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Study on Spectrum of Liver Dysfunction in Type-2 Diabetic Patients in Bangladesh

Harun-Or-Rashid, Mohd.

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Study on Spectrum of Liver Dysfunction in Type-2 Diabetic Patients in Bangladesh



A Thesis Submitted to the
Institute of Biological Sciences
University of Rajshahi for the Degree of
Doctor of Philosophy

By

Mohd. Harun-Or-Rashid

Institute of Biological Sciences
University of Rajshahi
Rajshahi-6205, Bangladesh

January, 2016

**Dedicated
To
My Beloved Parents, Wife and
Daughters**

Declaration

I do hereby declare that the material embodied in this entitle “**Study on Spectrum of Liver Dysfunction in Type-2 Diabetic Patients in Bangladesh**” prepared for submission in the Institute of Biological Sciences, University of Rajshahi, Bangladesh for the degree of Doctor of Philosophy are original research works of mine and have not been previously submitted anywhere for the awards of any degree.

Mohd. Harun-Or-Rashid
MBBS, MCPS (Medicine)
MD (Hepatology)

Department of Zoology



University of Rajshahi
Rajshahi-6205
Bangladesh

Certificate

This is to certify that the materials included in this thesis are the original research work conducted by Mohd. Harun-Or-Rashid. The thesis contains no material previously published or written by another person except when due reference is made in the text of the thesis.

Professor Dr. M. Nazrul Islam

Supervisor

Department of Zoology

University of Rajshahi

Rajshahi-6205, Bangladesh

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ABBREVIATION

2HABF	2 Hours After Breakfast
AD	Anno Domini
ALP	Alkaline Phosphates
ALT	Alaline Amino Transferase
AST	Aspertate Amino Transferase
BMI	Body Mass Index
BIRDEM	Bangladesh Institute of Research & Rehabilitation in Diabetes, Endocrine and Metabolic Disorders
CDC	Centre for Disease Control
CHD	Chronic Heart Disease
CRP	C-Reactive Protein
CT	Computed Tomography
DKA	Diabetic Ketoacidosis
DM	Diabetes Mellitus
ELISA	Enzyme-linked immune Sorbent Assay
FBS	Fasting Blood Sugar
RBS	Random Blood Sugar
FFA	Free Fatty Acid
GGT	Gamma Glutamyl Transferase
gm/dl	Gram/Decilitre
GULT-4	Glucose transporter type 4
HbA1c	Glycosylated Haemoglobin
HBS	Hepatobiliary System
HDL	High Density Lipoprotein
IGT	Impaired Glucose Tolerance

IL-6	Interleukin-6
kg /m ²	Kilogram/Square Metre
LDL	Low Density Lipoprotein
LFTs	Liver Function Tests
mmHg	Milimetre of Mercury
mmol/L	Milimole/Litre
MRI	Magnetic Resonance Imaging
NAFLD	Non Alcoholic Fatty Liver Disease
NASH	Non Alcoholic Steatohepatitis
NIDDM	Non Insulin Dependant Diabetes Mellitus
OHAs	Oral Hypoglycaemic Agent
SD	Standard Deviation
SPSS	Statical Package for the Social Science
TNF- α	Tumour Necrosis Factor- α
TZDs	Thiazolidinedione
U/L	Unit/Litre
UK	United Kingdom
USA	United State of America
USG	Ultrasonography
WHO	World Health Organization

ABSTRACT

This study was carried out in Rajshahi Diabetic Association Hospital, Department of Medicine, Rajshahi Medical College Hospital and Department of Hepatology, Bangubandhu Sheikh Mujib Medical University, Dhaka, over last two years to evaluate the liver function tests among type-2 diabetic patients and to see the extent and severity of abnormalities of liver function tests.

We have enrolled 100 (one hundred) diagnosed type-2 diabetic patients and 100 apparently healthy people. All of those study population were free from taking any hepatotoxic drugs and free from any preexisting liver disease. This exclusion was done thorough clinical examination and relevant investigations.

In our study mean serum bilirubin (mg/dl), Alanine Aminotransferase (U/L), Aspartate Aminotransferase (U/L), S albumin (gm/dl) level and prothrombin time (sec) were 0.7372 ± 0.3118 , 39.00 ± 24.21 , 26.42 ± 10.40 , 4.10 ± 0.513 , 16.46 ± 2.78 respectively among type-2 diabetic patients, where as 0.5063 ± 0.1831 , 28.26 ± 6.67 , 18.90 ± 4.75 , 4.12 ± 0.277 , 14.23 ± 1.04 respectively among normal people. All these difference were statistically significant. ($P=<0.05$, <0.001 , <0.001 , <0.05 and <0.01 respectively).

Serum Alkaline phosphate level was 89.61 ± 25.59 in type-2 diabetic patients and 96.83 ± 16.34 which was statistically not significant ($P=>0.05$). The prevalence of abnormal serum bilirubin, Alanine Aminotransferase, Aspartate Aminotransferase, Alkaline phosphatase, prothrombin time and S. albumin were 5.17%, 31.03%, 5.17%, 5.17%, 43.10% and 10.34%

respectively in type-2 diabetic patients and 00%, 02%, 00%, 02%, 03% and 00% respectively in normal people.

All the LFTs were mildly abnormal except 06 patients had moderate elevation of Alanine Aminotransferase, 08 patients had moderately prolonged PT, and 02 patients had moderately decreased S. albumin of type-2 Diabetic patients. All Abnormalities of normal people were mild.

A high proportion of patients with type-2 diabetes mellitus had abnormal liver function tests.

CHAPTER ONE

INTRODUCTION AND REVIEW OF LITERATURE

Diabetes mellitus (DM) is a common metabolic disorder characterized by hyperglycaemia due to absolute or relative deficiency of insulin. The world wide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million case in 1985 to 177 million in 2000, based on current trends >360 million individuals will have diabetes by the year 2030.¹ Diabetes mellitus is a growing health problem that causes significant morbidity and mortality.²

Although the prevalence of both type-1 and type-2 DM is increasing world wide, the prevalence of type-2 DM is rising much more rapidly. Type-2 DM is principally a disease of the middle-aged and elderly, typical age of onset is >40 years. In individuals >60 years the prevalence of DM 20.9% and over 70% of all case of diabetes occur after the age of 50 years.³

Type-2 DM has a strong genetic component. The concordance of type-2 DM in identical twins is between 70-90%. Individuals with parents with type-2 DM have an increased risk of diabetes, if both parents have type-2 DM, the risk approaches 40%¹. Epidemiological studies provide evidence that type-2 DM is associated over eating, especially when combined with obesity and under activity. The risk of developing type-2 diabetes increase tenfold in people with a body mass index >30 kg /m².³

This global pandemic principally involved type-2 diabetes to which several factors contribute including greater longevity, obesity, unsatisfactory diet,

sedentary lifestyle and increasing urbanization. Many cases of type-2 diabetes remain undetected. However both types of diabetes vary considerably around the world and is related to differences in genetic and environmental factors. The prevalence of known diabetes in Britain is around 2-3% but is higher in the Middle and Fareast (e.g. 12% in the Indian subcontinent).⁴

The prevalence, is lowest in rural areas of developing countries, is generally intermediate in developed countries and is highest in certain ethnic groups that have adopted western lifestyle patterns. The populations with the highest prevalence (Pima Indians in Arizona and Nauruans in Micronesia) have a high prevalence of obesity. The prevalence of diabetes is up to 4-6 folds higher in immigrant South Asians in the UK compared with European Caucasians, and a high prevalence is also noted in urban-dwelling South Asians.⁵

The recent WHO report on diabetes prevalence alarmed that diabetes has posed a serious threat to developing countries in respect to their existing health care service.⁶ Diabetes, hyperinsulinemia and coronary risk factors are more prevalent in Bangladeshis compared with other South Asian groups (Indian, Pakistani) settled in United Kingdom⁷, and with native populations.^{8,9} It has also been reported that Bangladeshis among the entire South Asian immigrants had highest mortality and attack rate from coronary heart disease.¹⁰

In the UK, the Health survey for England estimated the prevalence of self reported diabetes to be about 4.3% in men and 3.4% in women, the prevalence increases sharply with age in both sexes.⁵

It is estimated that more than 3 million people are now suffering from type-2 diabetes in Bangladesh. Some 40% of people with diabetes in Bangladesh are not able to support themselves productively because of complications related to their diabetes.¹¹

A high proportion of patients with diabetes mellitus in catchment population have abnormal liver function tests that may be marker for Nonalcoholic Steatohepatitis (NASH) and insulin resistance. Currently, routine liver function screening is not being advocated in type-2 diabetics but emerging evidence suggests that abnormal LFT may be a marker for metabolic syndrome and insulin resistance in type-2 diabetes. Such patients would thus warrant more intensive metabolic control particularly of their hyperglycaemia and dyslipidaemia and also their obesity and hypertension not only reduce cardiovascular risks attributed by their insulin resistance but also to prevent progression to significant hepatic dysfunction like cirrhosis and hepato-cellular carcinoma.¹²

RATIONALE

Type-2 DM is a complex condition characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and abnormal fat metabolism.¹ Type-2 DM is often associated with other medical disorders, particularly central (visceral) obesity, hypertension and dyslipidemia producing metabolic syndrome.³

Individuals with type-2 diabetes have a higher incidence of abnormalities in liver function test than individuals who do not have diabetes. Mild chronic elevations of transaminases often reflect underlying insulin resistance.¹³ As a result of insulin resistance in adipose tissue and obesity, free fatty acid (FFA) flux from adipocytes is increased, leading to increased lipid synthesis in hepatocytes. This lipid storage or steatosis in the liver may lead to non alcoholic fatty liver disease and abnormal liver function tests.³

The excess in free fatty acids found in the insulin-resistant state is known to be directly toxic to hepatocytes. Putative mechanisms include cell membrane disruption at high concentration, mitochondrial dysfunction, toxin formation, and activation and inhibition of key steps in the regulation of metabolism. Other potential explanation for elevated transaminase in insulin-resistance states include oxidative stress from reactive lipid per-oxidation, peroxisomal beta-oxidation, and recruited inflammatory cells. The insulin-resistance state is also characterized by an increase in pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), which may also contribute to hepatocellular injury.¹³

In preliminary studies, an increased frequency of specific TNF- α -promoter polymorphism was found in NASH patients, suggesting a possible genetic link or predisposition to fatty liver found in Insulin-resistant states. The above theories all attribute elevated transaminases to direct hepatocyte injury. It is also

hypothesized that elevation in Alanine Aminotransferase, a gluconeogenic enzyme whose gene transcription is suppressed by insulin, could indicate impairment in insulin signaling rather than purely hepatocyte injury.¹³

In a study in the USA in 2005 was found that type-2 diabetes is associated with a large number of liver disorders including elevated liver enzymes, fatty liver disease, cirrhosis, hepatocellular carcinoma and acute liver failure.¹⁴

Studies show a close interrelationship between liver dysfunction and type-2 DM, which is frequently overlooked. Thus patients may benefit from its early diagnosis and proper management. Some work is done in Bangladesh, but no such study is carried out in Rajshahi Medical College Hospital, Hepatology Department, Bangabandhu Sheikh Mujib Medical University, Dhaka or Rajshahi Diabetic Association Hospital. So, awareness is to be increased to protect the liver in patients suffering from type-2 DM and Liver Function Tests (LFTs) should be a routine test in type-2 DM.

HYPOTHESIS

Liver function tests are abnormal in type-2 diabetes mellitus patients.

AIMS AND OBJECTIVES

General

- To observe the hepatic dysfunction in type-2 diabetes mellitus as evidenced by liver function tests.

Specific

- To find out frequency of liver function test abnormalities in type-2 diabetes mellitus.
- To find out the extent and severity of abnormalities of liver function tests in type-2 diabetes mellitus.

REVIEW OF LITERATURE

Diabetes mellitus is a syndrome with disordered metabolism and inappropriate hyperglycaemia due to either a deficiency of insulin secretion or to a combination of insulin resistance and inadequate insulin secretion to compensate. Type-1 diabetes is due to pancreatic β -cells destruction predominantly by autoimmune process and the type-2 diabetes, the more prevalent form, results from insulin resistance with a defect in compensatory insulin secretion.¹⁵

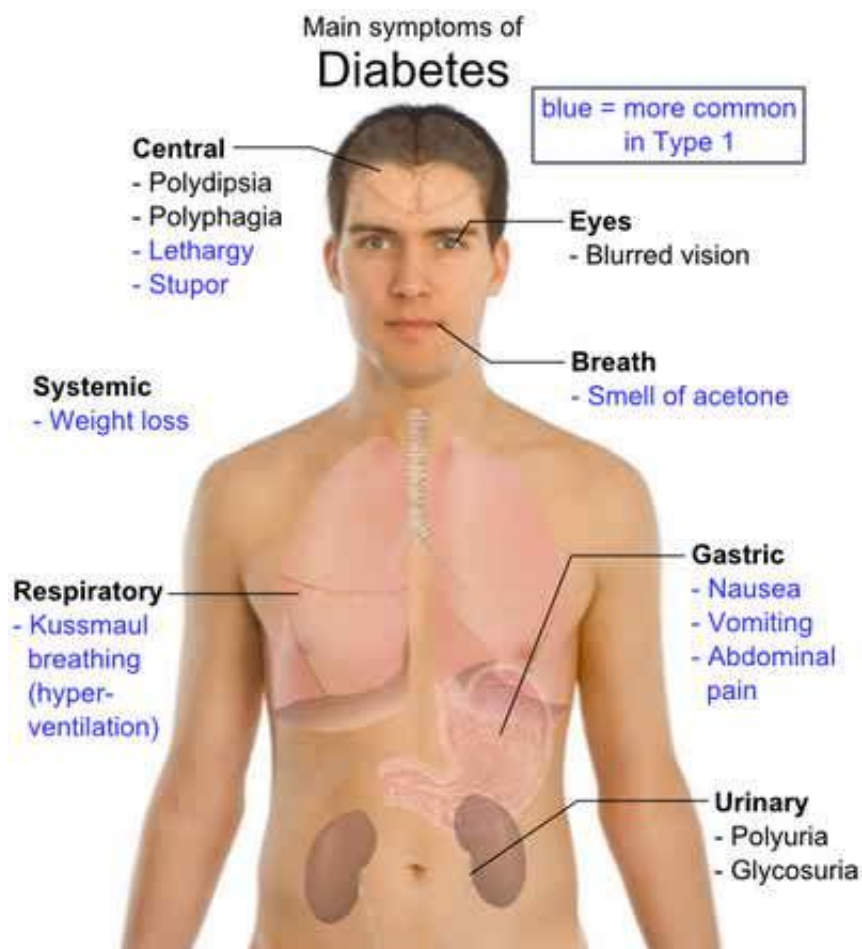


Fig 1.1 Main Symptoms of Diabetes

Historical Perspective of Diabetes Mellitus a Brief Review

Diabetes Mellitus is a disease, which was recognized in antiquity but its history has been characterized by numerous cycles of discovery, neglect and rediscovery.¹⁶ The term ‘diabetes’, which is Ionian Greek and means to ‘run through’ or a siphon; was first used by Aretaeus of Cappadocia in the 2nd century AD as a generic description for conditions causing increased urine output. Aretaeus wrote an accurate factual description of the condition that is instantly recognizable today and concluded that it was due to a fault in the kidney.¹⁶ The Roman physician, Galen (AD 131-201), like Aretaeus, thought diabetes to be rare disease and apparently encounter only two cases, employed alternative terms for diabetes, including diarrhea ruinous and dipsakos, the latter emphasizing the cardinal symptoms of excessive thirst and drinking.

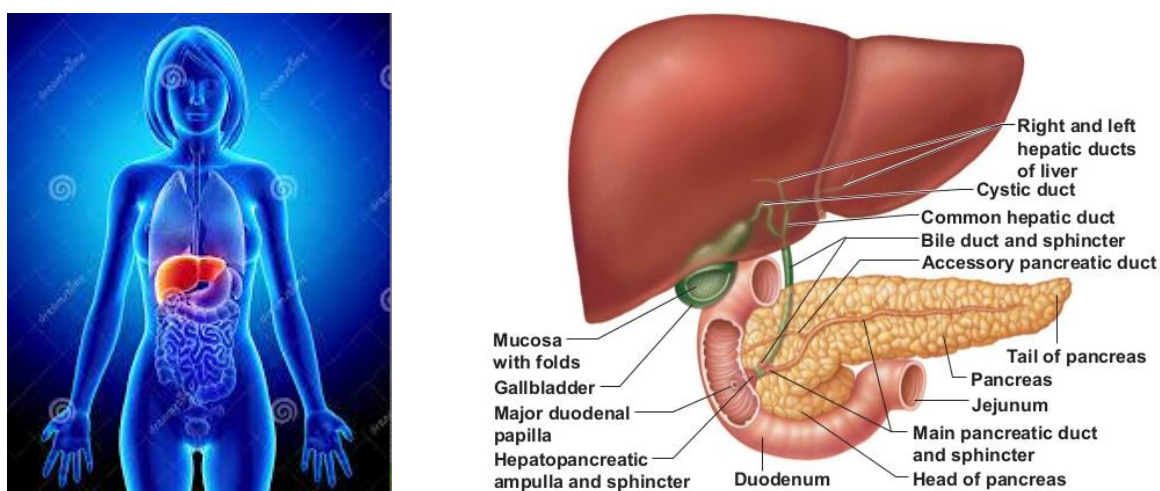


Fig 1.2 Anatomy of Liver and pancreas

Type-2 Diabetes Mellitus (DM) is a chronic, complex disorder which adversely affect both longevity and quality of life due to multiple, potential serious complications. Atherosclerosis is a primary cause of mortality, even within 1 year of diagnosis. It is common in middle aged and elderly individuals.¹⁷

Type-2 DM usually occurs after the age of 30 years. A strong genetic predisposition is evident. The pathogenesis is different from type-1 DM. Most individuals are obese/over-weight, having resistance to insulin action. Endogenous insulin production is usually adequate to avoid ketoacidosis, but DKA may occur with stress; exogenous insulin can be used to treat hyperglycaemia but is not required for survival.¹⁷

Type-2 diabetes has a more insidious onset than type-1 DM. Type-2 DM is often wrongly perceived as being a less serious disease, and its prognostic implications may be under-estimated. However, there is growing evidence that the pathophysiological features of type-2 DM are of profound importance in the initiation of a cluster of degenerative disease, including cardiovascular disorders.¹⁷

Epidemiology

High prevalence is seen in population who have changed from a traditional life style to a modern life style (e.g. American Indian, Pacific Islanders, Australian Aborigines and migrant Asian Indians) in whom the prevalence rate is up to 35% in adults.¹⁷ Although the prevalence of both type-1 and type-2 DM is increasing world wide, the prevalence of type-2 DM is rising much, more rapidly because of increasing obesity and reduced activity levels as countries become more industrialized. This is true in most countries, and 6 of the top 10 countries with the highest rate are in Asia. In the United States, the center for Disease control and prevention (CDC) estimated that 20.8 million person or 7% of the population had diabetes in 2005.¹

Increasing trend of diabetes prevalence throughout the world

The recent World Health Organization (WHO) report on diabetes prevalence alarmed that diabetes has posed a serious threat to entire population of the

world irrespective of stages of industrialization and development. The prevalence of diabetes for all age groups worldwide was estimated 2.8% in 2000 and to be 4.4% in 2030. The number of diabetic population was estimated to rise from 171 million in 2000 to 366 million in 2030. According to the recent report, the highest relative increase will occur in the Middle East, sub-Saharan Africa and India. The ten countries estimated to have the highest number of diabetic subjects in 2000 and in 2030.¹⁸

Table 1.1 List of countries with the highest numbers of estimated diabetic subjects for 2000-2030.

Ranking	2000		2030	
	Country	People with Diabetes (millions)	Country	People with Diabetes (millions)
1.	India	31.7	India	79.4
2.	China	20.8	China	42.3
3.	USA	17.7	USA	30.3
4.	Indonesia	8.4	Indonesia	21.3
5.	Japan	6.8	Japan	8.9
6.	Pakistan	5.2	Pakistan	13.9
7.	Russian Federation	4.6	Russian Federation	7.8
8.	Brazil	4.6	Brazil	11.3
9.	Italy	4.3	Italy	6.7
10.	Bangladesh	3.2	Bangladesh	11.1

The above information has taken from a journal published in American Diabetes Association.¹⁸

Increasing trend of diabetes prevalence in Bangladesh

In 2000, Bangladesh had 3.2 million people with diabetes and was listed at 10, which will occupy the 7th position with 11.1 million in 2030.¹⁸ Diabetes registry in the referral centers and diabetes survey at the community level

reflects the rapid increase of diabetes prevalence in the country. For example, only 389 diabetic subjects were registered at BIRDEM, a referral center, throughout the year 1960. This figure increased to 1181, 2363, 9641 and 15188 in the year 1970, 80, 90 and 2000, respectively. This increasing frequency of diabetes registry appears to be either increasing awareness of diabetes among people or real increase in diabetes prevalence in the community. Small diabetes surveys, at community level at different time point, proved an increasing prevalence of diabetes and impaired glucose tolerance.¹⁸ An increased prevalence of diabetes was found with 6.8% in the present survey in 2004 compared with 2.3% in the earlier survey in 1999.¹⁹ More than 10 million Bangladeshis will suffer from the disease in the year 2030. This is a conservative estimate because the trend of increasing prevalence will make this figure much higher. All these figures indicate that the magnitude of health problems related to diabetes in Bangladesh has been increasing rapidly.¹⁸

Subgroups of type-2 DM

On the basis of body weight, type-2 can be distributed into obese and non obese subtypes.¹⁷

Obese Type-2 DM

In the Western world up to 85% of type-2 patients are obese. These patients have insensitivity to endogenous insulin that is positively correlated with presence of an abdominal distribution of fat, producing an abnormally high waist-hip ratio. In addition distended adipocytes and over-nourished liver and muscle cells may also resist the deposition of glycogen and triglycerides in their storage depots. Hyperplasia of pancreatic β -cells is often present and probably accounts for the normal or exaggerated insulin responses to glucose and other stimuli seen in the milder forms of the disease. In more severe cases, secondary (but potentially reversible) failure of pancreatic β -cells secretion

may result after exposure to persistent hyperglycaemia. This phenomenon has been called desensitization. It is selective for glucose, and the β -cell recovers sensitivity to glucose stimulation once the sustained hyperglycaemia is corrected by any form of therapy, including diet, sulfonylureas and insulin.

A major cause of the observed resistance to insulin in target tissues of obese patients is believed to be a post receptor defect insulin action. This is associated with over-distended storage depots and a reduced ability to clear nutrients from the circulation after meals, consequent hyperinsulinism can further enhance insulin resistance by down regulation of insulin receptors. Furthermore, when hyperglycaemia becomes sustained, a specific glucose transporter protein in insulin target tissue also becomes down regulated after continuous activation. This contributes to further defects in post receptor insulin action. There by aggravating the hyperglycaemia. When overfeeding is corrected to the storage depots become less saturated and the cycle is interrupted. Insulin sensitivity improves and is further normalized by a reduction both the hyperinsulinism and the hyperglycaemia.¹⁷

Non-Obese Type-2 DM

Approximately 15% patients with type-2 are non-obese diabetics in western world. But in Bangladesh more than 70% patient are non-obese (as per present definition of obesity). In most of these patients, impaired insulin action at the post receptor level and an absence or delayed early phase of insulin release in response to glucose can be demonstrated.¹⁷

The hyperglycaemia in patients with non-obese type-2 often responds to dietary therapy or to oral hypoglycaemic agents (OHAs). Occasionally insulin therapy is required to achieve satisfactory glycaemic control even though it is not needed to prevent ketoacidosis.¹⁷

Diabetes Health Care Situation in Bangladesh

Diabetes Mellitus has become the significant threat to the developing countries in respect to their existing health care services.⁶ Diabetes, hyperinsulinemia and coronary risk factors are more prevalent in Bangladeshis compared with other South Asian migrants (Indian, Pakistani) settled in United Kingdom⁷ and with the native population^{8,9}. It has also been reported that Bangladeshis among the entire South Asian immigrants had highest mortality and attack rate from coronary heart disease (CHD).¹⁰

PATHOLOGY OF TYPE-2 DIABETES

Type-2 diabetes is a more complex condition than type-1 diabetes because there is a combination of resistance to the action of insulin in liver and muscle together with impaired pancreatic β -cell function leading to 'relative' insulin deficiency. Insulin resistance appears to come first, and leads to elevated insulin secretion in order to maintain normal blood glucose levels. However, in susceptible individuals the pancreatic β -cell are unable to sustain the increased demand for insulin and a slowly progressive insulin deficiency develops.²⁰

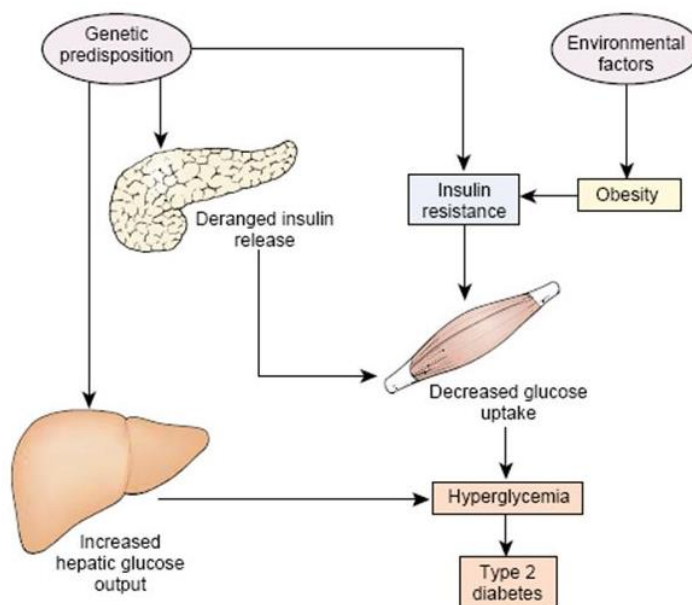


Fig 1.3 Pathogenesis of Type-2 DM

Insulin resistance in diabetes

In patient with type-2 diabetes excessive production of glucose in liver and under-utilization of glucose in skeletal muscle result from resistance to the action of insulin.²⁰

The precise molecular mechanism of insulin resistance is not clearly understood, but deficits in the post insulin receptor intracellular signaling pathways are believed to play a role.^{21, 22} Insulin resistance, which is usually present before the onset of diabetes, is determined by a number of factors, including genetics, age, obesity and later in the disease, hyperglycaemia itself. Excess visceral adiposity, dyslipidemia and hypertension often accompany insulin resistance. Other findings may include impaired fibrinolysis, increased platelet aggregation, vascular inflammation, endothelial dysfunction and premature atherosclerosis.²³ The inability to suppress hepatic glucose production is a major contributor to the fasting hyperglycaemia seen in diabetes.²⁴

The increase in lipolysis by adipose cells that are resistant to insulin and the subsequent increased levels of circulating free fatty acids also contribute to the pathogenesis of diabetes by impairing β -cells function, impairing glucose uptake in skeletal muscles and promoting glucose release from the liver. In addition to its role as a source of excess circulating free fatty acids, adipose tissue has emerged in the last decade as an endocrine organ. Adipose tissue is a source of a number of hormones (adipo-cytokines or “adipokines”) that appear to regulate insulin sensitivity (e.g., adiponectin, resistin), as well as appetite regulation (e.g., leptin), inflammation (e.g., tumor necrosis factor- α , interleukin-6) and coagulability (e.g., plasminogen activator inhibitor-1). Recent evidence suggests that the inflammatory cytokines are derived from infiltrating macrophages within adipose tissue beds rather than from the adipocytes themselves.²⁵

Type-2 diabetes often associated with ‘Insulin resistance syndrome’ or ‘metabolic syndrome’, characterized by the presence of the following criteria.²⁶

- Increased BMI
- Glucose intolerance
- Hyperlipidemia
- Hypertension

It has been shown that both hyperinsulinemia and insulin resistance predict the development of Noninsulin Dependent Diabetes Mellitus (NIDDM), insulin resistance is a more specific risk factor, since insulin concentration reflects both insulin resistance and secretion.²⁷ Inflammation is also associated with insulin resistance and prospective studies indicate that increased inflammation at base line is an independent risk factor for future development of type-2 DM.²⁸

The molecular basis for the link between inflammation and diabetes likely relates to the action of cytokines such as interleukin-6 (IL-6) and tumour necrosis factors TNF (α) which induce insulin resistance and stimulate acute phase inflammatory response.²⁹

Pancreatic β -cell failure

Islet β cells compensation for insulin resistance is sustained provided β cells are robust, resulting in long-term maintenance of normoglycaemia. Compensation processes, however, fail if there are genetic or acquired factors that result in susceptible β cells. The defect(s) create weak link(s) in the compensation process that promote β cell dysfunction by mechanisms with initiator roles that result in IGT and early type-2 diabetes. Hyperglycaemia, once established, promotes a further series of mechanisms, under the umbrella of glucotoxicity, that cause severe β cell failure and overt and late type-2 diabetes.³⁰

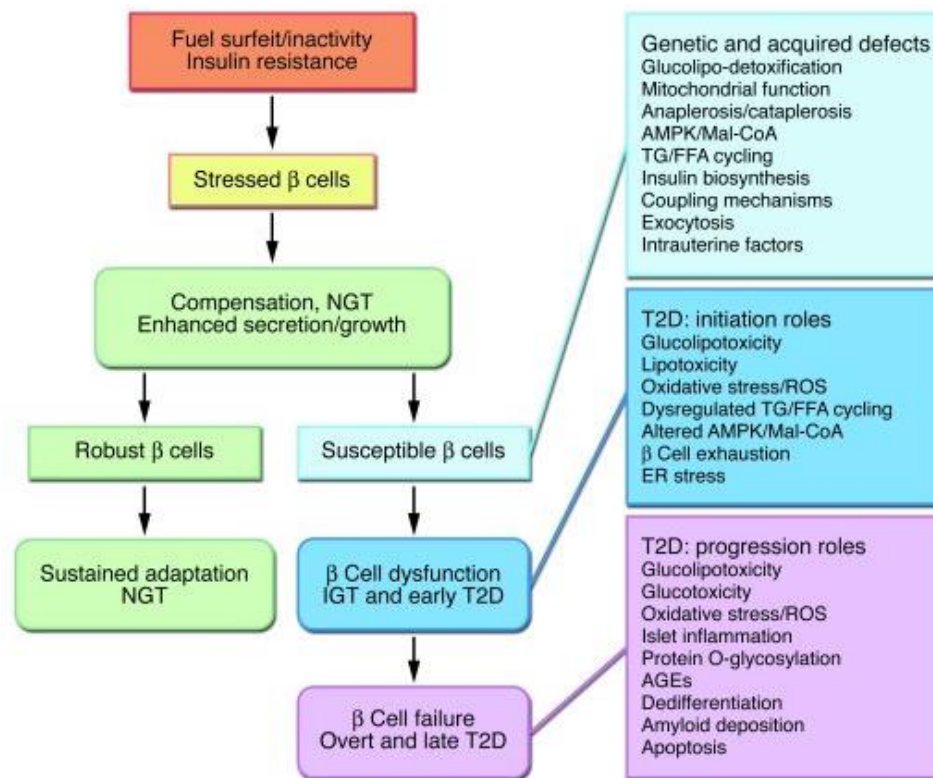


Fig 1.4 Mechanism of β -cell failure

Source: Clinical Therapeutics, 2003

The cause of progressive pancreatic β -cell failure is not completely understood but it appears to result from a number of factors, including genetic determinants, chronic inflammation, glucotoxicity and the deleterious effects of elevated levels of free-fatty acid on β -cells function, so called lipotoxicity.^{31, 32}

Environmental factors

Epidemiological studies provide evidence that type-2 diabetes is associated with overeating, especially when combined with obesity and under activity. Obesity probably acts as a diabetogenic factor only in those who are genetically predisposed both to insulin resistance and β -cell failure. Sweet foods rich in refined carbohydrate consumed frequently may increase the demand for insulin secretion, while high-fat foods may increase FFAs and exacerbate insulin resistance.²

MANAGEMENT OF TYPE-2 DIABETES

Diet

Diet therapy, although important for the prevention and the treatment of all stages of type-2 diabetes, continues to remain poorly understood and high controversial when obesity coexists with hyperglycaemia, as seen in the majority of individuals with type-2 diabetes, weight reduction is the major goal of dietary therapy. Traditional recommendations emphasize reduction of both the total and saturated fat content and replacement with complex carbohydrates to 50-55% of the dietary calories. In type-2 diabetic patients, such diets may cause marked postprandial hyperglycaemia. As there is considerable patient variability in the rate of glucose absorption, arduous attention to postprandial glucose monitoring and the addition of high fiber contents to the diet become critically important. Moreover, as the glycaemic response of the diet is also dependent upon the texture and content of other food stuffs in the diet as well as the rate of intestinal motility, the diet as well as the stage and duration of type-2 diabetes have to be considered on an individual basis.³³



Fig 1.5 Diabetic diet

Exercise

Exercise has been shown to be beneficial in the prevention of the onset of type-2 diabetes mellitus as well as in the improvement of glucose control as a result of enhanced insulin sensitivity (Decreased intra abdominal fat, an increase in insulin-sensitive glucose transporters (GLUT-4) in muscle, enhanced blood flow to insulin-sensitive tissues, and reduced free fatty acid levels appear to be the mechanisms by which exercise restores insulin sensitivity. In addition, exercise provides the added benefits of lowering blood pressure, improving myocardial performance, and lowering serum triglycerides while raising high density lipoprotein cholesterol levels.³³



Fig 1.6 Exercise

Drugs

Current therapeutic agents available for type-2 diabetes mellitus include sulfonylureas and related compounds, biguanides, thiazolidenediones, α -glucosidase inhibitors and insulin. In addition, several other classes of therapeutic agents will soon become available. A rational approach would be to begin with the agents particularly suited to the stage and nature of the disease, progressing, if necessary, to combination therapy. Pharmacological agents acting through different mechanisms of action should be chosen to improve glucose values while minimizing adverse effects.³³

COMPLICATIONS OF TYPE-2 DIABETES

If type-2 diabetes isn't well controlled, there are a number of serious or life-threatening complications you may experience, including.³⁴

Retinopathy

People with type-2 diabetes may already have abnormalities in the eyes related to the development of diabetes. Over time more and more people who initially do not have eye problems related to the disease will develop some form of eye problem. It is important to control not only sugars but blood pressure and cholesterol to prevent progression of eye disease. Fortunately, the vision loss isn't significant in most.³⁴

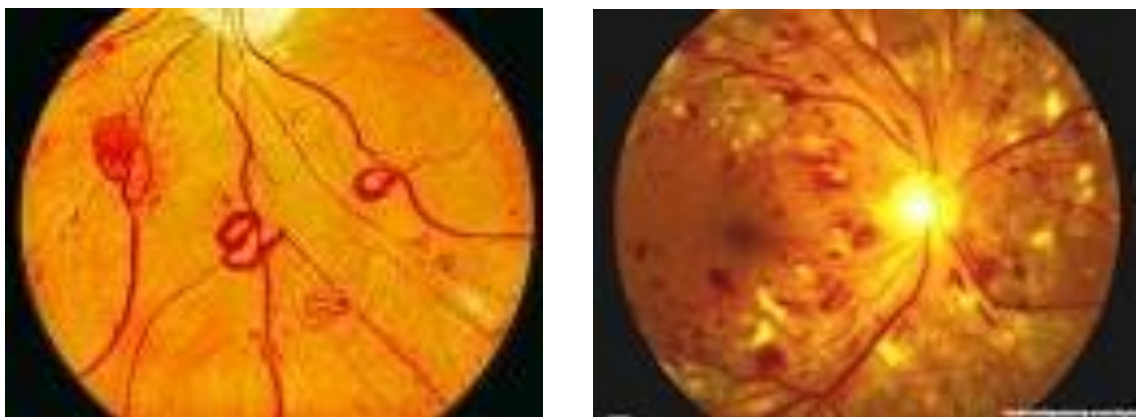


Fig 1.7 Diabetic retinopathy

Kidney Damage

The risk of kidney disease increases over time, meaning the longer you have diabetes the greater your risk. This complication carries significant risk of serious illness - such as kidney failure and heart disease.³⁴

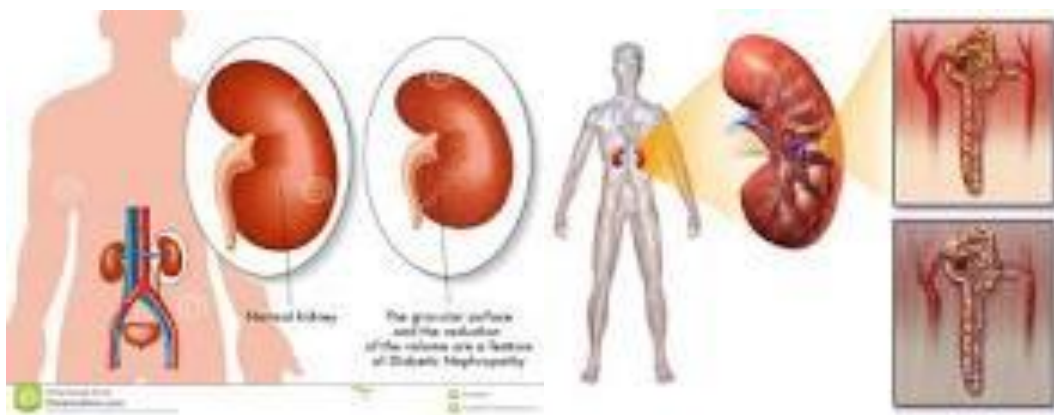


Fig 1.8 Diabetic nephropathy

Poor blood circulation and nerve damage

Damage to nerves and hardening of the arteries leads to decreased sensation and poor blood circulation in the feet. This can lead to increased infections and an increased risk of ulcers which heal poorly and can in turn significantly raises the risk of amputation. Damage to nerves may also lead to digestive problems, such as nausea, vomiting, and diarrhea.³⁴



Fig 1.9 Foot gangrene

Strategies to control the complications of type-2 diabetes

Different treatment strategies may be needed for different types of diabetic complications. Microvascular complications of type-2 diabetes are generally considered to be diabetic renal disease, retinopathy and neuropathy, because glucose is so strongly related to these diabetic complications. To prevent these complications, it is not really necessary to prevent type-2 diabetes, but merely to screen aggressively for type-2 diabetes and improve metabolic control markedly in diagnosed patients. On the other hand, for macrovascular complications of type-2 diabetes, glucose is not as strongly related; therefore, a broad-based set of interventions is needed. Improved glycaemic control may be important, although the relation between glycaemic control and macrovascular complications appears to be fairly modest. Other strategies to reduce macrovascular complications include prevention of type-2 diabetes and aggressive treatment of established cardiovascular risk factors in diabetic subjects; in addition, some people believe that therapy should be intensified in pre diabetic subjects as well. Lastly, prevention of macrovascular complications may include the use of diabetic agents that may preferentially improve cardiovascular risk factors, as some of the thiazolidinediones (TZDs) are thought to do.^{3,36} Regarding the management of diabetes mellitus, the goals of the therapy for type-1 or type-2 DM are to -

- a) Eliminate symptoms related to hyperglycaemia
- b) Reduce or eliminate the long-term microvascular and macrovascular complications of DM and
- c) Allow the patient to achieve normal life style as possible.

To reach these goals though glycaemic control is central to optimal diabetes therapy, comprehensive diabetes care of both type-1 and type-2 DM should also detect and manage diabetes mellitus specific complications and modify risk factors for diabetes mellitus associated disease.³⁷

Treatment goal

High Sensitivity C-reactive protein (CRP) value <1 mg/L

Glycaemic control – HbA1c <7%

Blood pressure <130/80 mmHg

Body mass index (BMI) 20 to 25 kg/m²

Waist/hip circumference ratio <1 in men and <0.9 in women.

Lipid profile (Fasting)

LDL <100 gm/dl (<2.6 mmol/L)

Triglycerides <150 mg/dl (<1.7 mmol/L)

HDL > 40 mg/dl (>1.1 mmol/L)

The Role of the Liver in Glucose Homeostasis

The liver plays a central and crucial role in the regulation of carbohydrate metabolism. Its normal functioning is essential for the maintenance of blood glucose levels and of a continued supply to organs that require a glucose energy source. This central role for the liver in glucose homeostasis offers a clue to the pathogenesis of glucose intolerance in liver diseases but little insight into the mechanisms of liver disease in diabetes mellitus. This review will draw on sources in the literature that address both pathogenetic directions.

An appreciation of the role of the liver in the regulation of carbohydrate homeostasis is essential to understanding the many physical and biochemical alterations that occur in the liver in the presence of diabetes and to understanding how liver disease may affect glucose metabolism. The liver uses glucose as a fuel and also has the ability to store it as glycogen and synthesize it from non carbohydrate precursors (gluconeogenesis). Mann and Magath demonstrated that a total hepatectomy in a dog results in death

within a few hours from hypoglycaemic shock,^{48,49} underscoring the important role the liver plays in maintaining normoglycaemia.³⁸

Liver diseases occurring as a consequence of diabetes mellitus-

- a) Glycogen deposition
- b) Steatosis and nonalcoholic steatohepatitis (NASH)
- c) Fibrosis and cirrhosis
- d) Hepatocellular carcinoma
- e) Acute liver failure
- f) Viral Hepatitis

Glycogen deposition

Excess glycogen accumulation in the liver is seen in 80% of diabetic patients. Glycogen synthesis in the liver is impaired in diabetes due to defective activation of glycogen synthase. However, studies attesting to this were usually performed on animals with recently induced diabetes. In patients with chronic diabetes, glycogen accumulation is seen, and it is postulated that long-standing insulin deficiency may actually facilitate synthase activity. This and enhanced gluconeogenesis may account for the net accumulation of glycogen in diabetes.

The mechanism of cytoplasmic glycogen deposition is uncertain but is perhaps related to the large variations in glucose concentration and frequent insulin dosing. No correlation between hepatic glycogen content and fasting blood glucose levels has been demonstrated. There is also no demonstrable association between the type of diabetes or the fat content of the hepatocytes and the presence of glycogen.³⁸



Fig 1.10 Glycogen deposition

Fatty Liver, Steatohepatitis

Hepatic fat accumulation is a well-recognized complication of diabetes with a reported frequency of 40–70%. Unfortunately, associated obesity is a frequently occurring confounding variable. Type-1 diabetes is not associated with fat accumulation if glycaemia is well controlled, but type-2 diabetes may have a 70% correlation regardless of blood glucose control.³⁸

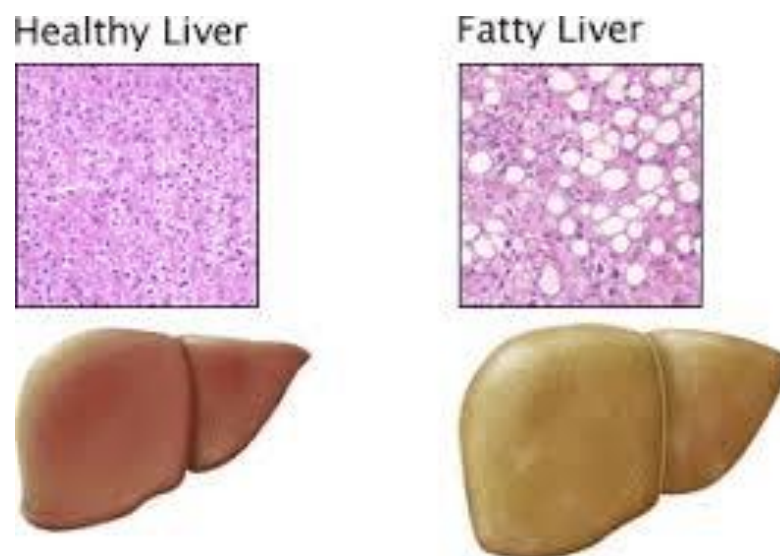


Fig 1.11 Normal and fatty liver

Cirrhosis

There is an increased incidence of cirrhosis in diabetic patients and conversely at least 80% of patients with cirrhosis have glucose intolerance.^{46,47} The reported prevalence of cirrhosis in diabetes varies widely. Diabetes increases the risk of steatohepatitis, which can be developed to chronic liver disease. Obesity is a significant confounding variable in determining the prevalence of cirrhosis in diabetes. Even with normal glucose tolerance, obesity can cause steatohepatitis and cirrhosis. Likewise, the lack of a clear understanding of diabetes to chronic liver disease in the past somewhat confounds these statistics.³⁸

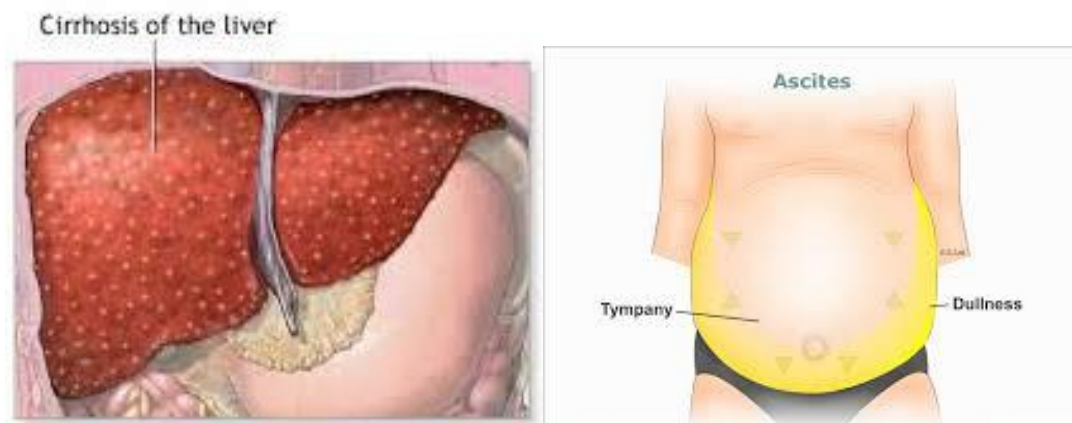


Fig 1.12 Cirrhotic liver and Ascites

Biliary Disease, Cholelithiasis, Cholecystitis

There is a reported increased incidence of cholelithiasis in diabetes mellitus, but obesity and hyperlipidemia may again be confounding variables. Several articles have reported a two- to threefold increased incidence of gallstones in diabetic patients, whereas others have failed to demonstrate a significant association.⁵⁰⁻⁵⁵

Gallbladder emptying abnormalities found in diabetic patients may predispose patients to cholelithiasis.⁵⁶ Secretion of lithogenic bile by the

liver in patients with type-2 diabetes probably predisposes them to forming gallstones, but this is likely a result of concomitant obesity rather than a result of the diabetes itself.⁵⁷ Increased biliary cholesterol saturation has not been demonstrated in insulin-dependent diabetic patients.

There is no indication in the literature that the natural history of gallstones is different in diabetic and nondiabetic individuals. The relative risk of mortality following acute cholecystitis is not significantly greater in diabetic patients than in the general population, and neither is the risk for serious complications. For that reason, prophylactic cholecystectomy cannot routinely be recommended for asymptomatic gallstones in patients with diabetes.⁵⁸ Any increase in mortality may be attributed to underlying renal or vascular disease. Patients with diabetes have comparable survival outcomes from laparoscopic or open cholecystectomy.⁵⁹

Abnormal liver enzymes

Elevation of serum alanine aminotransferase (ALT), while uncommon (0.5%) in apparently normal subjects, is common in patients with type-2 diabetes.³⁹

Hepatocellular carcinoma in diabetes

Numerous studies have confirmed a fourfold increased prevalence of hepatocellular carcinoma in patients with diabetes as well as an increased prevalence of diabetes in patients with hepatocellular carcinoma. It is not known whether the increased prevalence of hepatocellular carcinoma is unique to diabetes or the increased prevalence of cirrhosis, the precursor lesion of hepatocellular carcinoma. The pathogenic sequence of events leading to hepatocellular carcinoma appears to be insulin resistance, increased lipolysis, lipid accumulation in the hepatocytes, oxidative stress, and cell damage followed by fibrosis and cell proliferation, which are procarcinogenic.³⁹



Fig 1.13 Hepatocellular Carcinoma

Acute liver failure

The incidence of acute liver failure appears to be increased in patients with diabetes: It remains unclear whether it is diabetes, medications or some other factors that account for the increased risk of acute liver failure. Troglitazone was factored out in these studies.³⁹

Viral Hepatitis

There is no evidence in the literature that viral hepatitis has a worse prognosis in patients with diabetes. There is an increased prevalence of viral hepatitis in diabetes possibly due to an increased exposure to needles for the injection of insulin or for blood testing. Possible contamination of the platform in spring-loaded lancet devices may increase the risk of acquiring hepatitis B or C from these instruments. In 1996, hepatitis B outbreaks were noted in an Ohio nursing home and a New York hospital. Transmission was thought to be related to the use of spring-loaded devices for fingerstick glucose testing.^{60,61}

Diabetes is far more prevalent in patients with hepatitis C than in patients with other forms of viral hepatitis. In a study by Grimbirt and associates, 152 patients with hepatitis C and the same number with either hepatitis B or alcohol-induced liver disease were compared over the same period. Twenty-four percent of the patients with hepatitis C had diabetes compared with only 9% of the controls. The authors suggested a causative role of hepatitis C in the pathogenesis of diabetes.⁶²

Fraser and associates also found an association between chronic hepatitis C and the presence of impaired glucose control and reported that the prevalence of diabetes was much higher in hepatitis C than in the general population.⁶³ One hundred adults with cirrhosis were evaluated in a retrospective study. Of the 34 patients with hepatitis C, 50% had diabetes mellitus, as opposed to 9% of the 66 patients with cirrhosis unrelated to hepatitis C. The association has been described also by others and was thought to be statistically significant.⁶⁴⁻⁶⁶

Simo and associates also suggested that the hepatitis C virus may have a direct causative role in the development of diabetes. Most of their diabetic patients with hepatitis C had abnormal liver tests.⁶⁷

The association of diabetes with hepatitis C has also been investigated in posttransplantation patients, and there is a reported higher incidence of diabetes in liver transplant recipients with hepatitis C. This increased incidence appears to be significant, and the presence of the virus appears to be an independent risk factor.⁶⁸

Interferon therapy used to treat hepatitis B and C may induce hyperglycaemia, result in the development of type-2 diabetes, and necessitate increased insulin requirements in patients with type-1 diabetes.⁶⁹⁻⁷² Interferon therapy has resulted in the development of type-1 diabetes likely through the development of insulin autoantibodies.⁷³⁻⁷⁵ Fattovich and associates retrospectively studied 11,241 patients with chronic viral hepatitis who had undergone interferon therapy. However, only 10 patients developed de novo diabetes mellitus.⁷⁶ Interferon therapy also reportedly led to severe hypertriglyceridemia in a diabetic patient.⁷⁷

The hepatitis B vaccine effectively induces protective antibodies in most patients with diabetes.^{78,79} One study in children with type-1 diabetes concluded that children may not respond as well to the vaccination. This suggested that children should perhaps be vaccinated with four injections instead of three.⁸⁰

Liver function tests

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, bilirubin, albumin, and prothrombin time. Aminotransferases, such as Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST), measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. Alkaline phosphatase (AP), γ -glutamyl transpeptidase (GGT), and bilirubin act as markers of biliary function and cholestasis. Albumin and prothrombin time reflect liver synthetic function.¹³

LIVER FUNCTION TESTS INTERPRETATION

Severe, Moderate and Mild Elevation of Aminotransferases-

1. Severe (>20 times, 1000 U/L) : The Aspartate Aminotransferase and Alanine Aminotransferase levels are increased to some extent in almost all liver diseases. The highest elevations occur in severe viral hepatitis, drug or toxin induced hepatic necrosis and circulatory shock. Although enzyme levels may reflect the extent of hepatocellular necrosis they do not correlate with eventual outcome. In fact declining Aspartate Aminotransferase and Alanine Aminotransferase may indicate either recovery of poor prognosis in fulminant hepatic failure.⁴⁰

2. Moderate (3-20 times): The Aspartate Aminotransferase and Alanine Aminotransferase are moderately elevated in acute hepatitis, neonatal hepatitis, chronic hepatitis, autoimmune hepatitis, drug induced hepatitis, alcoholic hepatitis and acute biliary tract obstructions. The Alanine Aminotransferase is usually more frequently increased as compared to Aspartate Aminotransferase except in chronic liver disease. In uncomplicated acute viral hepatitis, the very high initial levels approach normal levels within 5 weeks of onset of illness and normal levels are obtained in 8 weeks in 75% of cases. For reasons, which are not, understood Aspartate Aminotransferase levels appear disproportionately low in patients with Wilson's disease.⁴⁰

3. Mild (1-3 times): These elevations are usually seen in sepsis induced neonatal hepatitis, extrahepatic biliary atresia (EHBA), fatty liver, cirrhosis, non alcoholic steatohepatitis (NASH), drug toxicity, myositis, duchenne muscular dystrophy and even after vigorous exercise. One third to one half

of healthy individuals with an isolated elevation of Alanine Aminotransferase on repeated testing have been found to be normal.⁴⁰

Prothrombin time

The results of this test may be expressed in sec or as a ratio of the plasma prothrombin time to control plasma time. Normal control usually is in the range of 9-11 seconds. A prolongation of more than 4 seconds is considered abnormal.⁴⁰ Prolongation >5 sec above control considered as marked elevation.⁴¹

CHAPTER TWO

MATERIALS AND METHODS

Type of study

Descriptive cross-sectional comparative study

Place of study

The study has been carried out in Rajshahi Medical College Hospital, Rajshahi Diabetic Association Hospital and Bangabandhu Sheikh Mujib Medical University, Dhaka.

Sample size

Sample size consists of consecutive 100 patients having type-2 Diabetes Mellitus with inclusion and exclusion criteria and 100 apparently healthy people.

Sample size is estimated by Formula-

$$n = \frac{Z^2 pq}{d^2}$$

Z = 1.96, at 95% confidence level

p = 6.8% = .068 (prevalence of type-2 DM in Bangladesh⁶)

q = 1-p = 0.932, d = 0.05 (5% acceptable error)

Duration of study: July 2012 to June 2014.

Inclusion criteria

- Age > 40 yrs
- Both sex
- Patients who fulfill the WHO criteria of type-2 Diabetes Mellitus⁸¹

WHO criteria of type-2 Diabetes Mellitus

*Fasting plasma glucose (FPG) \geq 126 mg/dl (7.0 mmol/l) OR
Symptoms (such as polyuria, polydipsia, unexplained weight loss) AND
a random plasma glucose \geq 200 mg/dl (11.1 mmol/l) OR
Plasma glucose \geq 200 mg/dl (11.1 mmol/l) 2 hours after a 75g glucose
load OR A1C \geq 6.5%*

Exclusion criteria

- Alcohol consumption
- Hepatotoxic drugs like – acetaminophen, NSAIDS, methotrexate, Amiodarone, Bleomycin, tamoxifen, sodium valproate, metformin, pioglitazone.
- Patients who are taking Insulin.
- Known other acute or chronic liver diseases.

Following investigations have been done in all patients

1. Blood sugar - fasting and postprandial with corresponding urine sugar
2. HbA_{1c}
3. Serum bilirubin
4. Serum alanine aminotransferase
5. Serum aspartate aminotransferase
6. Serum alkaline phosphatase
7. Prothrombin time
8. Serum albumin level
9. HBsAg (ELISA)
10. Anti HCV (ELISA)
11. Lipid profile
12. Ultrasonography of Hepato-biliary system

Sample procedure: Purposive sampling method.

Sample collection

The patients who have fulfilled both inclusion and exclusion criteria, have been enrolled in this study. Investigator himself collected the sample.

Procedure/data collection

This study have been carried out on 100 consecutive type-2 DM patients who have meet both inclusion and exclusion criteria and 100 apparently healthy people. Data have been collected by a prescribed data collection sheet through personal interview.

Descriptive data

Data of the patients have been compared and correlated to see the liver function tests abnormalities of the study patients.

Statistical analysis

Data have been analyzed with the help of SPSS software program and expressed as Mean \pm SD. P-value <0.05 have been considered significant.

Ethical Issue

Eligibility of each case has been assessed and identified and every patient and or responsible family member was asked for informed consent. They have been informed about the procedure and study goal. The eligible patient or family member has been informed that there is no extra cost to the patients for the investigations. They have also been informed that they are free to refuse to participate or to withdraw at any time without compromising their medical care. Patients name and age have been recorded initially in order to link their clinical and laboratory data. At enrollment they have been assigned a unique study identification number (ID), which used to level all study materials. Completed data collection forms and information linking patient personal identifiers to questionnaires have been kept by the principal investigator on a regular basis to which no one has got any access. The procedures in the protocol present no risk to participants.

CHAPTER THREE

RESULTS AND OBSERVATION

This study was intended to assess the abnormalities of liver function test findings and their extent and severity.

A total 200 persons were included in this study, 100 were type-2 diabetic patient considered as case group, and rest 100 apparently normal healthy people (without diabetes) were considered as control group. Both case and control group were free from any known liver diseases, exclude by clinical examination and investigations like viral markers and USG. They all were free from taking any kind of hepatotoxic drugs even oral hypoglycaemic drugs like Metformin, Pioglitazone, Rosiglitazone etc.

Liver function tests result among the Type-2 diabetic patients and normal people

Serum bilirubin (mg/dl) level of the type-2 diabetic patients and normal people were 0.7372 and 0.5063 respectively and standard deviation were ± 0.3218 and ± 0.1831 respectively. This difference in the serum bilirubin level are significant ($p < 0.05$).

Serum Alanine Aminotransferase (U/L) level of the type-2 diabetic patients and normal people were 39.00 and 28.26 respectively and standard deviation were ± 24.21 and ± 6.67 respectively. This difference are highly significant ($P < 0.001$).

Serum Aspartate Aminotransferase (U/L) level of the type-2 diabetic patients and normal people were 26.42 and 18.90 respectively and standard

deviations were ± 10.40 and ± 4.75 . This difference are highly significant ($P < 0.001$).

Serum Alkaline phosphatase (U/L) level of the type-2 diabetic patients and normal people were 89.61 and 96.83 respectively and standard deviation were ± 25.59 and ± 16.36 . These differences are not significant ($P > 0.05$).

Prothrombin time (second) of the type-2 diabetic patients and normal people were 16.46 and 14.23 respectively and standard deviations were ± 2.78 and ± 1.04 respectively. This differences are very significant ($P < 0.01$).

Serum albumin (g/dl) level of the type-2 diabetic patients and normal people were 4.10 and 4.12 respectively and standard deviations were ± 0.51 and ± 0.27 respectively. These difference are significant ($P < 0.05$) (Table 3.1).

Table 3.1 Liver function tests results of type-2 DM patients and control group

Liver function tests	Type-2 diabetic patient (n=100) (mean \pm SD)	Control group (n=100) (mean \pm SD)	P-value
Serum bilirubin	0.7372 \pm 0.3118	0.5063 \pm 0.1831	<0.05
ALT	39.00 \pm 24.21	28.26 \pm 6.67	<0.001
AST	26.42 \pm 10.40	18.90 \pm 4.75	<0.001
Serum Alkaline phosphatase	89.61 \pm 25.59	96.83 \pm 16.34	>0.05
Prothrombin time	16.46 \pm 2.78	14.23 \pm 1.04	<0.01
S.Albumin	4.10 \pm 0.513	4.12 \pm 0.277	<0.05

- Data are presented as mean and \pm SD
- n=Number of subject
- SD=Standard deviation

Data were analysed using the T-test and p-value are <0.05 for S.bilirubin, Alanine Aminotransferase, Aspartate Aminotransferase, PT, S.Albumin and >0.05 for SAP.

Frequency of LFTs abnormalities among type-2 diabetic patients and normal people

Among 100 type-2 diabetic patients, 58 had abnormal LFTs, of them 03 (5.17%) had raised S. bilirubin, 18 (31.03%) had raised Alanine Aminotransferase, 03 (5.17%) had raised Aspartate Aminotransferase, 03 (5.17%) had raised S. Alkaline phosphatase, 25 (43.10%) had prolonged Prothrombin time and 06 (10.34%) had decreased S. albumin level. On the other hand among healthy people none had any S. bilirubin, Aspartate Aminotransferase and Serum albumin abnormalities and 02 persons (02%) had raised Alanine Aminotransferase, 02 persons (02%) had raised Serum alkaline phosphatase, 03 persons (03%) had prolonged Prothrombin time (Table 3.2 and Appendix Figure I).

Table 3.2 Frequency of LFTs abnormalities among type-2 diabetic patients and control (n=100)

Liver function tests	Type-2 diabetic patients		Control groups	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Serum bilirubin	03	5.17	00	00
ALT	18	31.03	02	02
AST	03	5.17	00	00
Serum Alkaline phosphatase	03	5.17	02	02
Prothrombin time	25	43.10	03	03
S.Albumin	06	10.34	00	00

Severity of abnormal LFTs in type-2 diabetic patients

Three patients had abnormal Serum bilirubin, all were mildly elevated. Eighteen patients had abnormal ALT, among them 12 are mildly elevated and 06 are moderately elevated. Three patients had abnormal AST and 3 had abnormal Serum alkaline phosphatase, all had mild elevation.

Twenty five patients had prolonged Prothrombin time, of theme 17 were mildly prolonged and 08 were moderately prolonged. 06 patients had decreased Serum albumin, 04 were mildly decreased and 02 were moderately decreased (Table 3.3).

Table 3.3 Severity of liver function tests abnormalities in type-2 diabetic patients (n=58)

Liver function tests	Total abnormalities	Mild elevation/ decrease/ prolongation	Moderate elevation/ decrease/ prolongation	Severe elevation/ decrease/ prolongation
Serum bilirubin*	03	03	0	0
ALT*	18	12	06	0
AST*	03	03	0	0
Serum Alkaline phosphatase*	03	03	0	0
Prothrombin time**	25	17	08	0
S.Albumin***	06	04	02	0

*= Elevation, **=Prolongation, ***=Decrease

Glycaemic status of the study population

Mean Fasting blood sugar (mmol/L), blood sugar 2 HABF (mmol/L) and HbA_{1c} (%) level in type-2 diabetic patient were 7.33, 10.88, 7.79 respectively with SD ± 2.85 , ± 3.03 and ± 1.68 respectively. Mean fasting blood sugar (mmol/L), blood sugar 2HABF (mmol/L) and HbA_{1c} (%) level in normal people were 5.21, 8.78, and 5.69 respectively with SD ± 0.527 , ± 0.883 , ± 0.360 respectively (Table 3.4).

These differences in the plasma level of FBS, blood sugar 2HABF and HbA_{1c} between type-2 diabetic patient and normal people are significant ($P < 0.007$, $P < 0.014$ and $P < 0.002$ respectively).

Table 3.4 Glycaemic status of type-2 diabetic and control group

Tests	Type-2 diabetic patients (n=100) (mean \pm SD)	Control group (n=100) (mean \pm SD)	P-value
Fasting blood sugar (mmol/L)	7.33 \pm 2.85	5.21 \pm 0.5270	0.007
Blood sugar 2HABF(mmol/L)	10.88 \pm 3.03	6.78 \pm 0.883	0.014
HbA _{1c} (%)	7.79 \pm 1.68	5.69 \pm 0.360	0.002

Nutritional status of the study population

Among 100 type-2 diabetic patients, 70 (70%) people had normal BMI (18.5-24.9 kg/m²), 20 (20%) were overweight (BMI 25.0-29.9 kg/m²), 04 (04%) were obese (BMI>30.0kg/m²) and 06 (6%) were malnourished (BMI<18.5 kg/m²).

Among 100 normal people, 70 (70%) had normal BMI, 10 (10%) were overweight but 20% were obese but had no malnourished people (Table 3.5 and Appendix Figure II).

Table 3.5 Nutritional status of type-2 diabetic and control group expressed in BMI

	Case (n=100)		Control (n=100)	
	Number	Percentage (%)	Number	Percentage (%)
Normal	70	70	70	70
Over weight	20	20	10	10
Obese	4	4	20	20
Malnutrition	6	6	00	00

Age and sex distribution of the study population

In case group out of 100, 56 (56%) were male and 44 (44%) were female; in control group out of 100, 56 (56%) were male and 44 (44%) were female. Mean age of type-2 diabetic patients and normal healthy people were 54.06 and 55.30 years respectively.

Maximum age groups were between 41-50 years of age (50% and 60% respectively) and minimum age groups were between 71-80 years of age (04% and 00% respectively) (Table 3.6 and Appendix Figure III).

Table 3.6 Age and sex distribution of study population

Age group (yrs)	Case (n=100)			Control (n=100)		
	Male	Female	Total	Male	Female	Total
41-50	30	20	50	30	30	60
51-60	20	16	36	16	08	24
61-70	04	06	10	10	06	16
71-80	02	02	04	00	00	00
Total	56	44	100	56	44	100

Distribution of residence and occupation of study population

In case group out of 100 patients, 58 (58%) and 42 (42%) lived in urban and rural area respectively and in control group 60 (60%) and 40 (40%) lived in urban and rural area respectively.

In case group 18 (18%), 16 (16%), 30 (30%), 36 (36%) patients were Farmer, Businessman, Service holder and Housewife respectively, on the other hand in control group 16 (16%), 14 (14%), 50 (50%), 20 (20%) people were Farmer, Businessman, Service holder and Housewife respectively (Table 3.7 and Appendix Figure IV_a & IV_b).

Table 3.7 Residence and occupation of study population

A) Residence	Case		Control	
	No.	(%)	No.	(%)
Urban	58	58.0	60	60.0
Rural	42	42.0	40	40.0
Total	100	100.0	100	100.0
B) Occupation				
Farmer	18	18.0	16	16.0
Business	16	16.0	14	14.0
Service holder	30	30.0	50	50.0
Housewife	36	36.0	20	20.0
Total	100	100.0	100	100.0

Distribution of age, BMI, duration of DM, and glycaemic status among normal and abnormal LFTs groups

Mean age (years) of normal and abnormal LFTs group were 56.83 and 52.05 and SD were ± 10.16 and ± 8.01 respectively. The difference of age of these two groups was statistically significant ($P < 0.05$).

Mean BMI of normal and abnormal LFTs group were 22.24 and 23.57 respectively and standard deviation was ± 2.57 and ± 4.22 respectively. The difference of the BMI of these two groups was not significant. ($P > 0.05$).

Duration of diabetes (years) of normal and abnormal LFTs group were 5.29 and 4.08 respectively and SD was ± 4.01 and ± 4.18 respectively. The difference of the duration of diabetes was not significant ($P > 0.05$).

HbA1c (%) level of normal and abnormal LFTs groups were 7.74 and 7.82 respectively and SD were ± 1.73 and ± 1.66 . This difference was also not significant ($P > 0.05$).

Fasting blood sugar (m mol/L) level of normal and abnormal LFTs groups were 7.22 and 7.40 respectively and SD were ± 2.50 and ± 3.09 respectively. This difference was also not significant ($P > 0.05$).

Blood sugar 2 hours after breakfast (mmol/L) level of normal and abnormal LFTs groups were 10.81 and 10.93 respectively and SD were ± 2.86 and ± 3.17 respectively. This difference was also not significant (Table 3.8).

Table 3.8 Distribution of age, BMI, duration of diabetes and glycaemic status among normal and abnormal liver function test group

	Normal LFTs group (n=42) (mean±SD)	Abnormal LFTs group (n=58) (mean±SD)	P- value
Age (yrs)	56.83±10.16	52.05±8.09	<0.05
BMI (Kg/m ²)	22.24±2.57	23.57±4.22	>0.05
Duration of diabetes (years)	5.29±4.01	4.08±4.18	>0.05
HbA1C (%)	7.74±1.73	7.82±1.66	>0.05
FBS (mmol/L)	7.22±2.50	7.40±3.09	>0.05
2HABFS (mmol/L)	10.81±2.86	10.93±3.17	>0.05

Distribution of sex and residence of normal and abnormal LFTs groups

In normal LFTs group 23 (54.8%) and 19 (45.2%) were male and female respectively and in abnormal LFTs group 38 (65.5%) and 20 (34.5%) were male and female respectively.

In normal LFTs group 28 (66.6%) resided in urban and 14 (33.3%) in rural area and in abnormal LFTs group 30 (51.7%) resided in urban and 28 (48.3%) in rural area (Table 3.9 and Appendix Figure V_a-V_d).

Table 3.9 Sex and residence among normal and abnormal LFTs groups

A) Sex	Normal LFTs groups (n=42)		Abnormal LFTs groups (n=58)	
	No.	(%)	No.	(%)
Male	23	54.8	38	65.5
Female	19	45.2	20	34.5
Total	42	100.0	58	100.0
B) Residence				
Urban	28	66.6	30	51.7
Rural	14	33.3	28	48.3
Total	42	100.0	58	100.0

Distribution of USG findings of hepatobiliary system of Type-2 diabetic patients

Among 100 type-2 diabetic patients, 40 (40%) had normal, 34 (34%) had mild fatty change, 23 (23%) had moderate fatty change in liver; 03 had mild hepatomegaly. None had cirrhosis or hepatocellular carcinoma (Table 10 and Appendix Figure VI).

Table 3.10 USG of HBS in type-2 diabetic patients (n=100)

Findings	Frequency	Percentage (%)
Normal	40	40
Mild fatty change	34	34
Moderate fatty change	23	23
Mild hepatomegaly	03	03

CHAPTER FOUR

DISCUSSION

Type-2 diabetes mellitus is increasing throughout the world, particularly in Asia including Bangladesh.¹ Virtually the entire spectrum of liver disease is seen in patients with type-2 diabetes. This includes abnormal liver enzymes, non alcoholic fatty liver disease, cirrhosis, hepatocellular carcinoma and acute liver failure.¹⁴

This study was conducted regarding evaluation of liver function tests of type-2 diabetic patients among Bangladeshi population to increase awareness about the close interrelationship between liver function tests abnormalities and type-2 diabetes.

Elizabeth H. Harris¹³ showed in their study, 22.9% Alanine Aminotransferase and 10.2% serum bilirubin were elevated. In this study which were 31.03% and 5.17% respectively. Prevalence of Alanine Aminotransferase elevation was more and serum bilirubin elevation was less in this study.

Their study showed BMI>25 kg/m², poor diabetic control and the duration of diabetes 4 years had more elevated ALT, present study also showed abnormal LFTs group had more BMI, poor diabetic control, and less duration of diabetes than normal LFTs group.

Elizabeth H. Harris¹³ had done liver biopsy of 68 patients of type-2 diabetes having abnormal LFTs. Of the 68 patients 5 had normal liver histology and 63 patients with abnormal liver histology, 48 had fatty liver or steatosis with

non-specific inflammatory change, whereas 14 had evidence of fibrosis. In our study we did not done any liver biopsy.

In a larger study, Elizabeth H. Harris¹³ analyzed 18,825 non institutional patients within the United States with an over sampling of Africans Americans and Mexican Americans. Of the total sample 6.7% had type-2 diabetes, of these with type-2 diabetes; the prevalence of elevated Alanine Aminotransferase was 7.8%, compared to a 3.8% prevalence in those without diabetes.

In the present study, prevalence of elevated Alanine Aminotransferase is much more 31.03% in type-2 diabetes and more close 2% in normal people i.e. without diabetes.

Sherif Gonem, Afan Wall, Parijat De¹² also studied over 959 patients of type-2 DM and looked mainly for Alanine Aminotransferase, Alkaline phosphatase and Serum bilirubin levels. 151 (15.7%) had raised ALT, 100 (10.4%) had raised ALP and 37 (3.9%) had a raised bilirubin. In this study ALT 31.03% and Serum bilirubin 5.17%.

The combination of raised Serum bilirubin and ALT was seen in 10 patients (1%), Bilirubin and ALP was seen in 7 patients (0.7%) and both ALP and ALT were raised in 27 patients (2.8%) in their study.

In this study combination of raised Serum bilirubin and ALT was seen in 4 patients (4%) and Serum bilirubin and ALP was seen in 1 patient (1%) and both ALP and ALT were raised in 4 patients (4%). Beside these combination of raised ALT and prolonged PT was seen in 26 patient (26%) and combination of raised ALT and AST were seen in 6 patients (6%). Combination of raised ALT and decreased Serum Albumin was seen in 7 patients (7%).

Cusi and Kenneth⁴² in 2009 showed that approximately 70% person with type-2 diabetes mellitus had a fatty liver and the disease follows a more aggressive course with necroinflammation and fibrosis. New evidence suggests that it is not steatosis *per se* but the development of lipotoxicity-induced mitochondrial dysfunction and activation of inflammatory pathways that leads to progressive liver damage. Non alcoholic steatohepatitis is a leading cause of end-stage liver disease.

In the present study fatty change in liver was 57%, among them 34% had mild fatty change and 23% had moderate fatty change.

The prevalence of NAFLD in diabetes is estimated at 34-74% and in diabetes with obesity, at virtually 100%. While once considered a benign process, NASH has been found to lead in cirrhosis and in some cases to hepatocellular carcinoma. Of the patients with NAFLD 50% had NASH and 19% had cirrhosis at the time of diagnosis.¹⁴

Type-2 diabetic patients had 80% more fat than age, weight, and sex-matched non-diabetic subjects. S-Alanine Aminotransferase under estimates liver fat in type-2 diabetic patients.⁴³ We did not measure liver fat contain but diabetic patients of our study had 31% fatty liver and in normal people 13.3%.

Sidhartha Das⁴⁴ showed in 1999 that about one fourth of type-2 diabetes mellitus patients had a body mass index (BMI) below 19, i.e. low body weight type-2 DM in India. In this study 6% of type-2 DM patients were malnourished i.e. BMI <18.5 (kg/m²) and 70% were normal i.e. BMI within (18.5-24.5 kg/m²).

In a study in Mumbai, India in 2004, 49 out of 100 type-2 diabetic patients had fatty liver and 32 of these 49 patients underwent liver biopsy. 4 of 32 (12.5%) individuals had steatosis alone. Mild, moderate and severe NASH was present in 21/32 (65.5%), 4/32 (12.5%) and 3/32 (9.35%) respectively and fibrosis was present in 7/32 (21.8%) patients (four grade I and three grade II).⁴⁵

Shahid Ahmed, Nadir Ali, Zafor Abdullah, Mohammad Ihyas, Uzma Naeem⁴⁶ in 2006 in a study in Pakistan showed positive association of raised Alanine Aminotransferase in type-2 diabetic patients with high body mass index, recent onset of diabetes, fatty liver and poorly controlled diabetes. Our study was consistent with that study.

M.A. Meybodi, M. Afkhami-Ardekari and M. Rashidi⁴⁷ in 2005 in Iran showed in their study that elevated Alanine Aminotransferase and Aspartate Aminotransferase was found in 10.4% and 3.3% respectively which were less than our study. Mean age of patients were 58.8 ± 11.5 years which was consistent with our study but duration of diabetes and BMI were more than our study. Mean of Alanine Aminotransferase and Aspartate Aminotransferase were 24.67 ± 23 and 24.57 ± 15 respectively which were also less than our study.

Several studies showed abnormalities of liver function tests which reflect the excretory function (Serum Bilirubin, Serum Alkaline Phosphate) and hepatocytes injury (ALT and AST), but did not showed any synthetic function abnormalities i.e. prothrombin time, and Serum albumin level. We had also done tests, which reflect the synthetic function of liver. In this study of type-2 diabetes (10.34%) had low S. albumin level (4% had mild and 2% had moderate low) and 43.10% had prolonged prothrombin time (17 had mild and 08 had marked prolongation).

SUMMARY

Type-2 DM is a complex condition characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production and abnormal fat metabolism.² Although the prevalence of both type-1 and type-2 is increasing world wide, the prevalence of type-2 DM is rising much, more rapidly.³

As a result of insulin resistance in adipose tissue and obesity, free fatty acid flux from adipocytes is increased, leading to increased lipid synthesis in hepatocytes. This lipid storage or steatosis in the liver may lead to non-alcoholic fatty liver disease and abnormal liver function test.³

A total 200 persons were included in this study, of them 100 were diagnosed type-2 diabetes mellitus patients and 100 were apparently healthy people. All the study populations were free from pre-existing known liver diseases and also free from taking all kinds of hepatotoxic drugs even oral hypoglycaemic drugs like Metformin, Pioglitazone, Rosiglitazone etc. causing hepatocytes injury.

Our study showed most of the liver function tests was significantly abnormal in type-2 diabetic patients than normal healthy peoples. 57% patients of type-2 diabetes had fatty liver, which are about 3 fold more than normal people, which are 20%. None had cirrhosis or hepatocellular carcinoma. Ultrasonographically, though other imagings and liver biopsy not done.

Among 100 patients of type-2 DM, 42 patients had no LFTs abnormalities and 58 patients had some sought of LFTs abnormalities. Abnormal LFTs group patients had more BMI and poor status of glycaemic control but had less mean age than normal LFTs group patients.

CONCLUSION

A high proportion of patients with type-2 diabetes mellitus in our country have abnormal liver function tests that may be a marker of NAFLD, NASH and insulin resistance. Currently, routine liver function screening is not being advocated in type-2 diabetes but emerging evidence suggests that abnormal LFTs may be a marker of metabolic syndrome and insulin resistance in type-2 diabetes such patients would thus warrant more intensive glycaemic control, obesity, dyslipidemia and hypertension to be controlled that reduce cardiovascular risk and also to prevent progression to significant hepatic dysfunctions like cirrhosis and hepatocellular carcinoma.

RECOMMENDATIONS

Further larger scale study may be conducted. Liver biopsy and imaging may be done with liver function tests to see the other complications like non alcoholic steatohepatitis, Fibrosis, Cirrhosis, carcinoma etc.

LIMITATIONS OF THE STUDY

1. Small scale study was conducted for the time
2. Liver biopsy and other imaging like CT scan or MRI not done

CHAPTER FIVE

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APPENDICES

APPENDIX-1

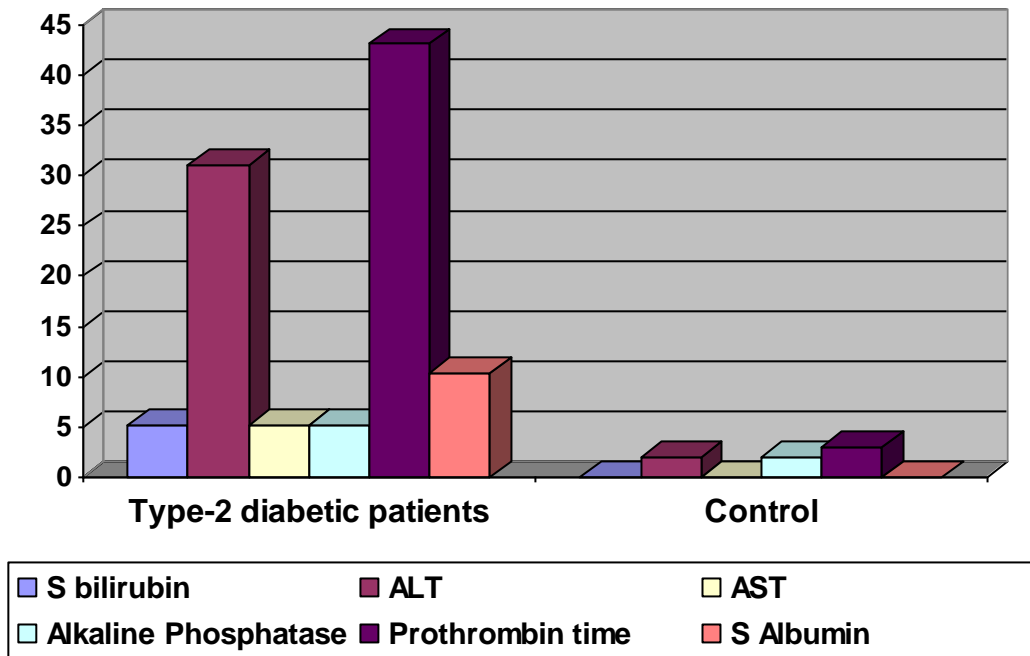


Figure I Frequency of LFTs abnormalities among type-2 diabetic patients and control (n=100)

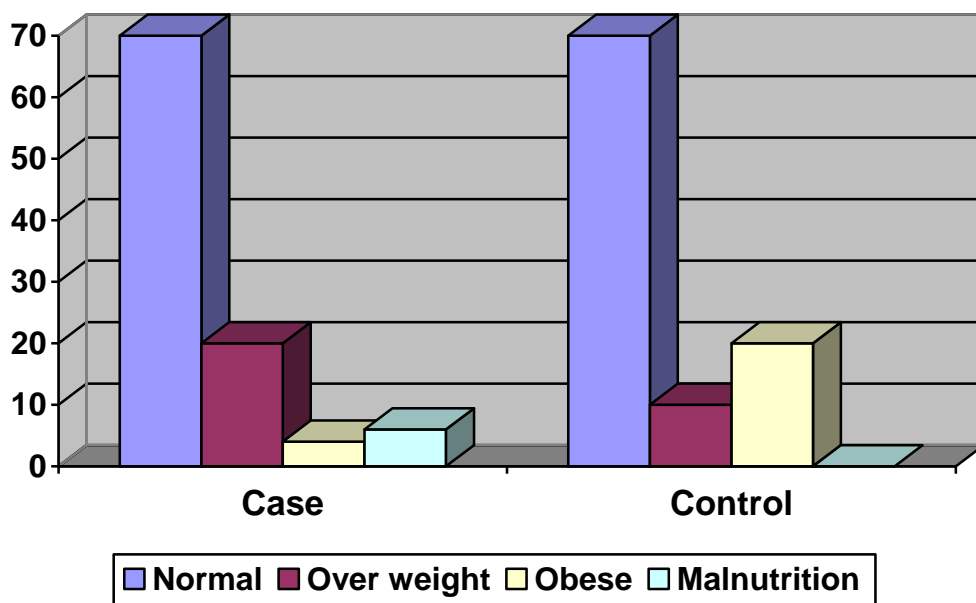


Figure II Nutritional status of type-2 diabetic and control group expressed in BMI

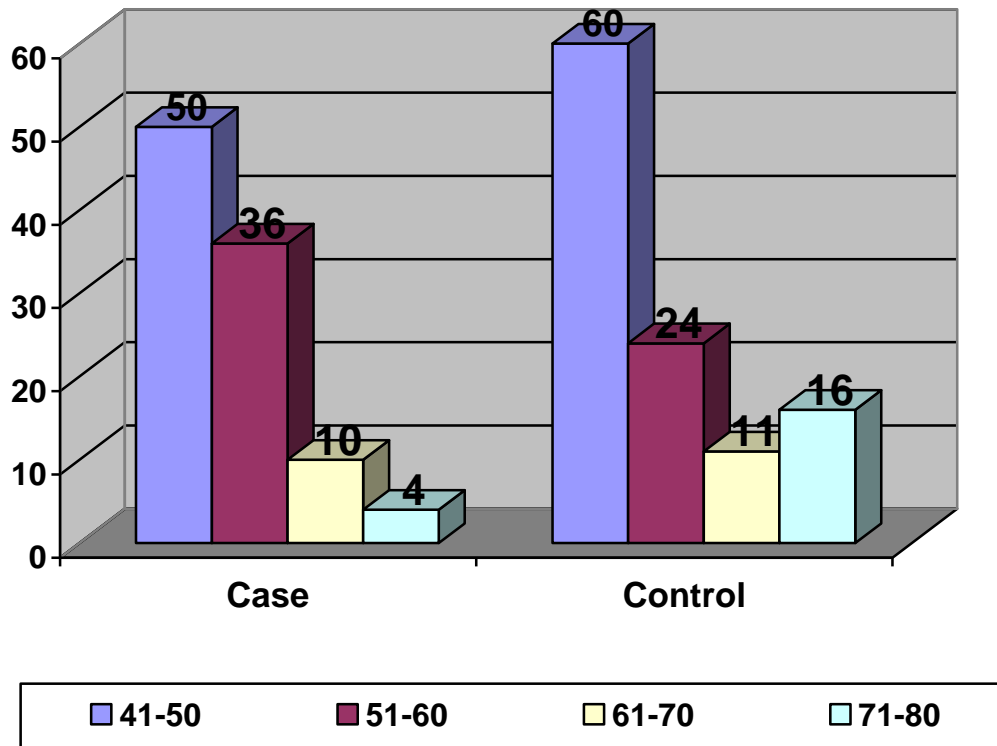


Figure III Age and sex distribution of study population

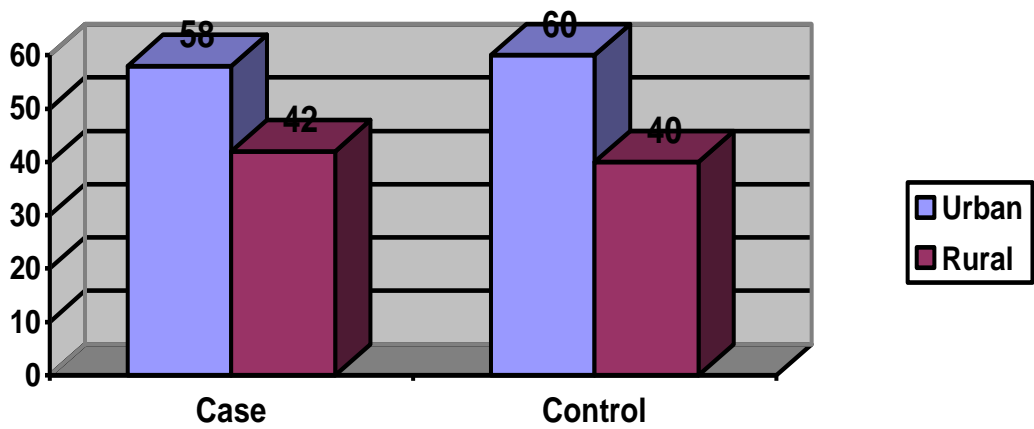


Figure IV_a Residence of study population

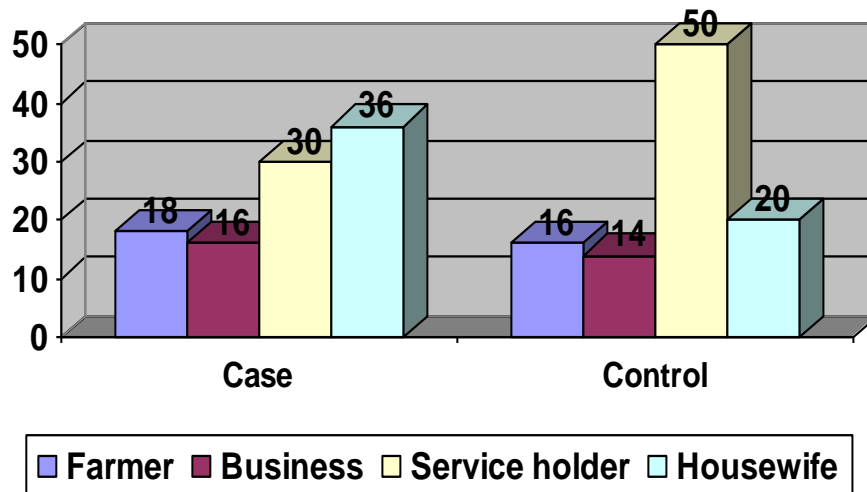


Figure IV_b Occupation of study population

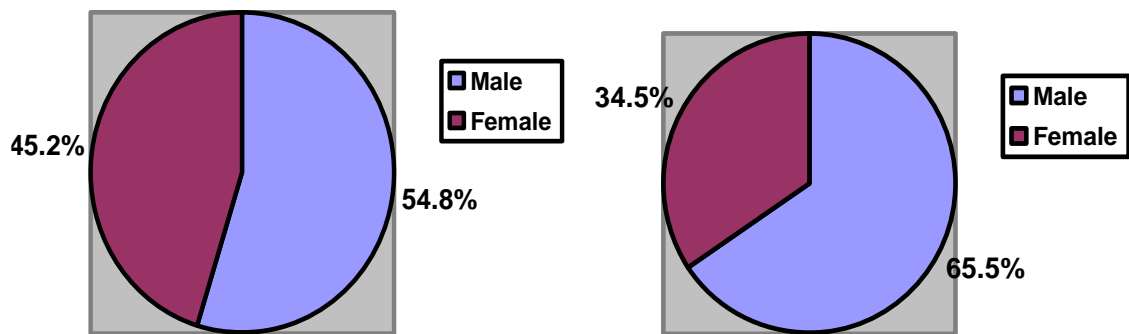


Figure V_a Normal LFTs group (n=42) Figure V_b: Abnormal LFTs group (n=58)

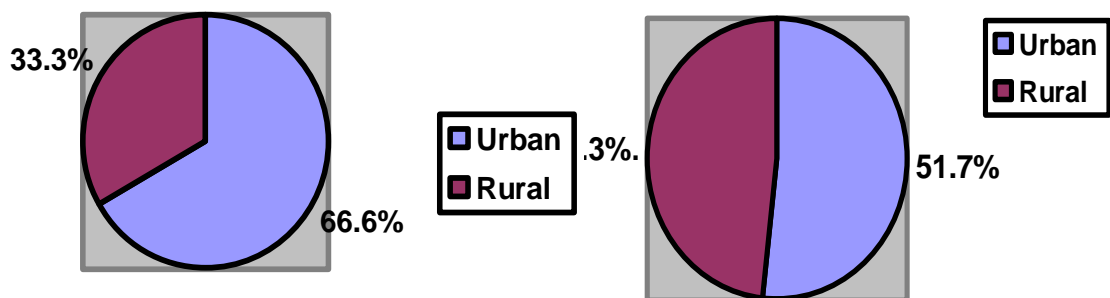


Figure V_c Normal LFTs group (n=42) Figure V_d Abnormal LFTs group (n=58)

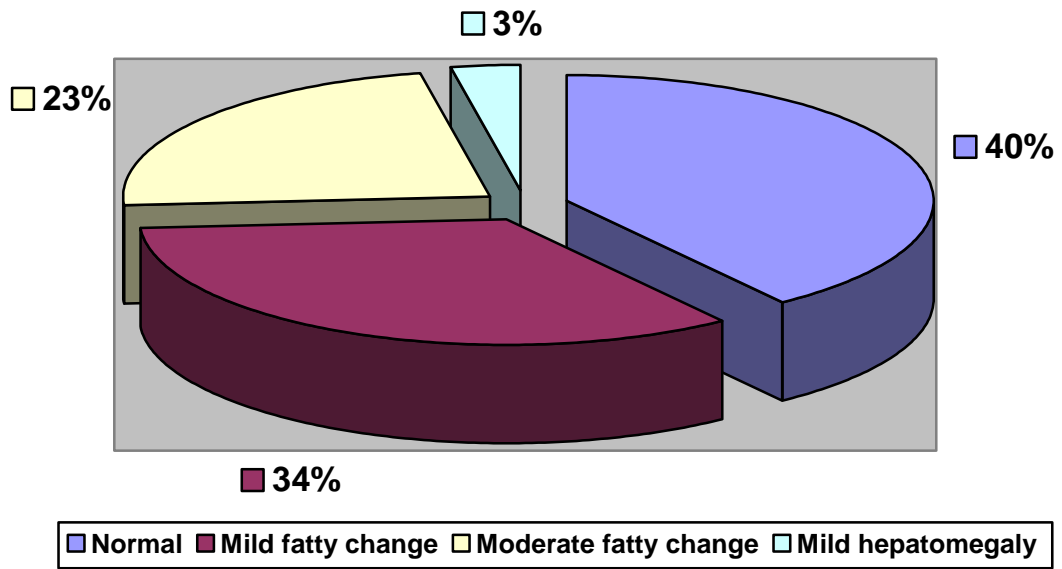


Figure VI USG of HBS in type-2 diabetic patients (n=100)

Appendix 2
Data Record Sheet

I.D. No: _____ Date: _____

MU: _____ Ward: _____ Bed: _____ MOPD Reg.No: _____

Particulars of the patient:

Name: _____

Age: _____ Sex: _____ Marital status: _____

Present Address: _____

Permanent Address: _____

Occupation: Farmer/Businessman/Service holder/Student/Housewife

Duration of diabetes mellitus: _____

At present anti diabetic therapy: _____

Personal history:

Alcohol Consumption/Drug Abuse:

Sexual Exposure: _____

Others: _____

Immunization history:

Vaccination against HBV

Vaccination against HCV

At present whether the patient is taking the following drugs or not:

- Acetaminophen,
- NSAIDS,
- Methotrexate,
- Amiodarone,
- Bleomycin,
- Tamoxifen
- Sodium Valproate

Physical Examination:

Appearance: _____ Pulse: _____

Body build: _____ Blood pressure: _____

Weight loss: _____ Temperature: _____

Weight gain: _____ Weight(kg): _____

Anaemia: _____ Height(meter): _____

Jaundice: _____ Waist: _____

Circumference: _____ Ascites: _____

Hepatomegally: _____

Splenomegally: _____

EXAMINATION OF OTHER SYSTEMS:

INVESTIGATIONS:

1. Blood Sugar-Fasting and 2 hrs after :
breakfast with CUS
2. HbA1c :
3. Serum Billirubin :
4. Serum Alanine Aminotransferase :
5. Serum Aspirate Aminotransferase :
6. Prothrombin time :
7. Serum Albumin level :
8. Lipid profile :
9. HBSAg :
10. Anti-HCV :
11. Ultrasonography of hepatobilliary :
system
12. CT, MRI of hepatobiliary system :
13. Serum alkaline phosphatase :

FOLLOW-UP

Appendix-3

(Bengali Consent Form separately attached on the folder)

গবেষণাধীন ব্যক্তির জ্ঞাপনোত্তর সম্মতিপত্র

আমি..... বয়স.....

পিতা/স্বামীর নাম..... ডায়াবেটিস ও যকৃত রোগে আক্রান্ত হয়ে রাজশাহী মেডিকেল কলেজ হাসপাতালের লিভার বিভাগের বিশেষজ্ঞ চিকিৎসকের অধীনে ভর্তি আছি। ডাঃ মোহাঃ হারুন-অর-রশীদ কর্তৃক জানতে পারি যে, কি কারণে এ রোগ হয়ে থাকে এবং এ রোগ থেকে কি কি জটিলতা হতে পারে। ডায়াবেটিস রোগে আক্রান্ত রোগীর লিভারে এর প্রভাব পড়ে বলে এ বিষয়ে তিনি আমাকে অবহিত করেছেন। লিভার রোগের বহিঃপ্রকাশ দেখে রোগের ধরণ এবং তীব্রতা সম্পর্কে যে পূর্বাভাস পাওয়া যায় সে সংক্রান্ত বিষয়ে গবেষণা কর্মে আমি স্বেচ্ছায় অংশগ্রহণ করতে ইচ্ছুক, আমাকে এ বলে আশ্বাস দেওয়া হয়েছে যে, এ গবেষণা কার্যক্রমে কোন পরীক্ষার জন্য আমার কোন শারীরিক বা মানসিক ক্ষতি হবে না। আমাকে আরও জানানো হয়েছে যে, এ গবেষণা ফলদায়ক হবে এবং ভবিষ্যতে আমাদের দেশে এ ধরণের পরীক্ষার রোগীরা যথেষ্ট উপকৃত হবে। আমি এ গবেষণা কার্যক্রম হতে নিজেকে প্রত্যাহার করার অধিকার রাখি। আমি এ গবেষণার জন্য কোন আর্থিক সুবিধা গ্রহণ করবো না। আমার ব্যক্তিগত গোপনীয়তা রক্ষা করা হবে। আমি স্বেচ্ছায়, স্বজ্ঞানে ও সানন্দে এ সম্মতিপত্রে স্বাক্ষর করলাম।

তারিখঃ.....

রোগীর স্বাক্ষর/বৃদ্ধাঙ্গুলের ছাপঃ

নামঃ

ঠিকানাঃ

গবেষকের স্বাক্ষর ও তারিখ

CONSENT FORM
(English version)

I am Mr./Ms..... age.....fathers/husband's name suffering from Diabetes and liver disease got admitted in Rajshahi Medical College Hospital under the supervision of liver specialist. I was informed from Dr. Mohd. Harun-Or-Rashid about the causes and complications of the diabetes. He informed me regarding the effect on liver resulting from disease. I wish to participate in the research work regarding the forecast of the types and intensity of the disease manifested liver dysfunction. I am fully convinced that during study I shall not suffer from any serious physical or psychological problem. If I am injured as a result of being in this study, treatment will be available. I am also informed that this study was carried out in the developing countries safely & my participation will bring fruitful result that will be beneficial for the most patients. I have rights to discontinue the participation of the study at any time. I shall not receive any financial benefit. My privacy and confidentiality will be safe guarded and my anonymity will be protected. I am willingly signing this consent form gladly with full conscience.

Date:

Signature of participant and date

Name:

Address:

Signature of researcher and date