride as shown in figure (3.13).

3.13. Correlation between glucose and LDL-cholesterol (LDL):

Significant correlation ($p=0.023$, $r=0.186$) was found between glucose and LDL-cholesterol as shown in figure (3.14).

3.14. Correlation between glucose and thyroid stimulating hormone:

Significant correlation (negative) was found between glucose and thyroid stimulating hormone (TSH) ($r= -0.272$, $p= 0.001$) as shown in figure (3.15).

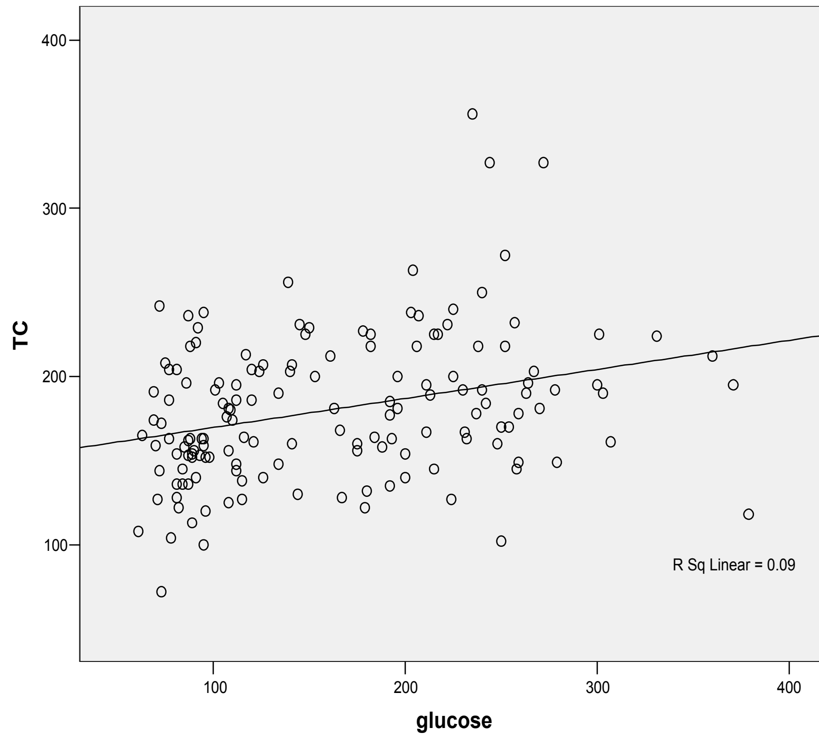


Figure 3.12: Correlation between glucose and total cholesterol (TC)

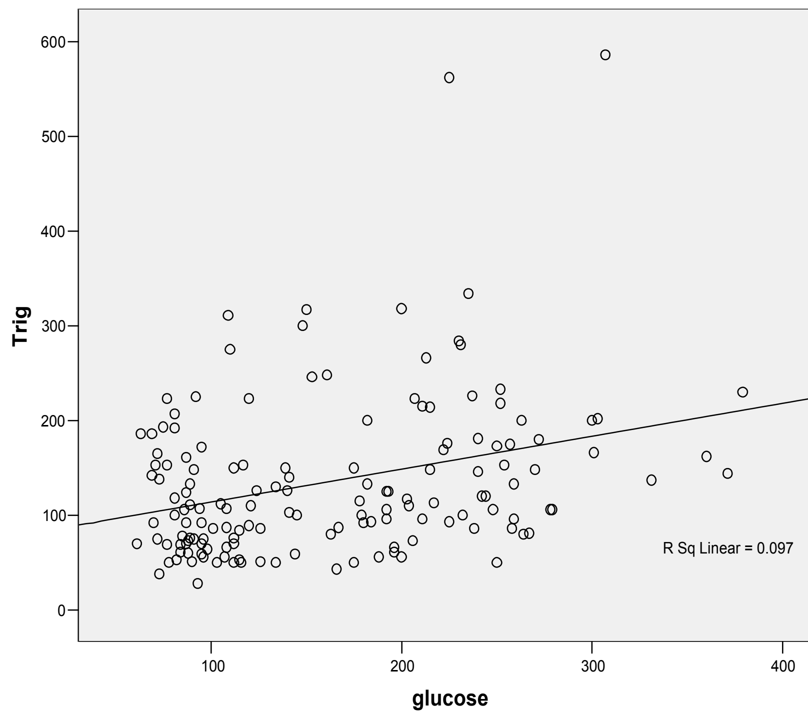


Figure 3.13: Correlation between glucose and triglyceride (TG)

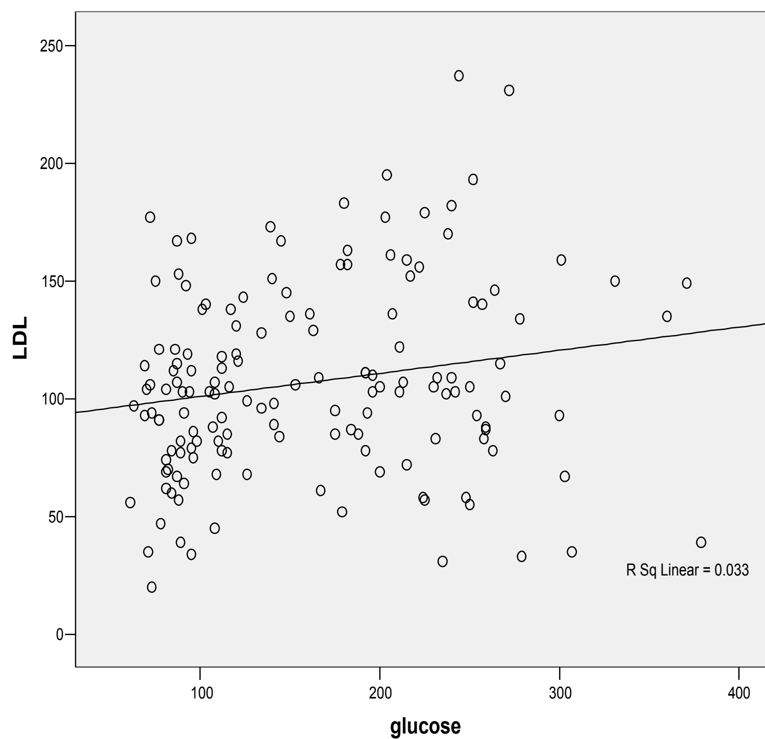


Figure 3.14: Correlation between glucose and LDL-cholesterol

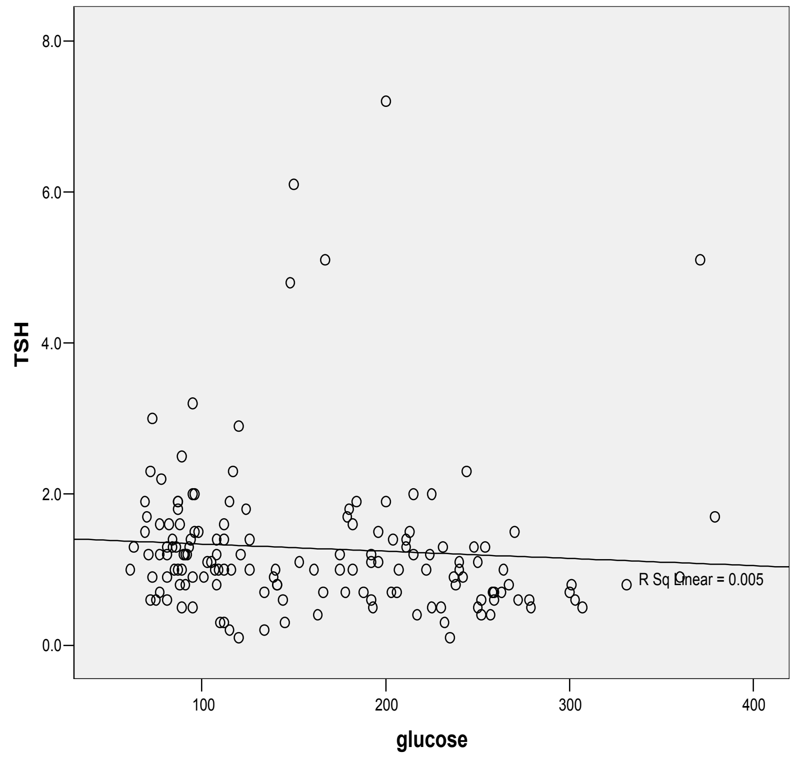


Figure 3.15: Correlation between glucose and TSH.

CHAPTER FOUR

DISCUSSION

4.1. Prevalence of thyroid dysfunction:

There are few studies on diabetes mellitus and thyroid dysfunction. They seem to indicate a higher occurrence of thyroid dysfunction among diabetic patients when compared with the general population. This study was concluded 13% of diabetic patients had thyroid dysfunction. Hyperthyroidism was the most common type of thyroid dysfunction. The high percentage of patients whose thyroid dysfunction was diagnosed, may justify routine thyroid function assessments of diabetic patients. The assessment of thyroid function by modern assays is both reliable and inexpensive. Screening for thyroid dysfunction is indicated in certain high-risk groups, such as diabetic patients. Many reports were also indicated a higher than normal prevalence of thyroid disorders in typeII diabetic patients with hypothyroidism being the most common disease (Kordonouri *et al.,* 2005; Patrecia, 2000; Tunbridge *et al*.,1997).

The availability of the highly sensitive immunoassay for serum TSH (with detection limit of <0.1 mlU/l) provides a major advance in the diagnosis of thyroid disorders. It is the most reliable and sensitive screening test for thyroid dysfunction and allows both hypothyroidism and hyperthyroidism to be diagnosed with certainty. In addition subclinical thyroid dysfunction can only be diagnosed by an abnormal TSH because the serum T_3 and T_4 are normal and, by definition, the patients are usually asymptomatic. In the present study the prevalence of thyroid dysfunction has been determined furthermore we wanted to evaluate the possible relationship between diabetes mellitus thyroid, dysfunction and lipid profile.

For the study purposes as shown in table (3.1) thyroid status has been suggested to be classified into five categories.

The present study showed prevalence of thyroid dysfunction (clinical or subclinical – hypothyroidism or hyperthyroidism was found to be 13 % and was highest in diabetic female 11%. All female patients were type I diabetes mellitus. When we compared our study with different studies done we found that, early studies reported prevalence of 5-6% of hypothyroidism or hyperthyroidism in patients with diabetes mellitus (Grey *et al*., 1980; Nabarro *et al*.,1979). Similaer to Wickham survey who found prevalence of 6.6% of thyroid dysfunction in diabetic community (Tunbridge *et al*., 1997).

Later , Perros *et al* (1995) found in their survey of type 1 and typeII , 31.4% of thyroid dysfunction in population with no previously diagnosed thyroid pathology. But Smithson (1998) found in his screening 5.5% of diabetic patients receiving community diabetes care.

In the present study 5% patients showed a TSH level exceeding 4.0 mlU/L with T_4 and T_3 within normal range indicating that they had developed sub clinical hypothyroidism. And 2% patients showed TSH level less than 0.4mlU/L but having high level of T_4 and T_3 which indicating that they had developed hyperthyroidism.

6% patients showed TSH level less than 0.4mlU/L but having normal T_4 and T_3 Which indicating that they had developed sub clinical hyperthyroidism and this finding was greater than what found in the Colorado Thyroid Disease Prevalence Study involving 25,862 subjects showed a prevalence of 2.1% (Canaris, *et al.*, 2000).

Hollowel *et al.*, (2002) reported in a population representative study of 17,353 people aged 12 and above and found that the prevalence of subclinical hyperthyroidism was only 0.7%.

In this study thyroid dysfunction was present in 13 subjects (13%). Five with subclinical hypothyroidism, though with marginally elevated TSH. This prevalence of thyroid dysfunction is in agreement with previous studies of young diabetic patients (Kenna *et al.*, 1990 ; Kontiainen *et al.*, 1990). The same finding , found in an extensive study of adult patients with IDDM demonstrating 13.4% to have clinical or subclinical thyroid dysfunction (Perros *et al.*, 1995). The study confirms that young diabetic patients have a higher prevalence of thyroid dysfunction. Similar prevalence was found in several other studies in young diabetic patients. It could be conclude that the subjects with diabetes mellitus had a much higher frequency (13%) of thyroid dysfunction than control subjects.

The indices of thyroid dysfunction in our diabetes mellitus patients were significantly different from those of control subjects. Impairment of thyroid function was significantly worse in NIDDM than IDDM as evaluated by TSH level. NIDDM have more impaired thyroid function than a control this is finding is different from that reported by Cardoso *et al* in African population (Cardoso *et al.*, 1995; Bernasconi *et al.*, 1984).

It is in agreement with others in that ,thyroid hormone levels were lower in diabetic population than control subjects this finding was reported by different authors (Patricia,2000;CastelandShah,1999;Steger and Rabe,1997Gilani *et al*.,1984;Giampietro *et al* .,1985).

Interestingly that all subhypothyroidism patients were females, reflecting the well-known sex difference in the prevalence of thyroid dysfunction. And this was reported by different authors (Trimarchi *et al*., 1984; Gilani *et al* 1984) . And this is in a good agreement with what has been published by Sukkar *et al.,* (2000) that all thyroid disturbances are more common in females than in males.

In this study subclinical hypothyroidism patients were elders having type I and this finding is in agreement with what has been reported by Jennal *et al*., 2002. explanation of this in addition to the autoimmune link between type1 diabetes and thyroid disease, diabetes and thyroid disease are more commonly found in the elderly (Patricia 2000; Jennal *et al* ., 2002).

With regard to obesity and diabetes mellitus, In this study we concluded that, obesity was a feature characterizing 62% of all the diabetic patients, and strong association with a family history (73%) of diabetes has been reported .Obesity is the most common and most expensive nutritional problem .A convenient and reliable indicator of body fat is the body mass index (BMI) which is the body weight (in kilogram) divided by square of the height (in meter); values above 25 are abnormal values of 25-30 are over weight and those with value> 30 are obese. As body weight increase, there is increasing insulin resistance (Ganong 2003).

4.2. Thyroid status:

This study was designed to compare the level of thyroid and thyroid related hormone between diabetic and non-diabetic population.

4.2.1. Serum thyroid stimulating hormone (TSH):

Significant difference (p=0.028) was found between diabetic and non diabetic population. The most important finding in this study was that TSH was lower in diabetic population (1.11± 0.94) compared to non diabetic group (1.44±0.64). This finding was explained by poorly diabetic control interfere with thyroid axis (Badman and Chowhury, 2002; Celania *et a*l., 1994; Mouradian *et al*.,1983). Also our finding was in agreement with level reported by Sathish and Mohan (2003). They reported, poorly controlled diabetes result in a low T_3 state and a loss of TSH response to TRH.

Regardless of glycemic control there is an absence of nocturnal TSH peak and in agreement with level reported by Steger and Rabe, 1997.

In diabetic subgroups (Type1 and TypeII) significant variation was found between them when this was compared with control subjects it was found that TypeII patients have lower TSH level, explanation of this finding is due to poorly glycemic control and this is in agreement with same other studies (Tunbridge *et al*.,1997 Smithson,2004) these studies which suggest that most of the TypeII diabetic patients were having poor glycemic control which interfere with thyroid axis and they might be approaching the pathology of Type1 . The same finding was reported by Kordonouri *et al*., 2005 who found elevated TSH level in type1 compared to typeII and reported that: type1 patients were considered to be autoimmune disease and they have increased level of thyroid auto antibodies which led to elevation of TSH.

Significant correlation ($r= -0.272$, $p= 0.001$) was found between glucose and thyroid stimulating hormone. The same finding was found by Cardoso *et al.* 1995.

4.2.2. Serum total thyroid hormones (T_4 and T_3):

4.2.2.1.Triiodothyronine (T_3):

Low level of T_3 (1.92± 0.61) in diabetic patients was found compared with non- diabetic subjects (2.11±0.53). Significant difference ($p=0.030$) was observed between them, this variation was found between diabetic subgroups ($p= 0.001$) compared to controlled subjects. This variation is due to poorly controlled diabetes which result in a low T_3 (Sathish and Mohan, 2003). This due to influence of diabetes mellitus at the conversion of thyroxine (T_4) to (T_3) in the peripheral tissues and this finding was in agreement with other studies (Castells,1984 and Shah1984 ;Gailani *et al.,*1984).They reported that alteration in thyroid hormones indicate the characteristic of low T_3 syndrome and this is due to marked hyperglycemia which decreased the activity and concentrations of hepatic T_4-5' deiodonase which a characteristic finding of low T_3 syndrome same conclusion was reported in other studies (Engin *et al*., 1999; Giampietrio *et al*., 1985). Our results indicated that the tendency to the low T_3 syndrome already described in adult diabetic patients is also identifiable in young particularly if poorly controlled (Badman and Chowdhury, 2002).

But recently other different findings were observed which concluded that (Yasmin *et al.,* 2006; Proces *et al.,* 2001) minor alteration in thyroid function is associated with diabetes mellitus.

4.2.2.2. Tetraiodothyronine (T_4):

No significant variation (p= 0.48) was observed in T_4 level between diabetic patients (102±22.0) and non-diabetic subjects (99.7±17.1).The same result (p= 0.64) was found between diabetic subgroups (TypeI and TypeII) and non-diabetic subjects. The same finding was observed by (Hamid *et a*l., 1998; Giampietro *et al.,* 1985) they found no variation in level of T_4 in diabetic patients and non-diabetic subject.

4.3. Glucose level:

Diabetic patients have higher level of blood glucose (199±65) compared with non diabetic subjects (87±14).Significant difference (p=0.001) was observed between diabetic and non diabetic subjects in blood glucose level and the same result (p=0.001) was found between diabetic subgroups (Type I and Type II) and control . This variation is due to absence or resistance of insulin, same results were found by (Yasmin *et al*., 2006; Abdelgader *et al*., 2006 ELbagir *et al*., 1996 Cardoso *et al*., 1995) In their studies of diabetic population in which they conclude that, the rest of diabetic population, the fasting blood glucose level is also elevated and this indicate poor control of diabetes mellitus.

4.4. Lipid profile:

The association between dyslipidemia and diabetes mellitus is well established. Although various lipoprotein abnormalities have been described in patients with diabetes mellitus elsewhere, there is limited information from African patients (kreisberg *et al.,*1998).

The results of the present study showed significantly increased levels of total cholesterol (p= 0.001) and LDL-C (p= 0.009) and high TG (p= 0.001) and a positive correlation (r=0.307, p= 0.001) and (r=0.186, p=0.023) and (r= 0.296, P=0.001) respectively between these parameters and glucose level ($p< 0.05$) which was associated with low HDL-C in diabetic subjects compared to controls. The same result ($p < 0.05$) was found between diabetic subgroups (Type1 and TypeII). These findings are in good agreement with others studies (Smaoui *et al*.,2004; Garg and Grundy, 1990; Dazien *et al.,* 1991; Howard, 1978)

In some studies concerned effect of diabetes mellitus on cholesterol metabolism it was concluded that total cholesterol and triglyceride were higher during uncontrolled hyperglycemia (Walden *et al*.,1984;Sosenco *et al*.,1980). The same finding was reported in the present study. This in agreement with Jacobs *et al.,* 2005 who studied

prevalence and control of dyslipidemia among persons with diabetes and he concluded that, cholesterol and LDL- cholesterol ,HDL-cholesterol and triglyceride are frequently abnormal in diabetic person. Same results have been reported by others (Chandalia *et al.,* 1999**)**

In fact, typeII diabetes is characterized not only by alteration in the glucose–insulin axis but marked features described as the diabetic dyslipidemia (Kreisberg, 1998). The most frequent alterations of lipid and lipoprotein profiles were the combination of elevated TGs (VLDL-TG), decreased clearance of TG-rich lipoproteins, and decreased high-density lipoproteins (HDL) most likely due to a low lipoprotein lipase activity which is well known in diabetic patients (Taskinen,2002). Lipoprotein lipase activity is depressed without sufficient insulin for adequate tissue levels. And this rise triglyceride and decrease removal of triglyceride in to the fat depots (Ganong, 2003). This diabetic dyslipidemia is associated with further increase of cardiovascular disease, This finding was in agreement with others studies (Chandalia *et al.,*1999).

Cholesterol level is usually elevated in diabetic person due to an increase in the plasma concentration of VLDL and LDL, which may be due to increase hepatic production of VLDL or decreased removal of VLDL and LDL from the circulation (Ganong , 2003).

4.5. Lipid profile and thyroid:

No significant ($p>0.05$) differences were found with regard to lipid profile nor any correlations($r = 0.0001$, $p>0.005$) between them and thyroid hormones. This was found when thyroid hormones and lipid profile were compared ,which can be explained by that majority (87%) of study subjects (diabetic patients) was euthyroid. and the metabolic disturbances of thyroid dysfunction in lipid profile at diabetic patients was not clear in this study. other group reported the same conclusion (EL Nobre *et al* ., 2002; Proces *et al* 2001)

CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

- The present study concludes that although thyroid dysfunction is not common among diabetic patients, considerable subclinical cases have been reported.
- If this subclinical thyroid dysfunction reachs clinical situation it can produce significant metabolic disturbances if not treated. Therefore, regular screening for thyroid abnormalities in all diabetic patients will allow early treatment of sub clinical thyroid dysfunction.
- A sensitive serum TSH assay is the best screening test for determination of thyroid dysfunction.
- Recognition and treatment of thyroid dysfunction is important in management of diabetic patients as this condition affects glycemic control.
- Plasma cholesterol and triglycerides are elevated in diabetic patients compared to non-diabetics and correlated closely with blood glucose.
- Control of diabetes is the most important factor that tends to normalize the lipids in diabetic patients.
- **Recommendations:**
- There is little information about diabetes and thyroid dysfunction in this country and also there is a need for baseline studies and there is still a great need for further research and studies in this field.
- The small size sample and the limited observation period do not allow definite conclusion from this data. So more comprehensive study with large population and long period would be more informative.
- We recommended that for further research the following will be done:
- for type I diabetic patients (autoimmune disease) it is helpful to determine if anti-thyroid peroxidase (TPOAb) and thyroglobulin antibodies (Tg Ab) are present because they are markers of thyroid autoimmune disease, and for type II diabetic patients, a TSH assay should be done.
- Ultrasound assessment.
- Glycosylated hemoglobin (HbA_{1c}).
- Periodic screening for dyslipidemia in all diabetic patients.

References

Abdelgadir, M. (2006). Clinical and Biochemical Features of Adult Diabetes Mellitus in Sudan. PhD thesis, Uppsala Dissertations from the Faculty of Medicine 144-47 . ISBN **91**-554-6542-0.

Aboud, B. A. (2000). Thyroid Function and Iodine Nutrition in Women During Pregnancy and in their Neonates. Study among Sudanese subjects. Doctor Degree in Medical Science, Uppsala University, Sweden.

Abusabib, M. (**2004**).Art, politics, and cultural identification in Sudan. Acta Unversitatis Upsaliensis . AESTH UPS **8**:21-56

Amos, A. F., D. J. McCarty, P. Zimmet, (1997). The rising global burden of diabetes and its complications. J. Diab. Med. **14:**7–85.

Arthur, H. and Herbert G. (1968). Diabetes mellitus and primary hypothyroidism. science direct. J. Metabolism. **17**(2): 108- 113.

Babiker, E. N. (1996). Iodine Supplementation in a Goiter Endamic Area. Doctor Degree in Medical Science, Uppsala University, Sweden.

Badman, M. and Chowdhury. (2002). Should thyroid function tests be done annually in all patients with diabetes? Diabetes UK .J. Diab Med. **19**: 1–18

Bani, I. A., Anokute, C. (1994). A pilot epidemiologic study of diabetes mellitus in the Sudan. 1: J R – Soc health. **114** (4):178 – 81.

Bernasconi, S. S., Vane, M., Nori, G., Siracusano M. F., Marcellini C., Butturini A., DeLuca, F. (1984). Serum TSH, T4, FT4, T3 and TBG in non-ketotic insulin-dependent diabetes mellitus subjects .J. Horm Res. **20**:213-217.

Betterle, C., Zanette F., Pedini B., Presotto F., Rapp, L. B., Monciotti C. M. (1984). Clinical and subclinical organ-specific autoimmune manifestations in type 1 (insulin-dependent) diabetic patients and their first degree relatives. J. Diabeto logia. **26**:431–436.

Brent, G. (1994). The molecular basis of thyroid hormone action. Med. **331**:922 – 926

Brook, C. G., and Marshal, N. J. (2001). Essential Endocrinology. 4th ed. The Black well science Ltd. United Kingdom. 78-96.

Canaris, G. J., Manowitz, N. R., Mayor, G. M., Ridgeway, E.C. (2000). The Colorado thyroid disease prevalence study. J. Arch Intern Med. **160**:526–534.

Cardoso, C., Ohovoride, A. E., Kuku, S. F. (1995). A study of thyroid Function and prevalence of Thyroid Autoantibodies in an African Diabetic population. J. Diabetes complic. **9**:37-41

Castells, S. (1994). Thyroid function in juvenile diabetes. J. Pediatr Clin North Am. **31**(3):623-634.

Cavalieri, R. R. (1999). The effects of nonthyroid disease and drugs on thyroid function tests.J. Medical Clinics of North America. **75**(1):27-39.

Celani, M. F., Bonati M. E., Stucci, N. (1994). Prevalence of abnormal thyrotropin concentrations measured by a sensitive assay in patients with type II diabetes. J. Diabetes Res. **27(1)**:15–25.

Chandalia, Ajgaonkar, Jayshree Bagrodia, P. S. Lamba and B. D. Punekar (1999). Lipid abnormalities in diabetes mellitus. INT. J. Diab. dev. Countries.19

Cook, A. R. (1901). Notes on the diabetes mellitus within Uganda, central Africa. J Trop Med. **4**:175-178.

Cooper, D. S. (2001). Subclinical hypothyroidism. N Engl. J. Med. **345**:260–265.

Danese, M. D., Ladenson P. W., Meinert C. L., Powe, N. R. (2000). Effect of thyroxine therapy on serum lipoproteins in patients with mild thyroid failure. J. Clin Endocrinol Metab. **85**:2993–3001.

Daniel, (2000). American thyroid association guide lines for detection of thyroid dysfunction. J. Arch inter Med. **160**:1573-1575.

Deman, F. H., Caberzas, M. C., Van barling, H. H., Erkelens, D. W.(1996). Triglyceride –rich lipoprotein in non- insulin- dependent diabetes mellitus. J Clini invest. **26**:89-108.

Dickman, T., Lansberg, P. J., Kastelein, J. J., Wierasinga, W. M. (1995). Prevalence and correction of hypothyroidism in a large cohort of patients referred for dyslipidaemia. J. Arch Intern Med. **155**:1490–1495.

Donckier, J. E. (2003). Endocrine disease and diabetes mellitus. Black well published company. **27** (1): 27-25.

Duntas, H., and Leonidas, M. (2002). Thyroid Disease and Lipids. J. Thyroid **12**(4):287-293.

Dzien, A., Lechleitner, M., Hopferwieiser, T., Drexel, H., Patsh, J. R., Braunstecine, H. (1991). Long –Term Effect of Intensified Insulin Treatment on Lipid parameters in diabetes Mellitus Type1. J. Klin Wochenschr. **69**:483 – 485.

Edward, R. (1980). RIA and IRMA methods. Netria diagnostic-test. 1st ed.

Edwards, R. (1996). Immunoassay, Essential Data. 1st ed. John wiley and sons Ltd .

EL Nobre, Z., Jorge, S., Pratas, C., Silva, and J. J. Castro, (2002). Profile of thyroid function in a population with typeII diabetes mellitus. J. Bio .Endocr **3**:298

Elamin, A, Eltahir A., Ismail, B., Tuvemo, T.(1992). Clinical pattern of childhood Type1 diabetes mellitus in the Sudan. J. Diabetes Res **35**:645-8.

Elamin, A., Omer, M. I. A., Hofvander, Y., Tuvemo, T. (1989). Prevalence of insulin dependent diabetes mellitus (IDDM) in school children in Khartoum, Sudan. J Diabetes Care. **12**:430-2.

Elamin, A., Omer, M. I., Zein, K., Tuvemo, T. (1992). Epidemiology of childhood type I diabetes in Sudan. J. Diabetes Care.15 (11):1556 – 1559.

Elbageir, M. N. (1997). Epidemamiology and Clinical course of Adult Diabetes in Sudan. Doctor Degree in Medical Science, Uppsala University, Sweden.

Elbagir, M. N, Eltom, M. ., Berne, C. (1998). Classification of newly-onset IDDIM and NIDDIM in subjects aged 25-45 years in Sudan. J. Intern Med. **244**(6):443-50.

Elbagir, M. N., Eltom, M. A., Elmahadi, E. M., Kadam, I. M., Berne, C. (1996). A population based study of the prevalence of diabetes and impaired glucose tolerance in adults in northern Sudan. J. Diabetes Care. **19**(10):1126-8.

Elbagir, M. N., Eltom, M. A., Elmahadi, E. M., Kadam, I. M., Berne, C. (1998). A high prevalence of diabetes mellitus and impaired glucose tolerance in the Danagla community in northern Sudan. J . Diabet Med. **15**(2):164-9.

Elbagir, M. N., Eltom, M. A., Mahadi, E. O., Berne, C. (1995). Pattern of long-term complications in Sudanese insulin-treated diabetic patients. J. Diabetes Res Clin Pract. **30**(1):59-67.

Elbagir, M. N., Eltom, M. A., Rosling, H., Berne, C. (1995). Glycaemic control of insulin dependent diabetes mellitus in Sudan: influence of insulin shortage. J Diabetes Res Clin Pract. **30**(1):43-52.

Elbagir, M. N., Etayeb, N. O., Eltom, M. A., Mahadi, E. O., Wikblad, K., Berne, C. (1999). Health related quality of life in insulin-treated diabetic patients in the Sudan .J. Diabetes Res Clin Pract. **46** (1): 65-73.

Elder, J., McClelland, A., O'Reilly, D. S., Packard, C. J., Series, J. J., Sheppard, J. (1990). The relationship between serum cholesterol and serum thyrotropin, thyroxine and tri-iodothyronine concentrations in suspected hypothyroidism. J. Ann Clin Biochem. **27(2)**:110–113.

Elizabeth, B. Scott, M. Grundy, and Mark J.(1982). Plasma lipids and diabetes mellitus in an adult community. J. American Epidemiology.**115** (5):657-663

Elmahadi, E. M. A., Salih, A. R., Mukhtar, E. (1989). Patterns of diabetes mellitus in the Sudan. J .Trop. Med. **4**:353-5.

El-Shazly, M., Abdel-Fattah, M., Zaki, A., Bedwani, R., Assad, S., Tognoni, G, Nicolucci. A. (2000). Health care for diabetic patients in developing countries. J Science Direct. Public Health. **114**(4):276-81.

Eltom, M., Hofvander, Y., Torelm, I., Fellstorm, B. (1984). Endemic Goiter in the Darfur Region. J. Acta. Med.Scand. **215**:467-475

Engin, G. Ney, Belgin. Aysen, Nur Kebap Ý, Esat ErenoÛ, (1999). Thyroid Disease in Diabetes Mellitus. J. Endocrino. Metab. **3**:119-122.

Franklyn, J. A., Daykin. J., Betteridge, J., Hughes, E. A., Holder, R., Jones, S. R. (1993). Thyroxine replacement therapy and circulating lipid concentrations. J Clin. Endocrinol. **38**:451–452.

Frazad, H., Azadeh, Z., Hadi, H., Fereidoun, A. (2006). The prospective association of general and central obesity variables with incident typeII diabetes in adults. Tehran Lipid and glucose study. J. Diab. research .**3724:1-6.**

Friedwal, W. T., Levy, R. I., and Fredrickson, D. S. (1972). Estimation of the concentration of low – density lipoprotein cholesterol in plasma without use of the preparative ultracentrifugem .J. clinical chem. .**18**:499 – 502.

Ganong, W. F. (2003). Review of Medical physiology. 21^{ed}. Lang MC, Graw Hill, Endocrine Function of the Pancreas. 336-58. Thyroid Gland. 320-330.

Garg, A., Grundy, S. M. (1990). Management of dyslipidemia in NIDDM. J. Diabetes Care. **13**(2):153– 169.

Giampietero, O., Miccoli, R., Lotti, M., Serri, M., Ferdeghini, M., Penno, G., Sampietro, T., Di Palma, L. (1985). Thyroid Function Evaluation in diabetes Mellitus. J. Nuclear Medicine and Allied sciences. **29**: 1-2

Gilani, B. B., Mac Gillivray, M. H., Voorhess, M. L., Mills, B. J., Riley, W. J., Mac Laren N. K. (1984). Thyroid hormone abnormalities at diagnosis of insulin-dependent diabetes mellitus in children. J. Pediatr. **105**(2):218-222.

Gray, R. S., Borsey, D. Q., Seth, J., Herd, R., Brown, N. S., Clarke, B. F. (1980). Prevalence of subclinical thyroid failure in insulin-dependent diabetes. J. Clin Endocrinol Metab. **50**(6):1034-7.

Gray ,R., Clarke, B.(1978). Primary autoimmune diabetes mellitus. J. Br Med .2:1715.

Gray, R., Smith, A.,Clarke,.(1981).Hypercholesterolaemia in diabetics with clinically unrecognised primary thyroid failure. J. Hormone Metab Res .**13**:508–510.

Greenspan, F. S., Gardner, D. G. (2004). Basic and Clinical Endocrinology. 7th ed. London. Lang Medical Books /Mc Graw Hill. 410:419.

Hamid, Sakineh, M., Farbod R., Fereidoun A. (1998). Association of Diabetes Meilitus ‘type II with Thyroid Dysfunction. Endocrine Research Center Tehran, Iran .

Hanadi, E. (2004). Study on maternal thyroid hormones levels in normal Sudanese pregnant women. To evaluate suspected thyroid abnormalities. M. Sc. Thesis in Physiology, Sudan, University,

Hansen, D, F. N., Bennedbæk1, L. K., Hansen, M. Høier-Madsen2, B. B., Jacobsen, and L. Hegedu,. (1999). Thyroid function, morphology and autoimmunity in young patients with insulin-dependent diabetes mellitus. J. Endocrinology. **140(6)** 512–518.

Hardman, J. G., and Limbird, L. E. (2001). The Pharmacological Basis of therapeutics. 10th ed. Miian: MCGW- Hill: 1563 - 1571.

Hassan, A. M. (1992). Studies on Simple Goiter in Omdurman, M. Sc. Thesis in Physiology, Khartoum University, Sudan.

Helfand, M. (2004). Screening for Subclinical Thyroid Dysfunction in Adults. J. Intern. Med. **140**:128-141.

Helfand, M., Redfern, C. C. (1998). Screening for thyroid disease .J. Ann Intern Med . **129**:144–158.

Henry, I.,Ulrike, K., Henri, W., Jan, L., demann, U., John, W. K. (2006). The spectrumof thyroid disorders in adult type 1 diabetes mellitus. J. diabetes. research. ISSN:1520-7560

Hollowell, J., Staehling, N., Flanders, D., Hannon, H., Gunter,W., Spencer, A., Braverman, L.(2002). Serum TSH, T_4, and Thyroid Antibodies in the United States Population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J. Clin. Endocrino. Metab. **87**(2):489–499

Howard, B. V. (1978). Lipoprotein metabolism in diabetes mellitus. J. Lipid Research. **28**: 613–628.

Hyden, J. M,. and Reaven, P. S. (2000). Cardiovascular disease in diabetes mellitus type (2). J. Lipid Research **.11**: 519 – 528.

International Diabetes Federation European Diabetes Policy Group, (1999). A desktop guide to Type II (Non- insulin-dependent) diabetes mellitus. J. Diabetes Metab.**16**:716–730.

International Diabetes Federation European Diabetes Policy Group. (1999). A desktop guide to type 1 (insulin-dependent) diabetes mellitus. J. Diabetes Metab **12**:345–378.

Jacobs, M. J., Thomas, K., Jose, R., Shaist, M., Gillert, J. L., Rolands Nathan, D. (2005). Prevalence and control of dyslipidemia among persons with diabetes in the united states .J. Diabetes.Research .Clini Prac. **70**:263-269.

Jennal L ,Johonson. MR, Daniel, S ,Duick (2002). Diabetes and Thyroid disease. J Diabetes spectrum .**15**(3):140 42.

Kambal, A. (1969). Endemic Goiter in Darfur, Sudan. M. Sc. Thesis Khartoum University. Sudan. 43.

Kek, P.; Ho, S. and Khoo, D. (2003). Subclinical Thyroid Disease. Singapore. J. Med. **44**(11): 595-600.

Kenna MJ, Herskowitz R & Wolfsdorf JI. (1990). Screening for thyroid disease in children with IDDM. J. Diabetes Care. **13**:801–803.

King, H., Aubert, R. E., Herman, W. H. (1998). Global burden of diabetes. Prevalence, numerical estimates, and projections. J. Diabetes Care. **21**:1414- 1431.

Klein, R. (1995). Hyperglycaemia and microvascular and macrovascular disease in diabetes .J. Diabetes Care. **18**: 258–268.

Knowler, W., Narayan, R. L., Hanson, R. G., Nelson, P. H., Bennett, J. T. (1995). Preventing non-insulin dependent Diabetes Mellitus. INT. J. diab.dev. **44**: 483–488.

Kontiainen S, Schlenzka A, Koskimies S, Rilva A Maenpaa J.(1990).Autoantibodies and autoimmune diseases in young diabetics. J. Diabetes Research .**13** :151–156.

Kordonouri, O., Hartmann, R., Deiss, D., Wilms, M., Gruters, A. (2005). Natural Course of autoimmune thyroidistis in type 1 diabetes: Association with gender, age, diabetes duration, and puberty. J. Arch, Dis child. **90**: 411 – 414.

Kozak, G. P., Coopan, R. (1985). Diabetes and other endocrinologic disorders. Diabetes Mellitus .12ed.784-816.

Kreisberg, R. A. (1998). Diabetic dyslipidemia. J. Cardiology. **82:**67-73, 85-86.

Laakso, (1999). Hyperglycaemia and cardiovascular disease in type II diabetes. J Diabetes Care. **48**: 937–942.

Ladenson, P. W., Singer, P. A., Ain, K. B., Bagchi, N., Bigos, S. T., Levy, E. G. (2000). American Thyroid Society guidelines for detection of thyroid dysfunction. J. Arch Intern Med.**160**: 1573–1575.

Longmore, M., Wilkinson, I., and Torok, E. (2001). Oxford Handbook of Clinical Medicine. 5th ed. Oxford University .

Mohn A, Di Michele S, Di Luzio R, Tumini S, Chiarelli F. (2002).The effect of subclinical hypothyroidism on metabolic control in children and adolescents with Type 1 diabetes mellitus .J . Diab. Med. **19**: 70-3.

Motala, A. A., Omar, M A., Pirie, F. J. (2003). Diabetes in Africa. Epidemiology of type 1 and type II diabetes in Africa. J. Cardiovasc Risk.**10** (2):77-83

Mouradian, M., Abourizk, N. (1983). Diabetes mellitus and thyroid disease. J. Diabetes Care. **6**: 512–520.

Nabarro, J. D. N., Mustaffa, B. E., Morris, M. J., Walport, M. J., Kurtz, A. B. (1979). Insulin deficient diabetes. Contrasts with other endocrine deficiencies.J Diabetologia . **16**: 5–12.

National Cholesterol Education Program (NCEP) (2001)**.** Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Downloaded from www. Jama. com at Queen Mary, London . JAMA.**285:**2486–2497.

Nerurkar, S. V., Retna, V. d., Taskar, S., Bhandarka, S. D. (1983). Lipid composition of plasma lipoprotein in treated diabetes. J . Medicine. **29(**4):201–2

Nessar, A. (2004). Advanced glycation endproducts — role in pathology of diabetic complications .J. Diabe Research and Clin Pract. **67**:3–21.

Pamela, C. ., Richard, A. (1994). Lippincott's illustrated Review, Biochemistry 2nd ed.

Papoz, L., Delcourt, C., Ponton, S. A., Lokrou, A., Darrack, R., Toure, I. A., Cuisinier, R. J. C. (1998). Clinical classification of diabetes in tropical West Africa. J Diabetes Res Clin P. **39**(3):219-27.

Patricia, W. (2000). Thyroid Disease and diabetes. J. Clinical Diabetes.**18** (1): 38.

Paul, W., Ladenson, P. A., Singer, K, B., Nandala, B. S., Thomas, B., Elliot, G., Levy, Steven, A., Smith, H.,(2000) American Thyroid Association Guide-lines for Detection of Thyroid Dysfunction. J. Arch Intern Med. **160:**1573-1575.

Perros, P., Crimmon, R., Shaw, G., Frier, (1995). Frequency of thyroid dysfunction in diabetic patients. value of annual screening. J. Diabet Med.**7**:622–627.

Peter, J. W. (1982). What is diabetes?J. British medical. 284.

Proces S.,Delgrange E Vander Borght (2001). Minor alteration in thyroid function tests associated with diabetes mellitus and obesity in patients without known thyroid illness. 1 Acta Clin Belg. **56**(2):86-90

Radetti, G., Drei, F., Franzellin, F., Pasquino, B., Mengarda, G. (1985). Thyroid function in type 1 juvenile diabetes mellitus: tendency to the low T_3 syndrome. Helv Paediatr Acta. **40**(6):461-6.

Rassul, F., Richter, V., Kullmann, E., Heberling, H., Homann, M., Zuk, U., Rotzsch, W., Lahmann, D. (1988). Lipoprotein Metabolism in patients with disordered thyroid gland functions. Dtsch. Z. verdant. Stoffweshselkr. **48**(2): 104-11.

Retnam, V. J., Nerurkar, S. V., Gupte, M., Bhandarkar, S., Taskar, S. P. (1983). Plasma lipids and lipoproteins in treated diabetics .J. Postgra Med. **29**(4):193-200

Riley, W. J., Maclaren, N. K., Lezotte., D. c., Spillar, R. and Rosenbloom, A. (1981). Thyroid autoimmunity in insulin-dependent diabetes mellitus: The case for routine screening. J. Pediatr. **98**: 835-854.

Robert, K. M., Daryl, K. G., Peter, A. M, Voctor, W. R. (2000). Thyroid hormones Harper's Biochemistry 25th Lange Medical Books.

Sathish, R., Mohan, V. (2003). Diabetes and Thyroid Disease. J. Dia. dev. Countries.**23**:120-123

Series,J. J., Biggart, E., Oreilly, D., Packard, C., Shepherd, J. (1988).Thyroid dysfunction and hypercholesterolaemia in the general population. J. Clin. Acta.**21** (3):172-217.

Shah, S. N. (1983). Thyroid disease in diabetes mellitus. An up-date. J. Assoc Physicians India. **32**(12): 1057-1059.

Sharma, B. K. (2001). Instrumental Method of chemical Analysis. 20th ed. Book Code No: CH3-20 / INDIA. Prakashan Media Ltd. 45-48

Smaoui, M,. Hammami, S., chaaba, R., Attia, N., Ben Hamda, K., Masmoudi, A. S., Masmoudi, S. Mahjoub, A. (2004). Lipids and lipoprotein concentration in Tunisian typeII diabetic patients. J. diabetes complic. **18**:258-263.

Smithson, M. J. (1998). Screening for thyroid dysfunction in a community population of diabetic patients. J. Diabetes. Med. **15**(2):148-50.

Sobngwi, E.,Mauvais, F., Vexiau, P.,Mbanya, J.,Gautier, J.(2001). Diabetes in Africans, epidemiology ,clinical specificities. J. Diabetes Metab . **27**(6): 628–634.

Sosenko, J. M., Breslow, J. L., Miettinen, O. S., Gabbay, K. H. (1980). Hyperglycemia and Plasma lipid levels. New England. J. Medicine. **12**(302): 650-654

Spandrio, S., Sleiman, I., Scalvini, T., Salvi, A., Di Stefano, O., Pagliaini, R. and Balestrier, G. P. (1993). Lipoprotein (a) in thyroid dysfunction before and after treatment . J. Horm. Metab. Res. **25**(11): 586-9.

Steger R., Rabe M (1997). The effect of diabetes mellitus on endocrine and reproductive function .J. Experimental biology and Medicine . **214**: 1-11

Sugrue, D. D., Mc Evoy, M., Drury, M. I. (1982). Thyroid disease in diabetics. J Postgrad Med.**58** (865): 680-684.

Sukkar, M. Y., EL- Munshied, H. A., and Hardui, M. S. (2000). Concise Human Physiology. 2nd ed, Blackwell Science Ltd. USA. 206-213.

Taskinen, M. R. (2002). Diabetic dyslipidemia. Atheroscler. Supp. **3**: 47 – 51.

The Diabetes Control and Complications Trial Research Group (1996). Influence of intensive diabetes treatment on quality-of-life outcomes in the Diabetes Control and Complications Trial. J. Diabetes Care; **19**: 195-203.

Tietz, N. W., Burtis, C. A., Ashwood, E. R., and Border, B. (2000). Tietz Fundamentals of Clinical Chemistry. 5th ed.

Trence, D. L., Morley, J. E., Handwerger, B. S. (1984). Polyglandular autoimmune syndromes. Am. J. Med. **77**: 107–116.

Trimarchi, F., Deluca, F. and Vanelli, M. (1984). Circulating thyroid antibodies and thyroid function studies in children and adolescents with insulin-dependent diabetes mellitus. Eur. J . Pediatr. **142**: 253-257.

Tunbridge, W. M. A., Evered, D. E., Hall, R., Appleton, D., Brewis, M., Clark, F., Evans, J., Bird, T., Smith, A. (1997). The spectrum of thyroid disease in the community: The Whickham Survey. J. Clin. Endocrinol Oxf. **7**:481-93.

Vanderpump, M. P., Tunbridge, W. M., French, J. M., Appleton, D., Bates, D., Clark, F. (1995). The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham survey. J .Clin Endocrinol. **43**: 55–68.

Vanderpump, M. P., Ahlquist, J. A., Franklyn, J. A., Clayton, R. N. (1996). Consensus statement for good practice and audit measures in the measurement of hypothyroidism and hyperthyroidism. J. Br Med. **313**: 539–544.

Walden, C. E., Knopp, R. H., WahI, P. W., Beach, K W., Strandness, E. (1984). Sex differences in the effect of diabetes mellitus on lipoprotein triglyceride and cholesterol concentrations. New England. J. Medicine.15(**311)**: 953-959

Williams, R. H. (2001). Textbook of Endocrinology. 8th ed. London: W. B. Saunders Company. 296-302.

Yasmin, T., **Ghafoor**, F., Malik, T., Ruhy, N. S., Khan, A. (2006). Pattern of thyroid Autoimmunity in Type 1 and type II Diabetes. JCPSP. **16** (12):751-754.

Appendix 1: Thyroid hormones, blood glucose, hemoglobin in diabetic patients.

No	T4nmol/L	T3 nmol/L	TSH mlU/L	Glu mg/dl	Hb %
1	87	1.5	.6	144	82
2	113	1.7	.6	192	68
3	104	2.0	1.2	215	95
4	107	1.4	.8	141	75
5	141	1.3	2.0	215	90
6	96	1.5	1.4	126	86
7	125	1.0	.8	91	89
8	73	1.0	1.0	109	82
9	118	2.3	.5	250	90
10	89	1.8	1.2	175	68
11	76	1.7	7.2	200	70
12	68	1.1	2.0	225	86
13	149	2.6	.7	188	84
14	123	2.0	1.5	96	84
15	105	2.0	.5	225	95
16	88	1.1	.8	108	95
17	127	1.6	.3	112	91
18	63	2.1	1.2	192	82
19	87	1.0	1.4	204	83
20	91	1.4	1.0	161	86
21	103	1.6	5.1	371	92
22	164	3.1	.3	110	74
23	111	1.5	1.2	121	95
24	82	1.4	.7	203	90
25	126	2.7	6.1	150	75
26	107	1.1	.1	120	86
27	85	1.7	.9	95	81
28	75	1.1	.9	242	80
29	113	2.2	.7	300	75
30	126	2.1	.5	307	90
31	96	1.3	.1	235	73
32	130	2.6	.3	232	81
33	91	1.9	4.8	148	73
34	105	1.3	1.8	124	75
35	113	2.8	.6	272	64
36	95	2.5	1.0	264	90
37	95	1.0	1.6	88	78
38	83	1.8	1.3	231	84
39	114	3.3	1.1	240	71
40	85	2.4	.4	163	82
41	97	1.4	1.1	196	69
42	95	1.3	1.1	250	58
43	77	1.6	.7	258	57
44	108	2.3	.5	279	80
45	85	2.5	.8	141	70
46	105	2.2	.6	278	78
47	132	2.4	.4	217	84
48	103	2.9	1.1	192	90
49	115	2.5	2.3	244	82
50	104	2.8	1.0	140	71

Appendix 2: Thyroid hormones, blood glucose, hemoglobin in diabetic patients.

No	T4 nmol/L	T3nmol/L	TSHmlU/L	Glu mg/dl	Hb %
51	79	1.3	1.3	254	78
52	141	2.3	.9	237	85
53	176	4.0	.2	115	67
54	83	2.4	.7	206	90
55	105	1.9	1.0	182	93
56	126	1.6	.8	301	84
57	94	2.3	1.6	182	83
58	140	2.2	.6	252	85
59	108	2.7	.8	238	85
60	97	2.8	1.0	126	71
61	75	1.9	.7	166	79
62	121	2.2	1.0	116	82
63	101	2.3	.7	134	113
64	121	2.0	.5	230	85
65	164	3.4	1.3	248	70
66	77	1.1	1.0	175	80
67	141	1.3	.4	257	90
68	113	2.3	.9	139	90
69	128	2.7	.9	360	82
70	90	2.1	1.9	184	92
71	82	1.3	1.1	103	72
72	75	1.8	.4	252	97
73	124	1.9	5.1	167	86
74	114	1.8	1.1	105	99
75	95	1.8	1.0	240	74
76	89	2.2	.8	331	81
77	73	1.8	.8	267	99
78	101	1.9	.5	193	86
79	79	1.6	.6	259	90
80	103	2.6	.7	259	80
81	71	1.4	1.5	213	83
82	86	2.4	1.5	270	99
83	109	2.6	1.3	211	73
84	106	2.0	1.0	222	92
85	95	2.2	1.5	196	98
86	94	1.0	1.8	180	67
87	127	3.1	.3	145	84
88	95	1.0	1.4	211	73
89	92	2.1	1.4	108	68
90	84	1.3	1.9	200	72
91	87	1.3	.7	178	74
92	80	1.9	1.0	207	82
93	101	1.7	1.4	112	74
94	99	1.4	.2	134	81
95	89	1.3	1.1	153	92
96	130	2.6	.7	263	79
97	91	1.6	1.7	379	83
98	84	1.3	1.7	179	105
99	106	1.5	1.2	224	104
100	84	1.7	.6	303	90

Appendix 3: Thyroid hormones, blood glucose, hemoglobin in non diabetic subject.

No	T4nmole/L	T3nmole/L	TSH mlU/L	Glu mg/dl	Hb%
101	95	1.7	1.2	92	92
102	115	2.4	1.5	69	103
103	91	1.9	.6	72	75
104	96	2.8	2.3	72	112
105	85	1.8	1.0	87	100
106	80	2.6	1.3	93	83
107	103	3.3	1.3	63	70
108	86	2.7	.6	75	84
109	118	2.3	.8	88	82
110	96	2.1	1.2	108	70
111	102	1.9	.9	101	82
112	118	2.2	1.2	81	77
113	102	2.7	1.2	91	95
114	106	2.4	1.3	81	52
115	98	2.2	1.3	86	92
116	87	2.1	.5	95	97
117	129	3.5	1.0	61	73
118	111	2.8	1.2	90	99
119	94	1.0	.5	89	78
120	58	1.2	2.9	120	81
121	100	2.1	2.3	117	83
122	100	2.5	1.9	87	65
123	102	1.9	1.9	115	70
124	57	1.4	1.4	94	56
125	88	2.0	1.7	70	59
126	83	2.1	3.2	95	57
127	77	2.3	1.6	77	83
128	88	2.8	3.0	73	79
129	84	2.0	2.5	89	83
130	135	2.7	1.4	84	90
131	101	1.1	1.6	82	102
132	76	2.2	.6	81	108
133	120	2.5	.7	77	109
134	100	1.9	1.0	89	62
135	113	2.0	1.2	71	102
136	104	2.2	.9	73	86
137	80	2.1	1.2	77	92
138	100	2.3	.9	81	69
139	92	2.0	1.9	87	70
140	102	2.1	1.3	84	68
141	107	1.8	2.0	95	72
142	105	1.5	1.0	85	74
143	110	.9	1.8	87	80
144	147	2.0	1.6	112	71
145	131	1.5	2.2	78	68
146	101	1.8	2.0	96	75
147	112	1.4	1.0	112	83
148	103	2.5	1.5	98	84
149	103	2.2	1.9	69	73
150	97	2.3	1.0	107	74

Appendix 4: Lipid profile (TC, HDL, TG, LDL) in diabetic patients

No	TC mg/dl	HDL mg/dl	TG mg/dl	LDL mg/dl
1	130	34	59	84
2	135	37	96	78
3	145	43	148	72
4	160	41	103	98
5	225	23	214	159
6	140	61	51	68
7	140	16	148	94
8	180	49	311	68
9	102	37	50	55
10	160	55	50	95
11	154	21	318	69
12	200	30	562	57
13	158	57	56	85
14	120	30	75	75
15	240	42	93	179
16	125	62	87	45
17	148	26	150	92
18	177	41	125	111
19	263	46	110	195
20	212	26	248	136
21	195	17	144	149
22	174	37	275	82
23	161	23	110	116
24	238	37	117	177
25	229	30	317	135
26	204	55	89	131
27	238	35	172	168
28	184	57	120	103
29	195	62	200	93
30	161	8	586	35
31	356	40	334	31
32	163	34	100	109
33	225	20	300	145
34	203	34	126	143
35	327	60	180	231
36	196	34	80	146
37	218	50	73	153
38	167	28	280	83
39	250	38	146	182
40	181	36	80	129
41	181	64	66	103
42	170	30	173	105
43	145	44	86	83
44	149	94	106	33
45	207	90	140	89
46	192	36	106	134
47	225	50	113	152
48	185	52	106	111
49	327	66	120	237
50	203	26	126	151

Appendix 5: Lipid profile (TC, HDL, TG, LDL) in diabetic patients

No	TC mg/dl	HDL mg/dl	TGmg/dl	LDLmg/dl
51	170	46	153	93
52	178	30	226	102
53	138	42	53	85
54	218	42	73	161
55	225	28	200	157
56	225	32	166	159
57	218	28	133	163
58	218	30	233	141
59	218	30	86	170
60	207	90	86	99
61	168	50	43	109
62	164	49	50	105
63	148	42	50	96
64	192	30	284	105
65	160	80	106	58
66	156	41	150	85
67	232	57	175	140
68	256	53	150	173
69	212	44	162	135
70	164	58	93	87
71	196	46	50	140
72	272	35	218	193
73	128	49	87	61
74	184	58	112	103
75	192	46	181	109
76	224	46	137	150
77	203	71	81	115
78	163	44	125	94
79	149	42	96	87
80	178	63	133	88
81	189	28	266	107
82	181	50	148	101
83	167	44	96	103
84	231	41	169	156
85	200	77	61	110
86	132	42	92	183
87	231	44	100	167
88	195	30	215	122
89	181	52	107	107
90	140	45	56	105
91	227	47	115	157
92	236	55	223	136
93	195	61	76	118
94	190	36	130	128
95	200	44	246	106
96	190	72	200	78
97	118	33	230	39
98	122	50	100	52
99	127	33	176	58
100	190	83	202	67

Appendix 6: Lipid profile (TC, HDL, TG, LDL) in non diabetic subject.

No	TC mg/dl	HDL mg/dl	TGmg/dl	LDLmg/dl
101	229	30	225	148
102	191	39	186	114
103	144	23	75	106
104	242	32	165	177
105	153	21	124	107
106	153	28	28	119
107	165	30	186	97
108	208	19	193	150
109	163	94	60	57
110	156	40	66	102
111	192	36	86	138
112	136	42	100	74
113	220	141	75	64
114	128	35	118	69
115	196	53	106	121
116	163	39	59	112
117	108	38	70	56
118	156	42	51	103
119	152	47	111	82
120	186	22	223	119
121	213	44	153	138
122	236	36	161	167
123	127	33	84	77
124	163	38	107	103
125	159	36	92	104
126	159	61	92	79
127	163	58	69	91
128	172	50	138	94
129	154	50	133	77
130	136	44	69	78
131	122	41	53	70
132	154	50	207	62
133	186	50	223	91
134	113	58	76	39
135	127	61	153	35
136	72	44	38	20
137	204	52	153	121
138	204	61	192	104
139	136	50	92	67
140	145	72	61	60
141	100	52	70	34
142	158	30	78	112
143	162	33	70	115
144	186	63	50	113
145	104	47	50	47
146	152	54	56	86
147	144	52	70	78
148	152	57	64	82
149	174	52	142	93
150	176	69	56	88

Appendix 7:

Sudan Academy of Sciences (SAS)
Atomic Energy council

Study Questionnaire

Evaluation of blood glucose, thyroid function among Sudanese diabetic patients.

Serial No:

Date:

Name:

Sex: **Age:** **Height:** **weight:**

Residence: **Social status:**

Disease history: ……………………………………………………

…………………………………………………………………

Type of diabetes: ……………………………………………………

Family History: ………………………………………………………

…………………………………………………………………

Treatment of Diabetes: ………………………………………………

…………………………………………………………………

Clinical investigation: ………………………………………………

…………………………………………………………………

Other diseases: ………………………………………………………

Appendix 8:Normal range of different measurements in human.

Measurement	Normal levels
Glucose (fasting)	Normal < 120 mg/dl Borderline :120-140 mg/dl Diabetic : above 140 mg/dl
T_4	50 – 150 nmol/L
T_3	0.8 – 3.0 nmol/L
TSH	0.4 – 4.0 mlU/L
Total cholesterol	< 200 mg/dL
Triglyceride	< 150 mg /dL
High – density Lipoprotein	> 55 (men) mg /dL
High – density lipoprotein	> 65 (women) mg/dL
Law – density lipoprotein	< 100 mg /dL
Hb	10.5- 14.9 g/dl